WEDNESDAY—MORNING SESSION

REPORT ON CANNED VEGETABLES AND VEGETABLE PRODUCTS

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

Owing to the possibility of confusion with other chapters, it is suggested that the name of Chapter XXXV, Vegetables and Vegetable Products, be changed to Processed Vegetable Products. This would include canned, dried, or frozen vegetables, and any methods for raw vegetables could be included in Chapter XII, Plants.

Certain methods in Chapter XXXV are obsolete or of little value, and others seem to belong more properly in other chapters. For example, the tentative method for physical examination and the official methods for volatile and fixed acids are obsolete, while the tentative method for the determination of sand and the official methods for microanalysis of tomato products seem more suitable for a chapter on extraneous material.

In his report on volatile and lactic acids in fruit and fruit products Hillig includes collaborative work on tomato products to show that, with proper preparation of samples as prescribed in his report, the methods are satisfactory for canned vegetables.

Fischbach reports on moisture in dried vegetables and shows that the standard method prescribed in Quartermaster Depot specifications for dried vegetables is sufficiently accurate for adoption as a tentative method, but suggests that it be further studied before it is adopted as an official method. His work on vapor pressure determinations for indicating moisture content gives considerable promise for the determination of the true moisture content of dried vegetables.

RECOMMENDATIONS*

It is recommended—

(1) That the tentative method for the physical examination of canned vegetables (**1**, p. 518)[1] be dropped.

(2) That the official method for the determination of volatile acids in canned vegetables (**10**, p. 519[1] and for volatile and fixed acidity in tomato products (**28, 29**, p. 522)* be dropped (final action).

(3) That the method for preparation of sample (**2**, p. 518)[1] and the methods for the determination of volatile and lactic acids in tomato and other vegetable products, recommended in the report of the Associate Referee on Volatile and Lactic Acids in Fruit and Fruit Products, be adopted as official (final action under suspension of the rules).

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* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 56 (1945).

[1] *Methods of Analysis, A.O.A.C.*, 1940.

(4) That the method for the determination of moisture in dried vegetables recommended by the Associate Referee be adopted as tentative.

(5) That studies of vapor pressure as an index of moisture content of dried vegetables be continued.

(6) That the tentative method for the determination of sand (**21**, p. 521)[1] and the official methods for the microanalysis of tomato products (**30–33**, pp. 522–524) be transferred to a chapter on extraneous material.

(7) That studies of methods for quality and fill of container be continued.

No report on quality-factors and fill of container was given, no associate referee having been appointed.

REPORT ON MOISTURE IN DRIED VEGETABLES

By Henry Fischbach (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

With the advent of the war the production of dehydrated vegetables became widespread. Quartermaster Corps specifications[1] for dehydrated carrots state that "Moisture determination shall be made by the vacuum oven method by drying two grams of the product at 70°C., for 6 hours in partial vacuum having a pressure of not over 100 mm. of mercury, or by any other method which gives equivalent results." A similar requirement appears in each of the other dehydrated vegetable specifications. Since most of the dehydrated vegetable pack is now purchased for the armed forces, drying 6 hours in vacuo at 70°C. is the present standard method of control of moisture content. However, details are lacking for preparation of the sample prior to the drying treatment. Particle size, time of grinding, and exposure to air are but a few of the generally recognized disturbing variables inherent in the preparation of the sample.

This laboratory has completed some initial experiments along these lines, the results of which suggest the adoption as a tentative method, of a procedure for determining moisture in dehydrated vegetables.*

DISCUSSION

Table 1 shows the loss in weight due to successively longer periods of heating of potatoes, carrots, onions, and beets. These vegetables were prepared in the manner previously described and noted as Sub A. In addition, a portion of the material used for Sub A was resifted to discard all material that passed a 30-mesh screen and designated Sub B. Unfortunately, Sub

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[1] C. Q. D. No. 73, July 18, 1942.
* To be published in the 6th Edition of *Methods of Analysis, A.O.A.C.*, 1945.

TABLE 1.—*Successive drying periods at 70°C. and at approximately 50 mm. pressure*

PRODUCT	SUB	MESH SIZE	WEIGHT	WEIGHT LOST AT 3 HOURS		WEIGHT LOST AT 4 HOURS		WEIGHT LOST AT 5 HOURS		WEIGHT LOST AT 6 HOURS	
			grams	*grams*	*per cent*	*gram*	*per cent*	*gram*	*per cent*	*gram*	*per cent*
Potatoes	A	20 & less	8.4988	0.6064	7.14	0.6296	7.41	0.6336	7.46	0.6453	7.59
Potatoes	B	20–30	7.2740	.4522	6.22	.4742	6.52	.4843	6.66	.4971	6.83
Carrots	A	20 & less	9.5174	.3868	4.06	.4076	4.28	.4258	4.47	.4391	4.61
Carrots	B	20–30	10.1556								
Onions	A	20 & less	8.2441	.3976	4.82	.4080	4.95	.4083	4.95	.4216	5.11
Onions	B	20–30	7.7440	.3654	4.72	.3768	4.87	.3815	4.93	.3927	4.07
Beets	A	20 & less	8.2116	.3271	3.98	.3429	4.18	.3565	4.34	.3669	4.47
Beets	B	20–30	8.5496	.2785	3.26	.2975	3.48	.3140	3.68	.3290	3.85

PRODUCT	SUB	MESH SIZE	WEIGHT	WEIGHT LOST AT 8 HOURS		WEIGHT LOST AT 12 HOURS		WEIGHT LOST AT 18 HOURS	
			grams	*gram*	*per cent*	*gram*	*per cent*	*gram*	*per cent*
Potatoes	A	20 & less	8.4988	0.6758	7.95	0.6973	8.20	0.7010	8.25
Potatoes	B	20–30	7.2740	.5276	7.25	.5499	7.56	.5600	7.70
Carrots	A	20 & less	9.5174	.4680	4.92	.4970	5.22	.5068	5.32
Carrots	B	20–30	10.1556						
Onions	A	20 & less	8.2441	.4370	5.30	.4496	5.45	.4546	5.51
Onions	B	20–30	7.7440	.4137	5.34	.4283	5.53	.4340	5.60
Beets	A	20 & less	8.2116	.3935	4.79	.4143	5.05	.4274	5.20
Beets	B	20–30	8.5496	.3590	4.20	.3880	4.54	.4090	4.78

B of the carrots was lost. Except for the onions the total loss in weight was slightly greater for Sub A than for Sub B. However, sufficient data are not available to show whether this was due to a higher moisture content in the material discarded for Sub B or a more rapid drying of the fine material in Sub A. One week later triplicate samples were taken of each vegetable from the same tightly closed bottles from which the Sub A's of Table 1 were obtained. These were heated successively for 6 and 12 hours. The results are given in Table 2. Comparison of the data in Table 2 with those for the Sub A's in Table 1 reveals that for the 6 hour heating period these determinations check to within 0.1 per cent—in most instances to less than 0.05 per cent.

Accordingly, the method discussed is recommended for adoption as a tentative method and further work is suggested.

TABLE 2.—*Successive drying periods at 70°C. and at approximately 50 mm. pressure* (Results obtained a week later than those shown in Table 1.)

PRODUCT	WEIGHT	WEIGHT LOST AT 6 HOURS		WEIGHT LOST AT 12 HOURS	
	grams	*gram*	*per cent*	*gram*	*per cent*
Potatoes	8.3236	0.6188	7.43	0.6666	8.01
Potatoes	7.9289	.6974	7.53	.6453	8.14
Potatoes	8.5305	.6435	7.54	.6816	7.99
Carrots	7.7119	.3540	4.59	.3926	5.09
Carrots	7.5513	.3473	4.60	.3874	5.13
Carrots	8.1903	.3739	4.57	.4101	5.01
Onions	6.5608	.3364	5.13	.3489	5.32
Onions	7.7034	.3956	5.14	.4129	5.36
Onions	7.5803	.3913	5.16	.4086	5.39
Beets	8.9170	.4013	4.50	.4463	5.01
Beets	7.4455	.3285	4.41	.3634	4.88
Beets	6.8415	.3046	4.45	.3382	4.94

VAPOR PRESSURE MEASUREMENTS FOR DETERMINING THE "DRYNESS" OF DEHYDRATED VEGETABLES

For the past two years this laboratory has experimented with the idea of substituting vapor pressure measurements for the conventional method for determining per cent moisture in dehydrated vegetables. The chief advantage of the vapor pressure technic is the use of the material in the physical form in which it is received and the more gentle handling of the material during the determination. As the sample is subjected to a temperature of only 35°C. at 0.05 mm. pressure, decomposition and oxidation are minimized. Only 1–2 hours is required for a determination. The disadvantage of this method is that it requires more complicated equipment

than that used in the proposed tentative method. However, it is anticipated that it will be possible to change the period of heating in the 70°C. vacuum oven to a more justifiable point than the present arbitrary 6 hours by means of sufficient vapor pressure data. For this reason alone the Associate Referee proposes the continuation of the vapor pressure measurements.

RECOMMENDATIONS†

It is recommended—

(1) That the procedure described, in which the prepared sample is heated for 6 hours at 70°C. in vacuum, be adopted as a tentative method.

(2) That study of the tentative method be continued.

(3) That the vapor pressures of dehydrated vegetables be studied for the purpose of fixing the time of heating in the 70°C. oven so that it will be more consistent with scientific fact.

No report on fill of container methods for foods, drugs, and cosmetics was given by the Referee.

No report on coffee and tea was given by the Referee.

REPORT ON COLORING MATTERS IN FOODS

By C. F. Jablonski (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Referee*

In accordance with recommendations of the Association, the Referee sent out a number of alimentary paste samples consisting of four subdivisions each to various collaborators with instructions to examine same for FD&C Yellow No. 5 (Tartrazine) by a submitted method. The principal aim of the Referee was to produce samples identical in appearance to standard products and endeavor to cover the slight deficiency with minute amount of color.

The composition of the samples was as follows:

No. 1, Macaroni.—Consisted of semoline, 90%; farina, 10%. FD&C Yellow No. 5, 1 p.p.m.

No. 2, Egg Noodles.—Consisted of flour, 95.5%; whole egg, 4.5%. FD&C Yellow No. 5, 1 p.p.m.

No. 3, Macaroni.—Consisted of semoline, 100%; no color added.

No. 4, Egg Noodles.—Consisted of product made according to standard specification and contained whole egg, 5.0%; no color added.

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† For report of Subcommittee C and action by the Association, see *This Journal*, 28, 56 (1945).

Results of collaborators on tartrazine

ANALYST	SAMPLE	WOOL DYEING	COUPLING
W. C. Woodfin	1	Positive	Positive (weak)
Atlanta Station	2	Positive	Positive (weak)
	3	Absent	Negative
	4	Absent	Negative
Phyllis B. Rokita	1	Present	Positive (weak)
Atlanta Station	2	Present	Positive (weak)
	3	Absent	Negative
	4	Absent	Negative
H. W. Haynes	1	Absent	Negative
Boston Station	2	Absent	Negative
	3	Absent	Negative
	4	Absent	Negative
C. S. Purcell	1	Negative	Negative
Boston Station	2	Negative	Negative
	3	Negative	Negative
	4	Negative	Negative
Harry J. Fisher	1	Positive	Negative
Agr. Expt. Sta.,	2	Positive (weak)	Negative
New Haven, Conn.	3	Negative	Negative
	4	Negative	Negative
James J. Winston	1	Positive	Slightly positive
Cereal Product Lab.	2	Positive	Slightly positive
New York	3	Negative	Negative
	4	Negative	Negative
Marie L. Offutt	1	Positive	Positive
New York Station	2	Positive	Positive
	3	Negative	Negative
	4	Negative	Negative
J. L. Hogan	1	Positive	Positive
New York Station	2	Positive	Weakly positive
	3	Negative	Negative
	4	Negative	Negative
J. W. Sanders	1	Positive	Positive (orange)
Philadelphia Station	2	Positive	Positive (orange)
	3	Negative	Negative
	4	Negative	Negative
L. W. Ferris*	1	Positive	—
Buffalo Station	2	Doubtful	—
	3	Negative	—
	4	Negative	—

* All the analysts from the Buffalo Station stated that the acid solution before the addition of wool was clear on Samples 1 and 3, but cloudy on Samples 2 and 4.

ANALYST	SAMPLE	WOOL DYEING	COUPLING
B. W. Barnes Buffalo Station	1	Positive	—
	2	Inconclusive	—
	3	Inconclusive	—
	4	Negative	—
H. M. Boggs Buffalo Station	1	Positive	—
	2	Positive	—
	3	Negative	—
	4	Negative	—
T. J. Klayder Buffalo Station	1	Negative	—
	2	Negative	—
	3	Negative	—
	4	Negative	—

COMMENTS OF COLLABORATORS

W. C. Woodfin.—The coupling reactions in each of two positive cases were faint and could only be detected by comparison with the two negative cases.

H. W. Haynes.—None of the samples gave a pink color on coupling, but yellowish to brownish.

C. S. Purcell.—The standard tartrazine solution of approximately identical intensity gave a distinct color on wool, but when coupled it produced an extremely faint pink, indicating that this concentration represents the limit of sensitivity.

Harry J. Fisher.—The coupling test gave the same reaction with all four samples. A straw color with no tint of pink or red.

James J. Winston.—The use of magnesium sulfate, silicotungstic acid solution, appears to be very instrumental in removing most of the magma.

Marie L. Offutt.—Method worked smoothly, with result that fairly clear solutions were obtained.

J. L. Hogan.—No particular difficulties were encountered with this method, however the coupling reactions were not of a pink, but of an orange shade.

J. W. Sanders.—Samples 1 and 2 presented difficulties in that the sampling reactions gave an orange or brownish orange color, unlike the pink color of authentic tartrazine.

L. W. Ferris.—The double dye test on No. 1 was very satisfactory but on No. 2 there was considerable yellow color present which was not taken up by the wool. However, the alphanaphthol test on the residue after removing the wool was positive, indicating the presence of a trace of tartrazine.

B. W. Barnes.—The coupling tests on Samples 2 and 3 produced a slight color but not distinct enough for positive identification. The solutions for the double dyeing test were a faint yellow color but only a small amount of the color was dyed onto the wool. The spot tests were indistinct.

H. M. Boggs.—It is important not to evaporate the solutions too low in the first wool fixation or a brownish yellow color (possibly from the carotene present) will be fixed on the wool, which confuses later tests.

DISCUSSION

The results reported by the collaborators can be considered satisfactory. Practically every collaborator was able to detect the dye on the fabric. It was the coupling test, however, that seemed to give considerable diffi-

culty. Almost every collaborator reports an orange or brownish orange coloration, which is evidence that the limit of sensitivity has been overstepped. Considering the fact that the maximum amount of dye which can possibly be extracted by following the directions is only 0.2 microgram, a portion of which is used for fabric dyeing, and considering further that another portion is occluded with the magma precipitate, it is indeed remarkable that almost every collaborator was able to note a change of color upon coupling.

The Referee is therefore of the opinion that the method proposed is quite adaptable to detect very small amounts of FD&C Yellow No. 5. This is of considerable importance since deficiencies of 0.5 per cent of egg solids or slightly inferior semolina flour may easily be improved with 1 or 2 p.p.m. of tartrazine. Another trouble phase that seems to have been eliminated to a large degree is the cumbersome emulsions, which hampered isolation and separation of the coloring matter. It may be advantageous to repeat these experiments, increasing the amount of dye to a point where a definite coupling reaction is obtained, this being of great importance in confirming the presence of tartrazine.

RECOMMENDATIONS*

It is recommended—

(1) That collaborative work be repeated on detection of small amounts of FD&C Yellow No. 5.

(2) That investigational work be continued on the quantitative separation of FD&C Yellow No. 5 (Tartrazine) and FD&C Yellow No. 6 (Sunset Yellow FCF).

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green SF Yellowish), FD&C Green No. 3 (Fast Green FCF), and FD&C Blue No. 1 (Brilliant Blue FCF).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow AB), FD&C Yellow No. 4 (Yellow OB), FD&C Orange No. 2 (Orange SS), and FD&C Red No. 32 (Oil Red XO).

(5) That collaborative work on analytical methods for coal tar colors certifiable for use in foods be conducted.

REPORT ON DAIRY PRODUCTS

By G. G. FRARY (State Chemical Laboratory, Vermillion, S. Dak.), *Referee*

For the 1945 revision of *Methods of Analysis, A.O.A.C.*, your Referee feels that all methods contained in the book should be examined with all

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 57 (1945).

the care permitted by the manifold duties that are necessary during these trying war times. Therefore, the Referee will make recommendations* in regard to the subject matter in Chapter XXII, p. 269, which covers dairy products.

Acidity.—This method, **4**, is essentially the same in the present book as when it first appeared in the 2nd edition. It is still a tentative method and should remain so until further studied and amended in keeping with the modern conceptions of acidimetry. Work reported by the Englishman, Barkworth,[1] and confirmed by work in this laboratory, shows the importance of concentration of the phenolphthalein indicator in the solution being titrated; also the effect of dilution of the milk sample. It is therefore recommended that the method for acidity be studied with particular reference to concentration of the indicator and effect of dilution of the milk. Slight editorial changes in the wording of the method as now written are recommended to the Revision Committee.

Total Solids.—The tentative method for determination of the approximate amount of total solids in milk calls for use of the lactometer. While it is not so stated, the instrument used in the great majority of cases is the Quevenne and the formula given presupposes the use of this instrument. In applying this method it is customary to obtain the Quevenne reading at any convenient temperature not too far from 60°F. and then use a temperature-correction table. It is also customary to use a table for obtaining from the figures for fat and hydrometer reading the final result in terms of total solids. Such tables have not been provided in the book. Since suitable reference tables may not be available in some laboratories it is recommended that the necessary tables be printed in the forthcoming revision. It is also suggested that the tables found on pages 144, 177, and 178 of Paul G. Heineman's book entitled "Milk," published by W. B. Saunders Co. in 1921, are suitable. The second of these tables was published by Shaw and Eccles in Bulletin 134, B.A.I., U. S. Department of Agriculture.

The official method for the determination of total solids in milk should be revised editorially so as to better conform to its use with evaporated milk. Therefore, the Referee concurs in the recommendation of the Associate Referee on this item.

Ash.—Methods for total solids and ash in milk and evaporated milk were studied under the Referee's direction in 1939, and the results were published in *This Journal*, **23**, 3, 453. The results of the 1939 study were satisfactory, and at that time it was expected that further studies would be made, hence this plan was recommended. However, conditions prevented carrying on the work. Since the methods used in the 1939 collab-

* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 57 (1945). The methods will be published in the 6th edition of *Methods of Analysis*, *A.O.A.C.*, 1945.
[1] *Dairy Ind.*, Vol. 5, 188 and 238 (1940).

orative work are believed now to be in general use, the Referee concurs in the current report of the Associate Referee and his recommendations as to ash and total solids in milk and evaporated milk.

Fat.—In the 5th edition of *Methods of Analysis, A.O.A.C.*, some editorial changes were made in the official extraction method for fat in milk, **20.** The method has long specified the use of the "Röhrig tube or similar apparatus." A broad construction of the phrase "or similar apparatus" has prevailed, for it is well known that use of the Mojonnier tube or flask has become standard practice in many laboratories, probably in most laboratories. This odd-shaped piece of apparatus has amply demonstrated its usefulness and practicability. Hence, the name should be inserted in the method when it is published in the 6th edition. The recommendation includes also the option of centrifuging instead of long standing and is made by the Associate Referee on Fat in Dairy Products, who also recommends some editorial change in the method for preparation of sample of evaporated milk. The recommendations are the result of an intensive study of the preparation of sample and determination of fat in evaporated milk, and the Referee concurs in these recommendations of the Associate Referee.

Formaldehyde in Milk.—Under the heading, "Preservatives—Official," **30,** the reader is referred to the chapter on "Preservatives and Artificial Sweeteners," XXXII, in the fifth edition. Among the tests there outlined for formaldehyde is the well-known Hehner test, which appears first to have been mentioned in print in an article appearing in *The Analyst* in 1895. The test is made by underlaying some of the suspected milk with commercial sulfuric acid, or pure sulfuric acid containing a trace of ferric chloride. A blue or violet color indicates formaldehyde. Fulton[1] has shown this test to be due to the reaction between formaldehyde and the tryptophan component of proteins, hence solutions of other proteins than those found in milk, e.g. egg albumin, may be used. Fulton showed that bromine is much better than ferric chloride as an oxidizing agent in this reaction and he states that as little as 1 p.p.m. of formaldehyde may be detected by use of this modification. David W. Horn, Bryn Mawr, Pa., has confirmed Fulton's work and compared[2] this sulfuric acid-bromine test with others often used. This matter is referred to the Referee on Preservatives and Artificial Sweeteners, with the request that the method be studied as to its applicability to milk and to its advantages over the present Hehner method.

Vitamin D in Milk.—It is the belief of the Referee that a separate chapter on vitamins should be included in the 6th edition of *Methods of Analysis, A.O.A.C.*, therefore it is recommended that the material in Chapter XXII relative to vitamin D in milk be transferred to such new chapter.

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[1] *J. Ind. Eng. Chem., Anal. Ed.,* 3, 199 (1931).
[2] Pub. Wagner Free Institute of Science, IV, part 1, 1944.

Phosphatase Test.—The Associate Referee is studying application of the phosphatase test to cheese in order to determine whether milk from which the cheese was made had been properly pasteurized. It is recommended that this study be continued.

Sampling Evaporated Milk.—The instructions for preparation of sample of evaporated milk, **66**, p. 289, include warming of the sample in an open dish and mixing in order to re-incorporate any fat which may have separated while in the can. Much more effective stirring can be accomplished and possible loss of moisture avoided by heating the milk in the can, with occasional thorough shaking. The Associate Referee on Fat in Milk and Evaporated Milk has made a study of this problem and is recommending the editing of the method to take care of this situation. The Referee concurs in this recommendation.

Butter.—In preparing copy for the fifth edition of *Methods of Analysis,* the editors inserted in paragraph **99**, the official method for moisture, the number of paragraph **96**, which is one of the two methods given for preparation of a butter sample for analysis. This has occasioned no little uncertainty as to whether a moisture analysis made on a sample prepared by the mechanical stirrer method, **97**, would be considered as conforming to the official method. It appears that the insertion of the one reference without the other must have been an oversight and leads to a comment on the lack of uniformity throughout the book in reference to prepared samples. In many cases methods are given for "Preparation of Sample." Then instructions for determining different components or factors on this prepared sample may direct to weigh a portion of the "prepared sample," or to weigh a portion of the "sample" and may give the number of the paragraph preceding, which contains a method for sample preparation (as in the case in point on butter analysis), or the instructions may refer only to "the sample" with no reference to preparation of the material to insure uniformity of composition in the portion under examination. The methods should be carefully edited in this respect and uniformly worded. Instructions should be definite and specific. This can be accomplished in either of two ways. On the page allotted to "Definitions of Terms and Explanatory Notes," which precedes the first chapter, a note could be inserted stating, in effect, that whenever a method is given for preparing a sample for analysis, only material so prepared shall be used. Then in the instructions for analysis the expression "prepared sample" should be used instead of "sample" and no reference given to the paragraph embodying the method of sample preparation. Or, the matter could be handled by referring, in the instructions for analytical procedure, to the "prepared sample" and give in each instance the reference by number to the paragraph or paragraphs which detail the sample preparation. This method is preferred by the Referee because it is specific. Also, some analysts might not read the general instructions in the front of the book.

It is recommended, therefore, that in **99,** p. 298, the method for moisture in butter, there be inserted after the figure 96, the number of the paragraph detailing the stirrer method of sample preparation, which is **98,** thus making it clear that a sample prepared by either method may be used.

The insertion into the methods of definite temperature factors for preparation of samples of butter for analysis has resulted in some confusion and almost necessary disregard for such specifications. In **96,** p. 293, the shaking method of sample preparation, no suggestion is made as to how the analyst shall determine the temperature of the butter which he is directed to "soften in a closed vessel," presumably the original sample container. Then, too, 35° may not be warm enough for some butters and may be warm enough to cause other butters to "oil off" to such an extent that re-emulsification cannot be accomplished. The sole purpose of the procedure is to obtain a uniform mixture, and it appears inadvisable to specify definite temperatures. It is recommended, therefore, that an Associate Referee on Preparation of Butter Samples be appointed with instructions to bring in a recommendation for simplification of the methods.

Mold in Butter.—The suggestion has been made, following study of the problems involved, that a separate chapter in *Methods of Analysis* be allotted to those methods that involve use of the microscope in determining minute amounts of materials. The Associate Referee on Mold Mycelia in butter has recommended that the methods for this determination be removed from the chapter on dairy products and included in such new chapter. With this recommendation the Referee concurs, as also in his recommendations for slight changes in the manipulations which the method involves.

Cheese.—It is recommended that studies on cheese sampling be continued.

Neutralizers.—The Associate Referee has carried on further studies on the detection of added neutralizers in skim milk through composition of the ash, particularly as to its alkalinity. He now recommends that the tentative method for detection of neutralizers be adopted as official, first action. In this recommendation the Referee concurs.

Lactic Acid in Dairy Products.—The Associate Referee recommends, after further collaborative study, that the present tentative method for lactic acid in dried skim milk and other dairy products be made official, first action. In this recommendation the Referee concurs.

Ice Cream.—The Associate Referee recommends that the tentative method for coloring matter in ice cream, **135,** p. 306, be corrected as to reference and made official, first action. In this recommendation the Referee concurs, also in the additional recommendations of this Associate Referee as to methods applicable to the examination of ice cream and other frozen desserts.

REPORT ON NEUTRALIZERS IN DAIRY PRODUCTS

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

At the 1941 meeting of the Association the Tillmans-Bohrmann method for the determination of the alkalinity of the ash of milk, as a means of detecting neutralization, was recommended for tentative adoption (*This Journal*, **25**, 610). Committee C approved this recommendation (*Ibid.*, 67).

In a paper "Composition of the Ash of Dried Skim Milk and its Relation to Neutralization" (*Ibid.*, **24**, 744) the Associate Referee showed that alkali added to reconstituted unneutralized dried skim milk in the form of calcium and sodium lactate could be quantitatively recovered through a determination of the alkalinity of the ash following the method referred to above.

This year two samples of dried skim milk, one of which had been neutralized, were submitted to 13 collaborators for determinations of the ash and alkalinity of the ash. The results are given in Table 1.

While the results on Sample 1 are not so satisfactory as those reported in the first reference for an unneutralized milk, in no case are they in excess of the maximum alkalinity found for unneutralized dried skim milk (32 samples), (*Ibid.*, **25**, 253).

The results obtained on Sample 2, a neutralized dried skim milk, show conclusively that the method is entirely satisfactory for the detection of neutralization.

Neutralization is also reflected in an increase in the percentage of ash found in the neutralized milk over that found in the unneutralized milk.

Considerable work has been done by A. H. Robertson and J. L. Perlman[1,2] in the Food Laboratory of the New York State Department of Agriculture and Markets, on the application of the ash alkalinity method to various dairy products, particularly to ice cream mixes. Good diagnostic results are reported. From their work it is evident that the method cannot be indiscriminately applied to dairy products depending solely on the base line material obtained on unneutralized dried skim milk for interpretative purposes. It will be necessary to obtain this base line material for each dairy product in order to determine whether or not a product has been neutralized.

The following is quoted from an abstract of a paper[3] on the application of the method by R. W. Kunkel and W. B. Combs of the University of

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[1] Circular 638, 1942.
[2] Reprinted from 17th Annual Report of the N. Y. State Assoc. of Milk Sanitarians, 1943.
[3] *J. Dairy Sci.*, **26**, 769 (1943).

TABLE 1.—*Ash and alkalinity of ash of dried skim milks*

COLLABORATOR	SAMPLE 1		SAMPLE 2	
	ASH	ALKALINITY 0.1 N ACID/100 GRAMS	ASH	ALKALINITY 0.1 N ACID/100 GRAMS
	per cent	*ml.*	*per cent*	*ml.*
1	7.77	55	9.56	360
	7.87	53	9.54	365
2	7.91	58	9.55	373
	8.05	57	9.73	370
3	7.7	75	9.5	378
	7.9	85	9.6	385
4	7.82	113	9.58	384
	7.84	110	9.51	386
5	7.80	55	9.51	355
	7.82	63	9.57	350
6	7.82	80	9.61	394
	7.85	84	9.59	397
7	—	—	9.66	387
			9.68	374
8	7.92	86	9.60	389
	7.94	93	9.60	391
9	7.97	84	9.61	415
	7.90	86	9.63	400
10	7.82	81	9.58	383
	7.86	82	9.56	386
11	7.86	110	9.58	389
	7.85	103	9.61	390
12	7.94	41	9.70	350
	7.92	40	9.60	358
13	7.87	96	9.62	371
	7.87	98	9.66	367

Minnesota, presented at the 1943 meeting of the American Dairy Science Association:

Samples of unneutralized dry skim milk were obtained from commercial drying plants to determine the approximate range of variation which may normally be expected in the ash alkalinity of skim milk. A total of 76 samples were obtained from

24 milk drying plants in Minnesota. These were analyzed and the averages of results obtained by duplicate analyses ranged from 76 to 127 and averaged 99.5 expressed as ml. of 0.10 N HCl per 100 grams of sample. Although the normal variation in ash alkalinity was considerable, a large percentage of the samples had an ash alkalinity within a comparatively narrow range. Thus, more than 50 percent of the samples had an ash alkalinity within the range 95 to 105. These samples of dry skim milk were obtained over a nine-months period from July 1942 to March 1943, and during this time no significant seasonal variations were apparent. . . . The method enabled detection of 0.01 percent neutralization if the ash alkalinity of the unneutralized skim milk was known. . . . Due to natural variations in the ash alkalinity of skim milk . . . addition of neutralizers in amounts sufficient to reduce the titratable acidity 0.10 percent may be possible without it being detected.

A titratable acidity of 0.10 per cent is equivalent to but 11 ml. expressed as 0.10 N lactic acid.

The alkalinity of the ash method for the detection of neutralizers is now tentative. The collaborative results previously reported, together with those submitted in this report along with the results obtained by other workers, justify its adoption as an official method. Inasmuch as the 6th Edition of *Methods of Analysis* is to appear next year, it is recommended* that the official action be made final for publication in that edition. The method has been tentative for 3 years.

The Associate Referee wishes to thank the following collaborators, all members of the Food and Drug Administration, for their splendid cooperation: Mae Carstensen, Washington, D. C.; L. L. Ramsey, Washington, D. C.; Leslie W. Ferris, Buffalo Station; H. I. Macomber, Baltimore Station; William Horwitz, Minneapolis Station; M. Tubis, Philadelphia Station; C. A. Wood, New York Station; L. H. McRoberts, San Francisco Station; Curtis Joiner, St. Louis Station; Menno D. Voth, Boston Station; Paul Mills, Seattle Station; and Robert D. Stanley, Chicago Station.

For report on mold mycelia in butter see report on dairy products under microanalytical methods for extraneous materials in foods and drugs, p. 225.

No report on lactose in milk was given by the Associate Referee.

REPORT ON FAT IN MILK

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

For some time the official method for the determination of fat in milk products has been in need of revision to provide for the use of the Mojon-

* For report of Subcommittee C and action of the Association, see *This Journal*, 25, 57 (1945).

nier extraction flask with its attendant benefits in technic. Under the present directions an emulsion frequently forms in the Röhrig tube when shaking is vigorous enough to assure complete extraction of the fat. These emulsions break with difficulty and often require long standing for separation of a reasonably clear upper liquid. This tempts the analyst to shake less vigorously to prevent emulsion formation with the attendant risk of a low result. The Mojonnier flask, adapted as it is to effectively breaking any emulsion by centrifuging, eliminates the temptation. Centrifuging also eliminates the necessity of waiting for the solvent layer to become clear.

Since a Mojonnier hand centrifuge was not available, light weight cups of reinforced, tempered Masonite were made* to hold the oddly shaped flask to fit a No. 239, 4-place head of a No. 2 model International Centrifuge. These cups were used in the tests later described. A trial showed that 600 r.p.m. was the maximum safe speed to break the emulsions without breaking the flask.

Cork stoppers are commonly used, since ordinary rubber stoppers have a tendency to swell in contact with ethyl ether and petroleum benzine. It is suggested, however, that "Neoprene" or other synthetic rubber stoppers unaffected by the usual fat solvents, if obtainable, be used with the Mojonnier flask, since these stoppers proved to be the most satisfactory in this work.

Several determinations were made to establish the time of centrifuging necessary to obtain an absolutely clear ether-fat layer and a residue which needed no "purification." The flasks were centrifuged 5, 10, and 15 minutes. The results (Table 1) show 15 minutes to be sufficient.

TABLE 1.—*Fat results before and after "purification"*

CENTRIFUGE TIME	FAT BEFORE "PURIFICATION"	FAT AFTER "PURIFICATION"
minutes	*per cent*	*per cent*
5	8.03	7.95
10	7.98	7.95
15	7.92	7.92

If the residue was not completely soluble in petroleum benzine, the extracted fat was "purified" by washing it from the flask with petroleum benzine and reweighing as prescribed in the official method. In order to insure an absolutely "purified" fat extraction, it is suggested that the extraction flasks be centrifuged for 20 minutes to make certain that there is no necessity for filtration or further "purification."

Another factor investigated was the optimum length of the shaking period. The official method specified 30 seconds. When, however, the

* By A. G. Sterling, instrument maker of the Food and Drug Administration.

shaking time was lengthened to one minute it was found by several comparative tests that as much as 0.04 per cent more fat was obtained as with only 30 seconds' shaking. The contents of the flask were in all cases shaken *very* vigorously, and the emulsions formed were without exception readily broken up by centrifuging.

These findings show the importance of providing in the method the use of the Mojonnier flask and the centrifuge as an option to the Röhrig tube, which cannot be centrifuged. Also that the directions for shaking should be more specific and the time of shaking longer. These changes in no way affect the fundamental procedure. The rewritten directions given below include a few statements taken from *Methods of Analysis A.O.A.C.*, 1940, p. 357, **24,** "Fat in Dried Milk Products," for the sake of clarity.

METHOD

Transfer 10 grams of sample to a Mojonnier fat-extraction flask or a Röhrig tube. Add 1.25 ml. of NH_4OH (2 ml. if sample is sour) and mix thoroughly. Add 10 ml. of alcohol and mix well. Add 25 ml. of ethyl ether, stopper extraction flask with cork or stopper of synthetic rubber unaffected by usual fat solvents, and shake very vigorously 1 minute. Add 25 ml. of petroleum benzine (redistilled slowly at a temperature below 65°) and repeat vigorous shaking. Centrifuge Mojonnier flask 20 minutes at approximately 600 r.p.m., or let it (or Röhrig tube) stand until upper liquid is practically clear. Decant ether solution into suitable flask or metal dish. Wash lip and stopper of extraction flask with mixture of equal parts of the two solvents and add washings to weighing flask or dish. Twice repeat extraction of liquid remaining in Mojonnier flask (or Röhrig tube), using 15 ml. of each solvent each time. Evaporate solvents on hot plate or steam bath at a temperature that effects complete evaporation, but not so high that spattering or vigorous bumping will result. Dry fat to constant weight in oven at temperature of boiling water. Weigh flask or dish. In weighing flask use a similar flask handled in same manner as counterpoise. Do not wipe flasks immediately before drying. Remove fat completely from the container with warm petroleum benzine, dry, and weigh as before. Deduct from total weight. Loss in weight = weight of fat. Correct weight of fat by a blank determination on reagents used.

OTHER DAIRY PRODUCTS

In the dairy products chapter the above general procedure for fat extraction is also used in the determination of fat in cream, various forms of concentrated milk, and in ice cream and cheese. The directions for the examination of these products vary with respect to preparation of sample; with the exception of evaporated milk, only editorial changes under the individual items are needed.

It is well known that separation of fat and other forms of inhomogeneity may occur as canned evaporated milk becomes old. In the present method for preparation of sample, *Methods of Analysis, A.O.A.C.*, 1940, 289, **66,** homogeneity is secured by mixing in an open dish and passing through a fine sieve. If separated fat is evident, such portion is warmed in the open to obtain a uniform emulsion. These directions first appeared in *Methods of Analysis, A.O.A.C.*, 1930, and no reports of study of the

method could be found in the proceedings of the Association since that time. Apparently the method acquired its official status by virtue of continued use. However, this is not unusual for methods of sample preparation which naturally do not lend themselves readily to collaboration.

The manipulations specified expose the milk to evaporation, which obviously might be unduly prolonged with a very inhomogeneous sample. The following method used in the industry provides for the mixing and warming of the sample in the unopened can. A few small lumps may be disregarded as it was found that their fat content was not higher than the liquid portion. The suggested method is recommended for adoption in place of **66 (a),** p. 289. The details follow:

Place the unopened can in a water bath at a temperature of ca 60°C.; remove, and shake the can vigorously every 15 minutes. At the end of 2 hours remove the can and allow to cool to room temperature. Remove the entire lid and thoroughly mix by stirring the contents in the can with a spoon or spatula. (If separation of fat which is non-emulsified is noticed, sample preparation has been inadequate.)

In par. **66 (b)** the word "mixture" should be substituted for "homogeneous mass."

With the sample prepared as suggested, a convenient method for rapidly weighing a portion for analysis is to half fill a 50 gram, wide-mouthed weighing bottle fitted with a one-hole cork or rubber stopper and a pipet with a large delivery opening. The cork and pipet are inserted, and the contents are weighed. Approximately 4.5 grams of evaporated milk is pipetted into a Mojonnier extraction flask, care being taken that none is deposited on the neck of the flask. The cork and pipet are replaced, and the contents are again weighed (a Bailey weighing buret or any other similar apparatus may also be used).

In testing the above procedures three cans of evaporated milk from three different batches submitted by a large evaporated milk company were used. Can No. 1 was treated according to the proposed method of preparation, and a fat analysis was made directly after treatment. Can No. 2 was accorded no preliminary treatment other than pouring back and forth, and an immediate fat determination was made. Can No. 3 was inverted, and also shaken several times a day for several days before analysis. The system of control employed by the manufacturer to check the fat content of the batches is as follows: The evaporated milk in the holding tank is analyzed just before canning. A can from this batch is then sent to the control laboratory and tested. For these particular samples additional duplicate fat determinations were made by two different analysts. There were thus four independent company analyses on each batch. Table 2 gives results reported by the Company and also those of the Associate Referee.

TABLE 2.—*Collaborative results*

	BY COMPANY					BY ASSOCIATE REFEREE			
CODE NO.	HOLDING TANK	CONTROL LABORATORY							
		1ST TEST	RECHECK	TESTS	AV.	1ST TEST	2ND TEST	3RD TEST	AV.
1	7.94	7.96	7.94	7.95	7.948	7.93	7.94	7.95	7.947
			7.95	7.95		7.95	7.96	7.95	
2	7.94	7.92	7.95	7.95	7.924	7.93	7.91	—	7.920
			7.94	7.95		7.90	7.94	—	
3	7.94	7.92	7.94	7.94	7.935	7.94	7.93	7.95	7.945
			7.94	7.93		7.94	7.93	7.98	

The best agreement was obtained with Can 1, where the sample was prepared by the method proposed for adoption.

The directions for weighing the charge under **69**, p. 289, should be changed as follows to conform to the general directions:

Transfer into a Mojonnier fat-extraction flask or Röhrig tube, by means of a weighing buret or similar device, 4.5–5.5 grams (ca 4.5 ml.) of the undiluted sample as prepared under **66(a)**. Add 1.25 ml. of NH_4OH and mix thoroughly. Add 4 ml. of water and proceed as directed under **20**, beginning "Add 10 ml. of alcohol."

RECOMMENDATIONS*

It is recommended—

(1) That the method for fat in milk, **20**, p. 272, for preparation of sample, **66 (a)** and **(b)**; and for determination of fat in evaporated milk, **69**, be revised as detailed in this report and their official status retained.

(2) That directions for fat in cream, sweet condensed milk, dried milk, malted milk, cheese, and ice cream be edited to conform to the revised general directions for determination of fat in milk.

No report on pasteurization of milk and cream was given by the Associate Referee.

REPORT ON SOLIDS AND ASH IN MILK AND EVAPORATED MILK

By GUY G. FRARY (State Chemical Laboratory, Vermillion, S. Dak.), *Associate Referee*

In 1939 the Referee reported the results of collaborative study of the official methods for total solids and ash in milk and in evaporated milk

* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 57 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

(*This Journal*, **23**, 453). At that time it was expected that further studies on the subject might be made. However, time has not permitted resumption of the work except by observation of routine laboratory work on inspection samples. As the methods now printed in *Methods of Analysis, A.O.A.C.*, 1940, call for the use of a larger portion of sample than has been found necessary and, in the case of ash in milk, call for wet oxidation by means of nitric acid, which often causes spattering with resultant loss, it is felt that the methods should be replaced by the procedure found satisfactory in the 1939 work. In reporting that work the following statement was made: "These figures (results obtained by collaborators) indicate that satisfactory results for total solids in milk and evaporated milk can be obtained by heating under prescribed conditions for a definite time period, and that ash in milk can be determined without use of the troublesome nitric acid. Heating the dish and milk on the steam bath before placing it in the oven is a definite aid."

Results obtained by use of the vacuum oven showed closest agreement among collaborators. However, these results in each case showed somewhat less solids than remained after drying at atmospheric pressure. As Hart (*This Journal*, **24**, 576) has pointed out, lactose begins to lose water of crystallization at lower temperature under reduced pressure than at atmospheric pressure. It appears, therefore, that milk products containing milk sugar should be dried at atmospheric pressure and at temperatures low enough to avoid caramelization.

The method used for total solids in milk is as follows:

Weigh 1.5–3 grams of prepared sample (*Methods of Analysis, A.O.A.C.*, 1940, 269, 2) into weighed flat-bottomed dish not less than 5 cm. in diameter, using nearer 5 grams and a Pt dish if ash is to be determined on same portion. Heat on steam bath 10–15 minutes, exposing maximum surface of bottom of dish to live steam; then heat for 3 hours in air oven at 98–100°C. Cool in desiccator, weigh quickly, and report percent residue as total solids.

The method for ash is as follows:

Into a suitable Pt dish weigh about 10 grams of prepared sample (*ibid.*) and evaporate to dryness on steam bath. Ignite in muffle at a temperature not higher than 550°C. until ash is free from carbon. Cool in desiccator, weigh, and calculate percent ash.

There is no reason why the same methods for total solids and ash may not be used for both milk and evaporated milk. The samples sent collaborators in 1939 were freshly canned evaporated milk. If a diluted sample of evaporated milk be used, the quantity weighed for the determinations should be approximately the same as in the case of raw milk. If the undiluted sample be used, the portion for analysis should be 1–1½ grams.

In the present official methods for solids in milk and evaporated milk there is no essential difference. The method for ash in milk, however, requires wet oxidation by nitric acid, while for evaporated milk it involves

only the ignition of the residue obtained in determination of total solids. The collaborative work in 1939 showed no advantage, but rather some disadvantage in using the acid. Better results were obtained without its use, and ignition of the dry solids in the muffle gave complete oxidation of carbon.

The Associate Referee suggests that action be taken to replace the present official method for ash in milk (*Methods of Analysis, A.O.A.C.*, 1940, 270) by a method such as that used in the collaborative study above mentioned.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of total solids in milk be corrected editorially to conform to the wording used in this report and that the method so corrected be made the method for total solids in evaporated milk by reference.

(2) That the method for the determination of ash in milk given in this report be adopted as tentative.

(3) That the present official method for the determination of ash in milk be dropped (first action).

(4) That the present official method for the determination of ash in evaporated milk be editorially corrected to fix a maximum ignition temperature of 550°C.

REPORT ON LACTIC ACID IN DRIED SKIM MILK

By Fred Hillig (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The collaborative results submitted to the Association on the determination of lactic acid by the colorimetric method (*This Journal*, **20**, 134; **25**, 255; **26**, 199) were so satisfactory that a recommendation was made that the method be tentatively adopted (*Ibid.*, **25**, 602), and Committee C approved this recommendation.

In order to study the application of the method when volatile acids (formic, acetic, and butyric) are present, samples containing these acids and also lactic acid in varying quantities unknown to the collaborators were submitted. Owing to the fact that this work was conducted in connection with the determination of volatile acids, the report was given in the "Report on Volatile Acids in Fish and Fish Products" (*Ibid.*, **27**, 237). Examination of the table will show that the results obtained by the collaborators were very satisfactory.

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 57 (1945).

This year two samples of dried skim milk were submitted to 12 collaborators. One of the samples had developed considerable lactic acid and had been neutralized. The results obtained by the collaborators are given in Table 1.

TABLE 1.—*Lactic acid in dried skim milk*

COLLABORATOR	SAMPLE 1	SAMPLE 2	COLLABORATOR	SAMPLE 1	SAMPLE 2
	per cent	*per cent*		*per cent*	*per cent*
1	0.03	0.37	7	—	0.38
	0.03	0.37			
		0.37	8	0.04	0.42
				0.04	
2	0.03	0.38			
	0.03	0.38	9	0.05	0.36
				0.04	
3	0.02	0.36			
	0.03	0.36	10	0.03	0.42
				0.03	0.42
4	0.04	0.40			
	0.04	0.40	11	0.05	0.39
5	0.04	0.41	12	0.03	0.41
				0.03	0.39
6	0.03	0.40			0.40
	0.03	0.41			

The results are very satisfactory.

The method for the determination of lactic acid in dairy products is now tentative. The collaborative results previously reported, together with those submitted in this report, justify its recognition as an official method. Inasmuch as the 6th edition of *Methods of Analysis* is to appear next year, it is recommended* that the official action be made final for publication in that edition. The method has been tentative for three years.

The Associate Referee wishes to thank the following collaborators, all members of the Food and Drug Administration, for their splendid cooperation: Mae Carstensen and L. L. Ramsey, Washington, D. C.; Leslie W. Ferris, Buffalo Station; H. I. Macomber, Baltimore Station; William Horwitz, Minneapolis Station; M. Tubis, Philadelphia Station; C. A. Wood, New York Station; L. H. McRoberts, San Francisco Station; Curtis Joiner, St. Louis Station; Menno D. Voth, Boston Station; and Paul Mills, Seattle Station.

No report on sampling and fat and moisture in cheese was given by the Associate Referee.

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 79 (1945).

A report by the Associate Referee on Frozen Desserts will appear in the next issue of *This Journal.* The method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

REPORT ON CHLORINE IN MILK

By W. H. King (Division of Laboratories, Louisiana State Health Department, New Orleans, La.), *Associate Referee*

The Rupp test[1] for free or loosely combined chlorine remaining after addition of hypochlorites and chloramines to milk has been studied by Hauser and the Associate Referee (see p. 417). Their conclusions are that this test is reliable for detection of preservative and germicidal quantities of chlorine added to milk in the form of hypochlorite solution. Inasmuch as Keister[2] found that the presence of more than 2.5 p.p.m. of copper in milk may cause a positive Rupp test, directions have been added to the method to make a quantitative determination of copper on milks exhibiting positive Rupp tests when the milk has been processed or handled in contact with copper-bearing equipment.

RECOMMENDATIONS*

Owing to the fact that the Rupp test has been in use for 25 years without published adverse comment other than Keister's (above) and because the Associate Referee has had favorable experience with the method, it is recommended that the Rupp test be adopted as tentative and studied collaboratively.

The paper entitled "Determination of Gelatine in Ice Cream," by Donald Mitchell, Edwin H. Shaw, Jr., and Guy G. Frary, was published in *This Journal*, **28**, 97 (1945).

REPORT ON EGGS AND EGG PRODUCTS

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

A report was received from only one associate referee, that on acids, who recommends the official adoption of methods for volatile and lactic acids. The collaborative work supports the recommendation, and in view of the fact that a revision of *Methods of Analysis* is to appear next year it

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[1] U. S. Dept. Agr. Bull. No. 1114 (1922).
[2] *Am. Jr. Pub. Health*, **15**, 781 (1925).
* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 57 (1945).

is recommended that the official action be made final to obviate the necessity of the designation "First Official" in the revision, and that the work be discontinued. Lepper, Bartram, and Hillig (*This Journal*, **27**, 204) found the methods for acids in eggs to be valuable in demonstrating decomposition of eggs in the liquid, frozen, and dried states.

At the meeting last year a method for salt in eggs was adopted as official, first action, as an alternative method. The original referee report supporting this action was published in *This Journal*, **26**, 352, and a consideration of the results shows that the method is one that should receive recognition as official, final action, and it is so recommended.*

The Referee on Eggs for 1943 (*Ibid.*, **25**, 365) recommended the tentative adoption of a method for unsaponifiable matter and the official adoption of a method for cholesterol in eggs. The latter was adopted as official, final action, last year (*Ibid.*, **27**, 88). In the examination of eggs the cholesterol is determined in the unsaponifiable matter separated by the procedure set forth in the tentative method so that the two determinations are, in fact, one procedure. It is not necessary to determine the quantity of unsaponifiable matter to obtain a cholesterol result. The previous referee did not regard the collaborative results on unsaponifiable matter sufficiently conclusive to recommend official recognition of the procedure as a determination for this constituent. Approximate results for unsaponifiable matter can, however, be obtained by the procedure if desired. Its use as a means of preparing unsaponifiable matter for subsequent determination of cholesterol has been demonstrated as suitable for official recognition. A revision of the method is recommended to provide for both directions, to be titled "Cholesterol—Official," with sub-headings for (a) *Separation of Unsaponifiable Matter* and (b) *Determination of Cholesterol.* In line six from end of the directions for determination of unsaponifiable matter beginning "which" and ending "follows" substitute the following: "An approximate determination of unsaponifiable matter can be obtained by collecting ether solutions in a flask previously dried and weighed as follows."

It is further recommended that the studies on added glycerol be continued.

No report on unsaponifiable matter and cholesterol was given, no associate referee having been appointed.

No report on added glycerol and salt was given by the Associate Referee.

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

REPORT ON ACID IN EGGS

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

The volatile and lactic acid content of certain foods may be significant to the regulatory chemist in detecting sophistication and also as an index of any retrograde changes involved in decomposition. A method for the determination of volatile acids has been proposed[1] and developed.[2] It has been applied to fish products and has been found of value in confirming decomposition.[3,4,5] A colorimetric method for the determination of lactic acid has been developed and applied to dairy,[6,7] tomato,[8] and fruit[9] products.

More recently these methods have been used to detect decomposition in liquid, frozen, and dried eggs. Report on an investigation to obtain fundamental data applicable to the solution of the problem of decomposition in eggs and egg products is given in a paper by Lepper, Bartram, and Hillig, entitled "Detection of Decomposition in Liquid, Frozen, and Dried Eggs."[10] In this paper methods for the determination of volatile and lactic acids are given in detail.

It is believed at this time, in order to make this report complete, that further information, not given in the previous paper,[10] should be submitted relative to the preliminary work performed in applying these two methods to dried eggs.

DETERMINATION OF VOLATILE FATTY ACIDS

The theory on which the method is based was developed in detail in a previous paper on the "Determination of Volatile Fatty Acids."[1] Tables are there given showing recoveries obtained on various mixtures of formic, acetic, propionic, butyric, and iso-butyric acids in pure solution. Knowing what acid or acids are present in a product, the quantity of each can be computed from simultaneous equations based on distillation data obtained from pure acids.

Previous papers[3,4,5] give curves showing fractional distillation of the volatile acids obtained from tuna fish, herring roe, and sardines. Such curves indicate the particular acid or acids present in each product.

The distillation apparatus employed in the work is described in a previous paper.[1]

In the course of examination of commercial dried eggs the odor of the

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

distillate indicated, in some cases, that butyric acid might be present. In order to verify the presence of butyric acid a considerable quantity of volatile acids was collected from one of these dried eggs, the formic acid present was destroyed with mercuric oxide, and the remaining volatile acids were recovered by distillation from a small volume of solution, saturated with magnesium sulfate. These acids were then subjected to fractional distillation following the procedure previously described.[3,4,5] The data are presented in Figure 1.

The values obtained from the titration of the various portions of distillate are represented by Curve No. 1 on the chart. This curve lies below the acetic acid line and above the line for propionic acid, thus indicating the presence of acetic acid and one or more fatty acids of higher molecular weight than acetic acid. The first 100 ml. fraction of distillate obtained in

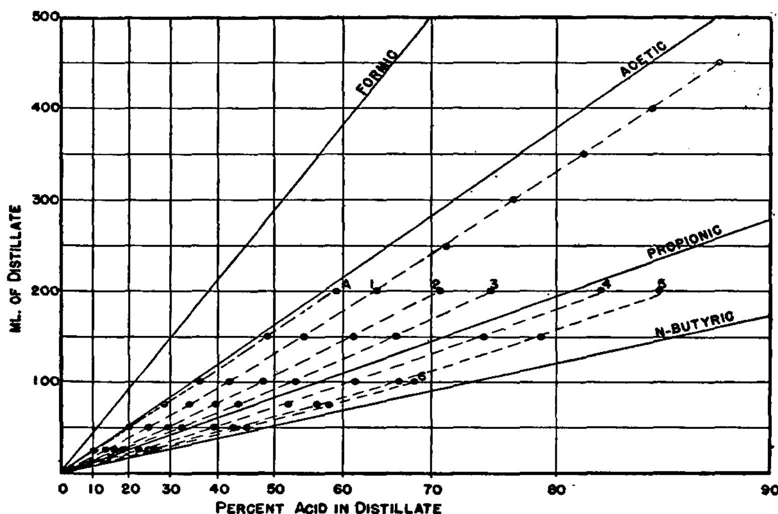

FIG. 1.—Fractionation of Volatile Acids from Dried Eggs.
A—Butyric removed—showed absence of propionic.
1—Distillation curve—formic removed.
Fractionation curves 5 & 6 show butyric highest detectable acid present.

preparing Curve 1 was then distilled, the various fractions were titrated, and the values were plotted as Curve 2. This process was repeated several times giving Curves 3, 4, 5, and 6. Since Curve 5 approaches, but never crosses, the butyric acid line, and a repetition of the process (Curve 6) does not materially change the slope, it follows that the highest detectable member of the series of acids present was butyric acid. It does not, however, prove the presence or absence of propionic acid. Curve A was pre-

pared from the distillation of the acids remaining in the flask after 500 ml. of distillate, used to prepare Curve 1, had been collected. Since the butyric acid had been eliminated in the first 500 ml. of distillate, and since the formic acid had been destroyed, Curve A should coincide with the acetic acid line, if propionic acid were absent. The fact that Curve A does coincide with the acetic acid line, within experimental error, indicates the absence of propionic acid.

In order to test the accuracy of the method as applied to egg products, varying quanties of formic, acetic, and butyric acids, either singly, or in combination with each other, were added to 25 gram portions of reconstituted egg powder. Recoveries obtained are shown in Table 1.

TABLE 1.—*Recovery of volatile acids (mg.) added to 25 grams of dried eggs*

SAMPLE NO.	FORMIC		ACETIC		BUTYRIC	
	ADDED	FOUND	ADDED	FOUND	ADDED	FOUND
1	0.46	0.32	6.0	7.2	—	—
2	0.90	0.69	11.9	12.8	—	—
3	2.3	2.3	17.9	17.5	—	—
4	4.6	4.9	20.9	20.6	—	—
5	6.8	6.4	23.9	23.7	—	—
6	9.1	8.8	26.9	26.5	—	—
7	13.7	13.0	29.9	31.1	—	—
8	18.2	17.7	35.8	34.7	—	—
9	27.3	26.6	23.9	23.8	—	—
10	4.6	4.4	17.9	20.1	4.4	4.5
11	6.8	6.3	23.9	22.9	13.2	13.9
12	9.1	8.8	29.9	29.5	22.0	18.1
13	13.7	14.6	35.8	34.2	44.0	43.5
14	—	—	35.8	33.8	—	—
15	—	—	—	—	52.8	50.4
16	27.3	25.9	—	—	—	—

It will be seen that the recoveries are quite satisfactory.

DETERMINATION OF LACTIC ACID

The colorimetric method for the determination of lactic acid in dairy products has been well established.[6,7] The only change necessary in the method in order to apply it to other food products is the preparation of the sample for ether extraction. Application of the method to some commercial samples of dried eggs, which had been judged organoleptically as unfit for human consumption, showed a quantity of lactic acid present often approaching, or exceeding, 1 per cent. On the other hand, dried eggs of good quality usually contained less than 50 mg. of lactic acid per 100 grams.[10]

In order to test the accuracy of the method, varying quantities of lactic

acid as lithium lactate were added to reconstituted dried egg powder. Recoveries are shown in Table 2.

TABLE 2.—*Recovery of lactic acid added to reconstituted dried egg powder*

ADDED	FOUND	ADDED	FOUND
2.0	2.0	20.0	18.6
5.0	4.6	40.0	38.8
10.0	9.1	50.0	48.4
15.0	14.5	100.0	96.6

The results are entirely satisfactory.

In order to test the accuracy of the two methods in the hands of various analysts, 3 samples of dried egg powder were submitted to 14 collaborators. The results are given in Table 3.

Examination of Table 3 shows that with a few exceptions there is remarkable uniformity in the results reported by the collaborators.

The volatile and lactic acid methods in principle are the same for all food products to which they are applicable, the only difference being in the preliminary preparation of the sample, either for ether extraction in the case of lactic acid, or for steam distillation in the case of volatile acids. The method for lactic acid as applied to dried skim milk is now tentative and the results this year are such as to justify a recommendation that it be made official, final action. The method for volatile acids has been subjected to a rather exhaustive collaborative study[11] with excellent results. In view of the satisfactory results previously obtained with these two methods and the excellent collaborative results reported in Table 3 it is believed a recommendation that the two methods be adopted as official for egg products is in order and it is so made.* Inasmuch as the 6th edition of *Methods of Analysis* is to appear next year, it is recommended that the official action be made final for publication in that edition.

The Associate Referee wishes to thank the following collaborators, all members of the Food and Drug Administration, for their splendid cooperation: Mae Carstensen, L. M. Beacham, and L. L. Ramsey, Washington, D. C.; Leslie W. Ferris, Buffalo, N. Y.; Jay V. Beck and Harold W. Gerritz, San Francisco Station; G. Kirsten, New York Station; Menno D. Voth, Boston Station; Paul Mills, Seattle Station; William Horwitz, Minneapolis Station, H. I. Macomber, Baltimore Station, George M. Johnson, St. Louis Station, and Harry Shuman, Philadelphia Station.

[11] *This Journal*, **27**, 237 (1944).
* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

TABLE 3.—*Analysis of dried eggs (results in percent)*

COLLAB-ORATOR	SAMPLE 1			SAMPLE 2			SAMPLE 3		
	FORMIC	ACETIC	LACTIC	F	A	L	F	A	L
1	0.007	0.05	0.03	0.16	0.09	0.22	0.21	0.13	0.25
	0.008	0.05	0.03	0.16	0.09	0.23	0.21	0.13	0.25
2	0.006	0.04	0.03	0.16	0.08	0.21	0.22	0.12	0.24
	0.008	0.04	0.03	0.16	0.09	0.21	0.22	0.12	0.25
3	0.007	0.04	0.03	0.16	0.10	0.22	0.22	0.12	0.22
	0.008	0.05	0.04	0.17	0.09	0.22	0.23	0.12	0.23
4	0.009	0.05	0.03	0.16	0.10	0.22	0.22	0.13	0.25
	0.009	0.05	0.03	0.16	0.10	0.23	0.22	0.12	0.25
5	0.002	0.05	0.03	0.16	0.09	0.23	0.22	0.12	0.26
	0.006	0.05	0.03	0.16	0.08	0.23	0.22	0.12	0.26
6	0.011	0.04	0.04	0.16	0.09	0.20	0.23	0.12	0.25
7	0.012	0.04	0.04	0.16	0.08	0.21	0.23	0.12	0.25
			0.03						
8	0.007	0.04	0.03	0.16	0.09	0.22	0.22	0.13	0.25
	0.009	0.04	0.03	0.16	0.09	0.22	0.22	0.13	0.25
9	0.010	0.03	0.03	0.16	0.08	0.24	0.23	0.12	0.24
	0.010	0.03	0.03	0.17	0.08				
	0.010	0.03	0.03	0.16	0.08	0.24	0.22	0.12	0.24
10							0.23	0.13	
				0.16	0.09	0.18	0.22	0.12	
	0.006	0.04	0.03	0.16	0.09	0.19	0.23	0.12	0.24
11							0.21	0.11	0.27
	0.009	0.03	0.03	0.16	0.08	0.24	0.22	0.12	
	0.009	0.03	0.03	0.16	0.08	0.24	0.21	0.12	0.27
12				0.16	0.10	0.23	0.22	0.14	0.26
	0.009	0.04	0.03	0.16	0.11	0.22	0.21	0.14	0.26
13	0.020	0.03	0.05	0.17	0.08	0.26	0.22	0.11	0.27
	0.010	0.03	0.05	0.17	0.08	0.26	0.23	0.11	0.25
14	0.012	0.03	0.03	0.17	0.10	0.24	0.23	0.13	0.27

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FOODS AND DRUGS

By HENRY WELCH (Food and Drug Administration, Federal Security Agency), *Referee*

During the past several years analysts of the Food and Drug Administration have developed a large number of procedures for the determination of extraneous material in food and drug products. These methods have had considerable use in regulatory work, and for a number of years many of them have been found to be of value. The methods which utilize a variety of procedures are, however, based upon one or more of three basic principles: (1) Solubility of the food or drug and the insolubility of the extraneous material; (2) The preferential wetting of insects, insect parts, rodent hairs, or other extraneous matter by oily liquids whereby they acquire a lighter specific gravity, which allows their separation by flotation methods; and (3) Selective sedimentation in water or in heavier-than-water liquids.

Some of these methods have been published.[1] On the other hand, many of the methods have only had a limited distribution. During the past two years the Microanalytical Division of the Food and Drug Administration has distributed to official analysts a "Manual of Microanalytical Methods" to be used in regulatory work, and one or two methods for the determination of extraneous material in food products have appeared in *Methods of Analysis, A. O. A. C.*, 1940. More recently the Microanalytical Division has published Food and Drug Circular No. 1, entitled "Microanalysis of Food and Drug Products," which will be made available to interested individuals. This circular discusses in considerable detail the theory and principles of the microanalysis of food and drug products.

At the present time there are approximately 100 procedures that deal with technics for the demonstration of extraneous material in food and drug products. These include methods for the analysis of drugs, beverages, and beverage materials; dairy products; nuts and nut products; canned foods, cereals, cereal products, and eggs; fruits, fruit products, and confectionery products; vegetable products, and spices, condiments, and miscellaneous methods. Each associate referee has submitted a report in his particular field.

It is recommended that these procedures be adopted as tentative methods and that they be included in a new chapter in *Methods of Analysis* under the title "Extraneous Materials in Foods and Drugs."*

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[1] Green, W. S., "A Method for the Detection of Filth in the Form of Insects and Other Extraneous Materials in Butter," *Food Ind.*, 7 (1), 441 (1935); Harris, K. L., "Foreign Matter in Corn Meal," *Cereal Chem.*, 18 (5), 655–661 (1941); Howard, B. J., "Fragment Count Methods for Tomato Products," *Canning Age*, 18 (9), 324–326 (1937); Howard, B. J., "Corn Ear Worm in Tomato Products," *Food Ind.*, 7 (7), 321–322 (1935); Wildman, J. D., "A Simple Method for Separating Certain Insects from Food Products," *Science*, 75 (1940), 168–169 (1932).

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis*, *A.O.A.C.*, 1945.

In *Methods of Analysis, A. O. A. C.*, 1940, there are included three methods: (1) Sand in Tomato Products—Tentative, page 521; (2) Microanalysis of Tomato Pulp, Purée, Sauce, and Paste—Official, page 522–3; and (3) Mold Mycelia in Butter—Tentative, page 300. These methods should logically be included in the chapter dealing with extraneous materials and it is so recommended.*

There are certain fundamental principles and types of apparatus that are used for a variety of microanalytical procedures. A statement that discusses basic apparatus and manipulations of general application to specific microanalytical methods is recommended for tentative adoption. This general statement should preface the chapter dealing with this subject.

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It should be pointed out that the proposed methods have resulted from the collaborative effort of the following individuals of the Food and Drug Administration:

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REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN DRUGS, BEVERAGES, AND BEVERAGE MATERIALS

By GEORGE L. KEENAN (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The methods now recommended for tentative action are those which have been used in most cases for several years by the Food and Drug Administration for the detection of extraneous materials.

The methods to be discussed in this report are those that have been devised for the detection of extraneous materials in (1) tea, leafy crude drugs, and condiments; (2) filth in cocoa, imitation cocoa, and cocoa substitutes; and (3) canned citrus juices.

Tea, leafy crude drugs, and condiments may be contaminated with both rodent and insect filth. The insect filth may be classified conveniently into that occurring on the growing plant, as in the field, and that which finds access during curing or storing of the plant materials. The rodent infestations are usually of the latter type. The procedure to remove this filth, and also sand, dirt, stones, etc., is based on the same general principles that have been used in other filth extraction methods. This method may be considered in two parts, that concerned with heavy filth, such as rodent and insect excreta; and the light filth, consisting of insects, insect fragments, and hairs. The heavy filth may be removed by treating the material in a percolator with chloroform. The light filth is floated off in mineral oil.

The filth recovered from the growing plant, that is field-infestation, may consist of soft-bodied insects or fragments from them, such as aphids, thrips, mites, whole insects or parts, and cast skins. Such filth debris occurs in the field and is not due to storage conditions.

The heavy filth that settles out by the use of chloroform occurs in the plant materials after they have been improperly stored and have been subjected to invasion by rodents and storage insects. The rodent infestation may assume the form of excreta pellets or fragments, or rodent hair fragments. The storage insects may consist of entire beetles, flies, etc., or fragments of them. Sand, stones, dirt, etc., will also come out in this separation and can be determined by the official method for the determination of ash insoluble in acid.

The filth encountered in cocoa, imitation cocoa, and cocoa substitutes may consist of a mixture of the light and heavy type, especially where imitation cocoa products may contain large percentages of cocoa shells. The latter may be the source of storage infestation consisting of rodent hairs and large numbers of insect fragments. The heavy filth, such as excreta pellets, can be separated with chloroform while insect fragments and rodent hairs can be extracted by the gasoline treatment.

In canned citrus juices, the use of decayed fruit in the finished product is indicated by mold contamination, detected microscopically. Off-grade fruit, faulty storage of sound fruit after it is picked, and moldy juice troughs and pipe lines may be responsible for such conditions. The determination of mold in the juice requires preliminary treatment, such as centrifuging for the purpose of segregating the mold, then mixing the mold residue with a viscous medium such as pectin solution so that a mold count can be made according to the official method.

Citrus juices may also be contaminated by insects and rodents. Both of these contaminations are due to faulty storage conditions, where the fruit has been held too long or was in an overripe condition or even decayed when stored. Such conditions attract vinegar flies and other insects. Improperly constructed bins also permit entrance of rodents that contaminate the fruit with hairs and excreta. Fly eggs and maggots may be separated with gasoline or by direct filtration through bolting cloth. Insect fragments and rodent contamination can be separated with castor oil.

It is recommended* that the methods for the detection of extraneous materials in tea, leafy crude drugs, condiments; canned citrus juices; and cocoa, imitation cocoa, and cocoa substitutes be adopted as tentative.

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN DAIRY PRODUCTS

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

Methods for the detection of extraneous materials in dairy products have been available for a number of years. The method for mold mycelia in butter[1,2] was adopted as official, first action, in 1940. This method has received critical attention and use by the Food and Drug Administration and the industry. Continued collaborative work has shown results similar to those reported previously. Some changes have been found desirable, such as the provision for (1) the use of other gums than those originally specified, (2) weighing the butter sample directly into a beaker, and (3) weighing the gum solution. These changes have been adopted in the interest of further standardization of procedure. It should be noted that by the adoption of a standard microscope alignment a further standardization of the method is achieved.

Since this method deals with extraneous materials it is recommended that it be transferred from the chapter on dairy products to the chapter on extraneous materials.*

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* For report of Subcommittee C and action of the Association, see *This Journal*, **28**, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

[1] *This Journal*, **20**, 93 (1937).

[2] *Ibid.*, **24**, 550.

The method for manure fragments in dairy products, which makes use of a staining procedure, was devised by Duggan.[3] He published collaborative results that showed that trained analysts secured results which closely approximated the actual proportion of manure fibers present. He concluded that the staining procedure alone will give acceptable results, but that the analyst's familiarity with authentic materials increases his efficiency. The method has been found useful in the examination of dairy products.

In regard to the methods for filth in cheese, it has been found necessary to provide a number of procedures in order to cope with the varying types of this product encountered. Greene[4] devised a method for filtering cheese which makes use of hydrochloric acid and alcohol. Spicer and Price[5] found that sodium citrate was superior to other reagents. Since then, work in the Food and Drug Administration has shown that no one procedure can be used for all cheeses.

Cheese may be contaminated with insect and/or rodent filth during the handling of the milk, during the manufacture of the cheese, or during curing. Where it is essential that an accurate picture of the amounts of such filth be obtained the procedure referred to previously[1] should be used. Where a number of samples are to be run, preliminary tests by one of the methods listed under the second reference cited may be found advantageous and more useful as a sorting procedure. Where the main purpose is to obtain sediment comparisons, one of the procedures given under this same reference may be used. In all cases, a sample of 225 grams should be used.

Fewer procedures have been found necessary for the examination of dried milks, evaporated milk, and condensed milk. These types may be contaminated with filth from the barn and utensils, and while the milk is en route to the processing plants; it may include insects, rodent hairs, nondescript dirt, and manure particles.

Two methods for testing cream for sediment are given. The first of these involves the use of sodium hexa-meta-phosphate and was devised by L. H. Chernoff. In routine work difficulty was encountered with the method when old, high-acid types of cream were tested. In an attempt to arrive at a generally applicable test collaborative work was done by four laboratories. Four methods were tried, and three types of cream were tested. The methods are given below:

Origin of Method	*Type of Test*
Chernoff	Simple filtration with sodium hexa-meta-phosphate.
Vandaveer-Goodman	Simple filtration with dilute HNO_3.
Keppel-Perlmutter	Simple filtration with dilute phosphoric acid.
Fine	Combination of flotation and filtration.

[3] *Ibid.*, 27, 331 (1944). [4] *Cheese Reporter*, 60, 1 (1936). [5] *J. Dairy Sci.*, 21, 1 (1938).

Three samples of each of the following cream types were tested: (1) sweet cream; (2) good sour cream with acidity less than 1 per cent; and (3) old or high-acid or demomposed cream.

The cream was picked up from local sources at four widely separated locations.

Practically no difficulty was encountered on any of the sweet or sour cream samples by any method. In the case of the old, high-acid, or decomposed cream, simple filtration methods were found to be unsatisfactory, since from 2 to 10 sediment pads were required for each sample. The Fine procedure on the other hand, required only one pad for most samples.

The results showed that in the old, high-acid, or decomposed cream some reaction had occurred which tended to prevent filtration. Further study of the end products of the aging of cream would undoubtedly yield information which would be useful in perfecting the methods of filtration. The Chernoff and the Fine procedures are included below. The following members of the Administration collaborated on the cream work: G. E. Keppel and S. H. Perlmutter of Minneapolis Station; J. S. Schurman and S. D. Fine of Cincinnati Station; F. M. Garfield of St. Louis Station; and J. J. Roe and T. C. Dunn of Denver Station.

RECOMMENDATIONS*

It is recommended—

(1) That the mold count method for butter be adopted as official, final action.

(2) That the following methods be adopted as tentative: Manure Fragments in Dairy Products, Filth in Cheese and Preparation of Sediment Pads from Cheese, Filth in Dried Milk and Preparation of Sediment Pads from Dried Milk, Filth in Evaporated and Condensed Milk and Preparation of Sediment Pads, Preparation of Sediment Pads from Cream, and Filth in Butter and Preparation of Sediment Pads from Butter.

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN NUTS AND NUT PRODUCTS

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

The following methods submitted for tentative action have been in use for some time by members of the Microanalytical Division and by various analysts in branch laboratories of the Food and Drug Administration.

Shelled Nuts.—Common adulterants in shelled nuts may consist of rodent excreta pellets, rodent hair fragments, insects and insect frag-

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

ments, and sand or dirt. The sand and dirt are determined by the standard procedure for insoluble inorganic residue. The other extraneous matter is extracted by means of the gasoline flotation method. A magnification of 20–40 diameters is used to determine this filth. An alternative procedure is given in which a 1 percent sodium oleate solution is used to determine the various forms of filth.

Peanut Butter.—The adulterants found in peanut butter may consist of water-insoluble inorganic residue, rodent excreta pellets, rodent and other animal hair fragments, insects and insect fragments, and decomposed and moldy peanut fragments.

The method involves the procedures for the extraction of the water-insoluble inorganic residue, designated as "WIIR," excreta, insect fragments and hairs, and the detection of decomposed and moldy peanut fragments.

It is recommended that these methods be considered as tentative methods.*

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN BAKED PRODUCTS, CEREALS, AND EGGS

By KENTON L. HARRIS (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The methods submitted for tentative use by the A.O.A.C. are closely related. They all involve a softening of the food material and its preparation so that the extraneous matter can be subsequently removed through the use of specific gravity or the surface properties of the material, or of both. There is also a similarity in the types of extraneous matter which may be found in various cereals and eggs. Bakery products and alimentary pastes may contain not only such filth as is introduced by these raw materials but additional contaminants which gain entrance during their preparation.

The cereals are subject to both field and storage investation by insects that contaminate the grain or flour with their whole bodies, fragments, and excreta pellets, and by rodents which leave their liquid and solid excreta in the food. Rodent hairs, insects, and insect parts all can be recovered by similar technics, which extract and concentrate this so-called "light filth," while the solid excreta or "heavy filth" must be removed by sedimentation.

Frozen or dried eggs may be contaminated with chicken manure, dirt, sand, metal fragments, splinters, insects and insect fragments, hairs, and

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

feathers, depending upon the condition of the eggs, method of manufacture, and storage conditions.

Enzymatic action has been found to be the least likely to alter the structure of the extraneous matter, while releasing it from the food. Pancreatin, which is active on proteins, fats, and carbohydrates, and which is obtainable as a standardized product, has been found to be almost universally applicable to cereal products. In some cases where enzymatic action is insufficient to prepare the food for subsequent filth determinations it has been found necessary either to digest with hydrochloric acid or extract the material in dilute alcohol solutions. Wherever possible the extraneous matter has been separated out by a procedure as simple and direct as it is practicable. For example, certain baked products are almost completely digested in pancreatin, and in these cases it is possible to simply filter the mixture through a comparatively fine bolting cloth to separate out the undigested extraneous matter. When some portions of the food cannot be digested, for example when fruit, nuts, or cocoanut are present, it is necessary to digest the material, separate out the undigested large pieces of food, and then remove the extraneous matter. Alimentary pastes fall into the first category, that is, completely susceptible to digestion; however, because of their dense, hard nature, it is necessary to boil them in dilute acid before using pancreatin. Heavy filth can be separated from comminuted cereal material by a difference in the specific gravity of the filth and the cereal. In this connection chloroform or a mixture of chloroform and carbon tetrachloride has been found to be the most useful liquid. The residues from the extraction of heavy filth can be directly used to obtain insects and rodent hairs, and the methods here involved are similar to those used for bakery products except that because of the powdery nature of the food the procedures are simpler. It should be noted that the presence of bran or chaff presents considerable difficulty in these extractions, and when they are present it is necessary to handle them by somewhat modified procedures, which have been written into the methods.

Three separate procedures are given for the separation of extraneous matter from eggs. The first two involve the digestion of eggs and subsequent separation of the solid extraneous matter, and the third method concerns the separation of chicken excrement. It should be noted that the phosphoric acid and sodium hydroxide procedures are intended for use on dried whole egg. These procedures also are applicable to frozen whole eggs, but they are not intended for use with dried egg albumen.

The method for chicken excrement depends upon the formation of a blue color by the reduction of arsenophosphotungstic acid by uric acid in the presence of alkaline cyanide. This test is used ordinarily for colorimetric quantitative estimation of uric acid in blood and urine. The method, involving centrifugation after suitable dilution, isolates the actual

number of urate fragments to show the presence of manure, but it does not measure the actual amount of uric acid. The uric acid is found in the sediment along with egg shell fragments, chalaza, meat bits, shell membrane, plant, insect and dirt fragments, sand, and rodent hairs. Frozen eggs should be examined as soon as possible after thawing because as decomposition proceeds the amount of egg material found in the sediment increases and renders examination difficult and sometimes impossible.

It is recommended* that these methods be included as tentative methods of analysis for the A.O.A.C.

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FRUITS AND FRUIT PRODUCTS; AND CONFECTIONERY, SUGARS, SIRUPS, MOLASSES, AND HONEY

By W. G. HELSEL (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Apple butter is sometimes prepared from rotten or insect-damaged fruit, and in some cases apple chops contaminated with rodent filth are used. Methods have been devised for detecting this filth in the dried apple products and also in the apple butter.

The detection of rot in canned and frozen berries involves a careful macroscopic examination of the berries under water. If the berries are frozen they must be thawed, and the water must be practically free from color. It is frequently necessary to confirm the presence of rot by making a microscopic examination for mold filaments in the berry tissue. The berries are then pulped, and a mold count is made on the pulp. In the case of frozen strawberries some work has been done to correlate the mold count on the pulp with the amount of rot in the whole berries. It is advisable to use at least four 1-quart samples of strawberries and pulp half of each of them at once. If the mold count on this pulp is high, the usual macroscopic examination of the whole berries should be made.

Berries may be infested in the field with various coleopterous and lepidopterous insects, usually in the larval stages. Some of these insects are still present when the fruit is canned or frozen, and the method of detection consists of a mashing or breaking of the fruit, followed by a gasoline extraction. This method will not recover maggots, which are frequently found in blueberries and cherries. Maggots will sink in water and are recovered from the bottom of the pan after the fruit tissue has been decanted. Maggots are more easily detected against a dark background, such as a black pan.

Fig and prune pastes may contain considerable insect and rodent filth,

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

which may be detected by thinning the paste with water and making the usual extractions with castor oil instead of gasoline. Mincemeat may also contain insect and rodent filth, which may be detected by making the the usual gasoline extraction with 80 per cent alcohol instead of water.

Candy may be filthy because it has been made from contaminated raw materials, or because it has become contaminated with rodent, insect, or other filth during or after manufacture. If the contamination has come from the raw materials the filfth will be incorporated more or less generally throughout the mass of the candy, while storage contaminations will usually show on the surface. Because of the many varieties of candy, several methods are described.

Refined sugar, if stored under proper conditions, will contain little if any filth, but occasionally storage contamination does occur. Raw or partially refined sugar, which is sometimes used for manufactured products, is more subject to contamination. The method recommended is for the detection of water-insoluble filth in sugars of both types.

The filth frequently found in sirups and molasses and in honey is usually similar to that in unrefined sugar.

It is recommended* that these methods be adopted as tentative methods.

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN VEGETABLE PRODUCTS

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

A group of 26 methods are used for the detection or recovery of extraneous material in vegetable products. Some of these methods have been used by analysts for many years, and others are of more recent origin. All have proved satisfactory for the purpose for which they are intended.

One of the most common types of decomposition found in vegetable products is caused by mold. This decomposed material may be included in the finished product due to careless handling or preparation of the vegetables. Tomatoes are especially subject to decay, and because of the finely divided condition of some comminuted tomato products the rot is not apparent to the consumer. Even puréed infant foods, which should be prepared with special care, are sometimes made from partly decayed raw material. Several methods for the detection of rot have been devised.

Nearly all vegetables are attacked by insects, most of them having several species of such pests. Unless proper care is taken in preparing the vegetables for packing, whole insects, fragments, or excreta may be included in the finished product. Green, leafy vegetables are especially sub-

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* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

ject to attack by aphids and thrips and to a lesser extent by caterpillars. Caterpillars, such as the corn ear worm and pin worm, are very destructive to tomatoes in certain sections of the country, and the former also attacks sweet corn in all sections. In addition to worms, tomatoes are also attacked by the maggots of fruit flies and other flies, but only if they have been crushed or are beginning to decay. Beans and peas while in the field are sometimes infested with weevils, and after they are dry they are sometimes completely destroyed by these insects if they are not properly protected. Mushrooms are attacked by several insects when in the fresh condition, the most serious pest being the fungus gnat. After they have been dried a number of storage insects may destroy the mushrooms if they are not properly stored.

One of the most disgusting types of filth found in food products is of rodent origin. While vegetable products are not as prone to rodent infestation as some other foods, they are sometimes infested if protective measures are not taken. Since evidence of rodent infestation, especially hairs, is difficult for the consumer to detect, it is seldom noticed.

Methods for the detection of all of these types of extraneous materials in vegetable products are recommended* as tentative methods.

The mold, yeast, and spore count methods for tomato products are already in *Methods of Analysis, A.O.A.C.*, Chapter **XXXV**, paragraphs **31** and **32.** It is recommended that these methods be removed from this chapter and placed under extraneous materials, since they are microscopic methods for the detection of decomposed material. They have been rewritten somewhat to conform to present procedures.

The method for sand in tomato products, paragraph **21,** belongs under extraneous material, and it is recommended that it also be transferred to this chapter.

REPORT ON MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN SPICES, CONDIMENTS, AND MISCELLANEOUS PRODUCTS

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

Methods are submitted for the determination of various types of filth in spices and condiments. The kinds of filth generally encountered are those from insect and rodent infestations and the presence of sand or soil. Contamination by insects and rodents is evidenced in ground spices and other condiments by the presence of excreta and parts of insects, and the

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* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

urine stains, hairs, and excreta of rodents. Metal fragments and pieces of other foreign material may also find their way into a product if adequate attention is not paid to the basic principles of sanitation. Sand and soil may be incorporated in these food products if the natural plant materials are improperly cleaned or washed.

These methods are primarily an objective means of determining the use of unfit ingredients in the preparation of spices and condiments, and the existence of insanitary conditions whereby such products may become contaminated with filth. Many of these methods involve the general principle of extraction by means of immiscible liquids such as water-gasoline and water-oil mixtures, in which the filth elements are separated from the spice tissues and are trapped off in the gasoline or oil layer. Others involve separation by means of such liquids as chloroform and carbon tetrachloride, which take account of the differences in specific gravity between the spice tissues and the filth elements.

A microsopic mold-count method is submitted for the determination of rot in ground capsicums such as red or cayenne peppers, chili powder, paprika, etc. This method involves the suspension of 10 grams of the sample in an alkaline clarifying medium by means of the Waring Blendor and a counting procedure with the Howard mold-counting cell used for tomato products.

Methods also are included for the detection of urine stains of rodents and other mammals based on the presence of urea in the stains.

These methods, which have been used by Food and Drug analysts for many years, have given consistent results. It is recommended* that they be adopted as tentative methods.

REPORT ON DECOMPOSITION IN FOODS

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

The initial report of a general Referee in this field seems to call for a few tentative generalizations. The major task is the development of new chemical methods, and the adaptation of published procedures of analysis, to the detection and the measurement, if possible, of the extent of decomposition in foods. The chief advantage of such tests is their objective nature, in contrast to the highly subjective organoleptic examination. Of course, all such chemical methods must correlate with impartial organoleptic judgment.

Decomposition in food—a topic which makes most people want to get farther away—is a condition normally decided by organoleptic examina-

* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, **59** (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

tion by the senses of sight and smell, and sometimes of taste when the appearance or odor is not so bad as to discourage a taste. The great number of factors which enter into one's likes and dislikes of a smell and taste make for as many opinions as there are "organoleptors"—a fancy word not yet in the dictionary, but used in some professional circles to denote those who have trained their eyes, noses, and tongues to examine food for its fitness. The tendency of expert organoleptors to disagree on the fitness of some foods for human consumption has created a need for objective chemical tests which will be sufficiently consistent to lend scientific support to the subjective judgment.

But before the chemist can even begin his work the experts must tell him whether or not his authentic samples are decomposed and, when possible, how decomposed each one is. If the expert calls a sample "bad" today and "good" tomorrow, or if a sample actually is bad today and good tomorrow as judged by a panel of experts, then the chemist may have lost his battle before he starts. This somewhat confusing state of organoleptic affairs will not be pursued further here. For this discussion the assumption is made that experimental material of unambiguous organoleptic condition will be available to the chemist. Perhaps the most important need for chemical criteria of decomposition is in the situation where rotten material has been so processed that even the expert cannot smell out its evil history, let alone the average consumer.

The chemistry of food decomposition is sufficiently complex without complicating it further at this time by including the chemistry of extraneous filth in food. Preservatives and other contaminants which may be objectionable will likewise be excluded from this discussion.

Decomposition in food is usually the result of microbiological action; an exception would be the deterioration of improperly blanched frozen foods as a result of chemical changes brought about by the enzymes normally present. Guilty microorganisms are chiefly bacteria, yeasts, and molds. For regulatory needs then a knowledge of their metabolic processes would seem to be a prerequisite; however, except for a few generalizations of limited value, the chemical behavior of microscopic life in foods is positively unpredictable. All accurate metabolic studies on microorganisms have been made with pure cultures of a single strain, and the experiments have been carried out under controlled conditions which will never be found in the microbiology of commercial foods, where the little understood phenomenon of symbiosis is always present and is perhaps the biggest factor in the control of the chemical changes, especially in determining the final end products of decomposition. In addition, variations in such physical conditions as *p*H, temperature, and humidity alter the course of events in a microorganism's life cycle. High acidity inhibits many bacteria, but often favors molds. Thus a changing flora may exist at varying degrees of decomposition. Bacteria, yeasts, and molds may

develop simultaneously, and each may have its own metabolic pathways. In recent years the similarity in nutrition between microorganisms and higher animals is being recognized; *i.e.*, the same foods—carbohydrates, proteins, and fats—are metabolized in both cases. Many microorganisms require one or more of the same vitamins for growth that are required by humans, and yet there are numerous differences in end products of metabolism. Whereas bacteria are usually considered as being chiefly occupied with the breakdown of more complex materials into simpler molecules, these microorganisms also have great synthetic capacities; *i.e.*, they propagate at tremendous rates and their cells are made up of protoplasm and other biological compounds just as complicated as our own bodily substances. However, the mass of their cells is infinitesimal in comparison with the mass of their metabolic products. Microorganisms apparently generate enzymes to do everything that the human chemical mechanism can, and in many cases they can synthesize many compounds that the human cannot.

Even though the preceding statements indicate that general chemical tests for decomposition are not to be expected, in actual practice some generalizations are now recognized and no doubt there are others which are not yet recognized. Some of these hold true often enough to be used for regulatory purposes. It is our task to find the useful regularities in microbiological metabolism and to establish the experimental conditions by which they can be applied.

The preferred principle in the development of chemical tests for decomposition in foods is to measure one or more of the compounds which together are responsible for the smell or taste that causes the organoleptor to conclude that the sample is unfit for food. This simple principle has been very difficult to put into practice, chiefly because the organs of smell and taste are far more sensitive than the present chemical tests; yet, by definition, odoriferous compounds must have some volatility. Therefore it should be possible by using high vacuum apparatus to separate them unchanged from the non-volatile compounds.

The next best alternative is to find and measure a compound that is always present in decomposition, but may not be an important factor in the organoleptic judgment. In practice the chances are that several tests will be tried before a promising one is found, and then a substantial number of authentic samples must be analyzed before it can be accepted. The most favorable case would be a compound which is present only as a result of decomposition; *i.e.*, one which is entirely absent in a food that shows no detectable decomposition. This premise eliminates that bugaboo of "natural variation," which is always casting doubt on the reliability of a test that depends upon the change in concentration of a compound that is also present in the absolutely fresh product. Such a test also suffers from the serious disadvantage that, in a product which may contain a

variable and unknown amount of decomposed material, there is little hope of getting usable data.

The numerous published procedures that claim to measure the freshness of certain foods were all based on a very few classes of volatile chemical compounds, such as the lower fatty acids, ammonia, and the methylamines, or hydrogen sulfide. In general, each succeeding publication was an effort either to improve or to simplify the technic, or to get a better correlation of the analytical data with the organoleptic condition of the samples. Only when the individual acids or bases were measured did a procedure develop which has maximum usefulness; *e.g.*, the technic for the volatile fatty acids,[1] which has been applied to fish[2] and eggs.[3] Its regulatory value has not been thoroughly explored. Lactic acid has also been useful in eggs[3] and dairy products.[4,5] A test which may prove to be acceptable but which has a more limited scope, is the trimethylamine content of salt water fish.[6] Recently a revised method for hydrogen sulfide in eggs[7] has been found to correlate with organoleptic examination. These few examples represent about all of the reliable chemical criteria for decomposition available, and their applications are by no means universal. Thus the need for new tests is obvious, and with the relatively recent progress in methods of organic analysis and the increased availability of physical instruments with special applications to the measurement of organic compounds, the opportunities for future discoveries are great.

If one were asked to explain the poor showing of chemical methods in this field, the answer might be that the chief factor has been the lack of specific quantitative tests for those decomposition products which "the nose knows" are there. A contributory factor may well have been the wishful effort to find a simple and universal indicator of decomposition. Certainly the chemical complexity of foods and the equal complexity of microbiological changes do not make the task easier. Yet such a complex situation is not at all hopeless, for there are some generalizations; but even in a single type of food, say fish, vegetables, or fruits, it is probably impossible to find one method which will detect all cases of decomposition. However, a test that will catch the majority of "bad" samples and find no false positives would be a useful advance. This is the goal for each type of food, starting with the most urgent and important ones.

Fundamentally, what must a chemical criterion of decomposition do? From the chemist's viewpoint any chemical measurement which will correlate with the organoleptic classification of any food, amid all the variations in geography and climate, and especially the great variety of microorganisms which may be responsible for its decomposition can be used as a criterion. It must be admitted that in practice such a test is indeed

[1] *This Journal*, **25**, 176 (1942). [2] *Ibid.*, **21**, 688 (1938).
[3] *Ibid.*, **27**, 204 (1944). [4] *Ibid.*, **20**, 130 (1937). [5] *Ibid.*, **25**, 253 (1942).
[6] *J. Biol. Bd. Can.*, **3**, 77 (1937).
[7] *U. S. Egg Poultry Mag.*, **49**, 308 (1943).

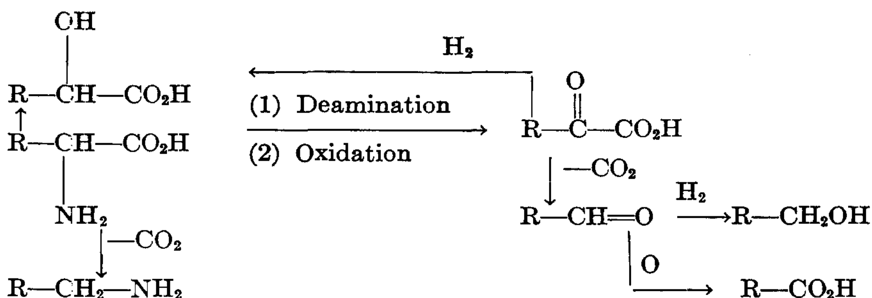
rare. Perhaps the most rigorous requirement which an acceptable test must fulfill is that it never gives a false positive. Theoretically, this possibility is never entirely eliminated from a method, for there is always the chance of an exception. If a second test can be found, even though it be longer and more difficult, to serve as a check on the first, then the chances of a "double false positive" are lessened to the vanishing point. The corroborating test must either measure a different compound than the first or not be subject to the same predictable interferences as the first. Of course, a test which did not completely satisfy these requirements might still be very useful to supplement factory inspection or in other special cases, provided an unjustified interpretation were not put upon the analytical figures. Some tests would be good enough on one lot of uniformly spoiled food, but would become worthless when that food was mixed with, say, ten times as much absolutely sound material.

In developing a new chemical criterion of decomposition, what general strategy should be followed? First available information can be utilized, not only that which is known about microbiological decomposition, but also that which has been discovered about the metabolic products of foods in higher animals, for after all there are some similarities. In some instances the elegant and refined analytical methods for the constituents of blood and urine can perhaps be adapted to this purpose. New reagents and new instruments are constantly appearing. When these can be used without making the test too involved in detail or without requiring too fancy a gadget, then this approach may lead to the goal most quickly.

A second strategy could be based on the premise that there are unrecognized products of either microbiological catabolism or anabolism. Without any preconceived opinion on what specific compounds are there, experiments to isolate these products from a typical decomposed food could be initiated. Such an approach is a long-term project indeed; it is "the hard way"; but it is believed that the answer to some of these problems will come only from this source.

There is a third possible strategy which may be quickest to bear fruit. Much academic effort has gone into the investigation of the mechanisms of bacterial metabolism. A use may be found for a few of the discoveries on the catabolic fate of carbohydrates, fats, and proteins. A superficial examination of carbohydrate end products, other than those now being used as criteria, does not reveal anything very promising. Fats likewise do not seem to be generally useful. The proteins of foods have some promise; in decomposition they are first hydrolyzed to their constituent amino acids, which are in turn further metabolized to yield products that have not been reported to occur in undecomposed natural products. Some of these amino acid degradation products have been, and others could be, determined with great sensitivity and considerable specificity. A brief outline of the known reactions which amino acids undergo may aid in the

explanation of this chemical approach to decomposition, in which one tries to think of what compounds he would expect to find, and then tests for these. Such an approach assumes that suitable tests are available for the expected compounds; if they are not, then the analyst must either devise a method or forfeit his chances of solving a problem. Some of the chemical transformations which microorganisms can produce in amino acids are illustrated in the accompanying diagram.



"R" in these formulas represents that portion of the amino acid molecule which is not present in the amino and acidic groups; *e.g.*, in alanine "R" is "CH_3." Which of the above reactions will take place depends not only upon the types of microorganisms which are present, but also upon the properties of the medium. The basic amines (RCH_2NH_2) are in general formed only in decomposition of protein at a neutral or alkaline *p*H. Whenever the keto acids (RCOCOOH) are formed, they are converted to other compounds so fast that their maximum concentration is hardly enough to detect their presence. In other words, the keto acids are far more reactive than the amino acids from which they are derived.

Those amino acids whose degradation products are most likely to be useful in detecting decomposition in foods are tyrosine, phenylalanine, tryptophane, and histidine, each of which contains a characteristic group for which sensitive specific tests are available. These characteristic groups will almost invariably remain in the degradation products, and can be measured quantitatively in some cases.

Changes in vitamin content or changes in some of the more abundant constituents of food, which result from the action of microorganisms, have been suggested as a measure of decomposition. However, the natural variation in these constituents severely limits their general utility for regulatory needs.

As far as organic compounds are concerned, the belief that the so-called end products of microbiological action in foods are static is an illusion; the microorganism which creates them may be unable to use them, but soon another one will appear on the scene and convert these previous end

products into something else, usually more simple. If nature is allowed to finish the job, organic compounds will eventually become carbon dioxide, water, and inorganic nitrogen. Thus one can never expect to find a high concentration of any end product in an uncontrolled fermentation; the objective should be to find a substance which is present in measurable quantity as a decomposition product at the time of analysis.

These general remarks are not intended to suggest that any two foods will undergo the same type of decomposition; indeed it may be necessary to hunt for a test to apply to each kind of food. However, acetic acid does seem to be a general product of all decomposition, although in some food control problems it is not the whole answer.

The future of chemical criteria of decomposition in foods is unlimited; little has been done, but the tools for success seem to be awaiting application. However, the complexity of the problem and the necessary precautions will not permit a rapid solution. The process of developing a new test is slow and laborious, and the consequent strain of slow progress and occasional retreat requires a somewhat optimistic mental attitude, as well as plenty of perserverance and patience.

The Referee* concurs in the recommendation of the Associate Referee that the method for volatile acids be adopted as official, final action, for eggs, fish and other marine products, fruits, and vegetables, and that a method for lactic acid be adopted as official for eggs, vegetables, dairy products, and fruits.

For further study of chemical criteria of decomposition it is recommended that associate referees be appointed in the following fields: (1) dairy products, (2) fish and other marine products, and (3) fruits and fruit products.

REPORT ON VOLATILE ACIDS IN FISH PRODUCTS

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

In the report last year[1] a résumé was given of the volatile acid investigations as applied to fish products. Attention was called to a paper[2] in which a method was proposed for the quantitative determination of individual volatile acids. The method calls for the use of an apparatus previously described.[3] In the proposed procedure distillation data on volatile acids of known purity must be obtained by each analyst using his own apparatus. These distillation data are necessary for use in setting up the

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* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

[1] *This Journal*, 27, 237 (1944).

[2] *Ibid.*, 25, 176 (1942).

[3] *Ibid.*, 21, 684 (1938).

simultaneous equations that are employed in computing the quantity of volatile acid or acids present. Calibration data obtained by 20 collaborators on their own apparatus were included in last year's report. A table showing recoveries obtained by 16 collaborators from various mixtures (quantities unknown to collaborators) of pure solutions of formic, acetic, and butyric acids was also included in the report. Some of the mixtures contained lactic acid. The results obtained were very satisfactory.

The revised volatile acid method[2] was first applied to egg products for the purpose of detecting decomposition. In a paper on "Detection of Decomposition in Liquid, Frozen, and Dried Eggs"[4] the method is described in detail.

Once the sample is prepared for distillation the method is the same for all food products to which it is applicable. Directions for the preparation of sample for fish and fish products are recommended for adoption.*

TABLE 1.—*Formic and acetic acids in tuna fish*
(Mg./100 grams)

CAN NO.	CLASS 0		CLASS 1		CLASS 2		CLASS 3		CLASS 4	
	FORMIC	ACETIC	FORMIC	ACETIC	FORMIC	ACETIC	FORMIC	ACETIC	FORMIC	ACETIC
1	Trace	16	Trace	18	2	24	15	50	14	65
2	Trace	15	Trace	21	4	24	19	71	16	76
3	Trace	16	Trace	14	4	24	10	47	27	91
4	Trace	14	Trace	17	5	27	14	62	17	74
5	Trace	19	Trace	16	2	21	20	81	21	66
6	Trace	13	Trace	14	3	19	15	66	22	81
7	Trace	16	Trace	18	6	34	15	59	16	59
8	Trace	17	Trace	19	3	19	14	62	23	81
9	Trace	16	Trace	18	4	25	15	73	28	96
10	Trace	16	Trace	18	3	21	16	54	19	72
Av.	—	16	—	17	4	24	15	63	20	76
Max.	—	19	—	21	6	34	20	81	28	96
Min.	—	13	—	14	2	19	10	47	14	59

Late in the summer of 1943 authentic packs of tuna fish, sardines, and mackerel, showing varying classes of decomposition, were prepared at Los Angeles. The results of work on these and other authentic packs of fish are not yet ready for final publication. However, in order to show the applicability of the method to the detection of decomposition in fish products, analysis of one of the tuna packs is given in this report. The data are presented in Table 1. Class 0 is prime fish, and the other classes progressively indicate spoilage.

[4] *Ibid.*, 27, 204 (1944).
* Details of the methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

It will be seen that as decomposition progresses the quantity of formic and acetic acid increases. The data indicate that volatile acids can be used as an index of decomposition in fish products.

Seven samples of canned fish, consisting of one sample of mackerel; three samples of California sardines, one of which contained tomato sauce; and three samples of tuna fish, were submitted to collaborators for the determination of volatile acids. The results are given in Table 2.

TABLE 2.—*Formic and acetic acids in several varieties of canned fish* (Mg./100 grams)

COLLABORATOR	CALIF. MACKEREL	CALIF. SARDINE	CALIF. SARDINE	CALIF. SARDINE, TOMATO SAUCE ADDED	TUNA	TUNA	TUNA
			Formic Acid				
1	10	Trace	28	33	Trace	6	27
	9		30	36		6	31
2	8	Trace	28	31	Trace	6	25
	8		28	32		6	28
3	11	Trace	34	39	Trace	8	31
	12		34	40		8	33
4	9	Trace	30	32	Trace	6	28
	9		29	33		6	28
			Acetic acid				
1	53	12	92	105	19	27	109
	54	12	94	105	19	27	112
2	50	11	91	102	19	24	108
	51	12	94	102	20	25	109
3	60	13	92	108	19	31	108
	58	12	95	110	20	32	110
4	52	12	93	106	19	27	108
	52	12	93	109	18	26	108

The volatile acid method has been subjected to a rather exhaustive collaborative study,[1] and excellent results have been obtained. In view of the satisfactory data previously reported and the excellent collaborative results reported in Table 2, it is believed that a recommendation that the method be made official is in order and it is so made. Inasmuch as the 6th edition of *Methods of Analysis, A.O.A.C.*, is to appear next year, it is

recommended* that the official action be made final for publication in that edition.

The Associate Referee wishes to thank the collaborators, Mae Carstensen, L. L. Ramsey, and L. M. Beacham, of the Food and Drug Administration, for their cooperation.

REPORT ON GELATIN, DESSERT PREPARATIONS, AND MIXES

By S. C. ROWE (*Referee*) and E. F. STEAGALL (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

For many years various methods have been used in the analysis of gelatin, gelatin desserts, starch pudding powders, pie fillings, etc. Only part of these methods is available in *Methods of Analysis, A.O.A.C.*, 1940, although Federal purchase specifications for these commodities require that the methods of the A.O.A.C. shall be used. Unfortunately, it is also not clear which methods should be used since they are not presented in any one place in the book. Therefore, it is considered that there is real need for a chapter in the 6th edition which will group together all the reliable methods formerly used and the new methods of comparatively recent development that are applicable to this work. Since methods for plain gelatin will be included in the proposed new chapter, there would appear to be no need for those now found in the chapter on Meat and Meat Products. Accordingly, it is recommended† that paragraphs **59–63**, inclusive, pp. 386, 387, be transferred to the new chapter, paragraphs **64–68** be transferred to Chapter **XXIX**, and paragraph **70** be transferred to Chapter **XXXII**. It is recommended that paragraph **69** be dropped because of improved methods for the determination of jelly strength. It is also recommended that the following methods, which have proved sufficiently accurate to warrant their adoption as tentative, be included in a separate chapter under the heading "Gelatin, Dessert Preparations, and Mixes."

No report on fish and other marine products was given by the Referee.

No report on total solids and ether extract was given by the Associate Referee.

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* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 59 (1945).
† For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 59 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

REPORT ON GUMS IN FOODS

By F. LESLIE HART (Food and Drug Administration, Federal Security Agency, Los Angeles, California), *Referee*

Methods of Analysis, A.O.A.C. is scheduled for its 6th edition in 1945. A review of methods listed in the 5th edition, and of methods and changes recommended since the publication of that edition is therefore indicated.

MAYONNAISE AND FRENCH DRESSING

A method for detection of gums in mayonnaise and French dressing is given as tentative in Chapter **XXXIII, 55.** There is some ambiguity in this method in the interpretation of results. This is due apparently to precipitation of traces of starch or other carbohydrates in the spices present. Associate Referee Fine, in his report, amends the method to remove this ambiguity. He also recommends deleting confirmatory tests 3 and 4. The Referee agrees with this recommendation.

SOFT CURD CHEESE

A method for detection of gums in soft curd cheese is given as tentative in Chapter **XXII, 127.** This method has been further studied by Associate Referee Gnagy. He shows that a more nearly complete separation of casein occurs at its iso-electric point, and has refined the method so as to separate larger amounts of gum. The Referee agrees with his recommendation.

Note 6, page xii, of *Methods of Analysis, A.O.A.C.*, 1940, states that "x%" means "X grams of substance is dissolved in water and made up to 100 ml." Gnagy has revised paragraph **127,** Chapter **XXII,** to accord with this statement.

Since this method has been adopted as tentative the Federal Security Agency (7 FR 10755) (SRAFDC 2, Federal Security Agency 19-515, 19.520) has promulgated standards for cream cheese and for Neufchatel cheese. These standards permit up to 0.5 per cent of certain gums (or gelatin). A quantitative method for determination and identification of these stabilizers is therefore indicated for future study by the Associate Referee on Soft Curd Cheese.

STARCHY FOODS

This new topic has been studied by Associate Referee Redfern. He reports a very promising method for detection of gums in starchy salad dressings. Redfern first removes starches and gums by extracting the defatted, neutralized salad dressing with calcium chloride solution, removes the starch by precipitation as starch-iodide, and precipitates the gums in the filtrate with alcohol. He recommends collaborative study next year. The Referee agrees.

ICE CREAM

This topic was assigned to the Referee for further study, but no work was done during the past year. Algin, alone, or in combination with other gums, or with gelatin, is used as a stabilizer in the manufacture of ice cream and ice milk. Algin is converted to alginic acid by trichloracetic acid, and hence is precipitated along with proteins; other gums are not affected; if tannic acid is used as a protein precipitant, algin, along with other gums, is found in the filtrate. It is hoped that a method will be devised, through use of these differential reactions, to distinguish algin from other gums.

OTHER FOOD PRODUCTS

One method, Gum and Dextrin in Wines, Chapter **XV, 35,** has been deleted, since addition of these products to wine is not at present a commercial practice. A method for detection of agar agar in meat products was adopted as tentative (*This Journal,* **25,** 93), and as official (*Ibid.,* **27,** 90).

Gums are occasionally used as adulterants in jams and jellies, and as stabilizers in beverage bases and imitation beverage bases. These products offer the problem of separating gums from pectin. Gums are also used in conjunction with cocoa or chocolate in chocolate milk, chocolate beverage bases, bakery products, etc. All of these subjects give opportunity for future study by the Association.

RECOMMENDATIONS*

It is recommended—

(1) That the editorial revisions of the tentative method for detection of gums in mayonnaise and French dressing by the Associate Referee be adopted.

(2) That the method proposed by the Associate Referee for detection of gums in soft curd cheeses be adopted as tentative, and that the present tentative method (**127,** p. 305) be dropped.

(3) That the method for detection of agar agar in meat products (*This Journal,* **25,** 93), made official (first action) in 1943 (*Ibid.,* **27,** 90) be adopted as official (final action).

(4) That studies be undertaken on the identification and quantitative estimation of gums in soft curd cheese.

(5) That the method for detection of gums in starchy foods submitted by the Associate Referee be studied collaboratively.

(6) That studies be undertaken on the detection of gums in jams, beverage bases, and other fruit products.

(7) That studies be undertaken on the detection of gums in cacao products.

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* For report of Subcommittee C and action by the Association, see *This Journal,* **28,** 59 (1945).

(8) That studies of methods for the detection of gums in frozen desserts be continued.

REPORT ON GUMS IN SOFT CURD CHEESE

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

The Associate Referee, on recommendation of the Committee, submitted the tentative method for the detection of gums in soft curd cheese to further collaborative study.

The control of *p*H as shown in the method given in the Associate Referee's report, published in *This Journal*, **25**, 725, showed much promise. Accordingly, the tentative method given in *Methods of Analysis*, 1940, 305, **127–130**, was rewritten to incorporate this principle and to clarify certain other points which caused the Associate Referee and his collaborators trouble. However, the main principle of the method as evolved by F. Leslie Hart and published as the tentative method was not changed. The modified method with samples and instructions was submitted to three collaborators by the Associate Referee.

EXPERIMENTAL WORK

1. Description of Samples and Method.—A fine-grained, soft curd cheese, free from gum, was secured from a local creamery, and 100 grams portions were weighed into small glass jars. The jars were separated into three sample groups. No gum was added to jars in sample No. 3, 0.05 gram of locust seed gum was added to each jar of sample No. 2, and 0.1 gram of the same gum was added to each jar of sample No. 1. One jar from each of the three samples was sent to each collaborator but the collaborator neither knew which jar contained the gum nor the quantity of gum. In addition, another jar (sample X), was sent to each collaborator, who was informed that the jar contained no added gum and was asked to analyze the cheese in that jar first so he would become familiar with the results obtained from an authentic cheese with no added gum.

2. Method.—The method sent out to the collaborators was essentially the same as the method recommended in this report for adoption as tentative. It has been edited and clarified to accord with the suggestions and findings of the collaborators, which were corroborated by the work of the Associate Referee. Editing involved changes in time periods and speed of centrifuging, and minor changes in technic to secure better precision. A paragraph was inserted to cover the procedure to be used in case protein has been precipitated with the gum. The manipulation was simplified at one place by precipitating in the centrifuge bottle instead of first transferring to a beaker. The Benedict test was standardized and the aliquot

taken for the test was cut in half. The collaborators were asked to weigh the dried alcohol precipitate with a view to a possible quantitative determination. This was left out of the method recommended.

The collaborators were (1) B. H. Gnagy, Department of Health, Los Angeles, Calif.; (2) R. D. Dye, Bureau of Water and Power, Los Angeles, Calif., and (3) Sam D. Fine, Food and Drug Administration, Cincinnati, Ohio.

The collaborative results are shown in Table 1.

COMMENTS BY COLLABORATORS

B. H. Gnagy.—The casein tube reading helps in concluding that a sample may contain added gum. It should be of value in using the method for the first time.

Some difficulty was experienced in removing the dried gum from dish. This trouble was overcome by adding 20 ml. of hot water to the gum and allowing to stand for about 20 minutes at the temperature of boiling water.

R. D. Dye.—I found the casein tube reading to be of real value in concluding whether a sample contained added gum or not. I think the casein tube would be helpful to a chemist using the method for the first time. Under "Preparation of Sample" some difficulty was experienced in getting all the curd in solution following adjustment of pH to 9.0. Perhaps instructions to stir vigorously and smash the curd with a stirring rod with continual application of heat would be helpful.

No. 3 sample could be classed as doubtful as it was negative according to the casein tube and weight test and positive (faintly) by the Benedict test. Perhaps instructions to disregard a faint positive Benedict test would be helpful.

Sam D. Fine.—The casein tube reading assisted in concluding whether or not a sample contained added gum. I believe that the casein tube reading would be of value to a chemist using the method for the first time. As 500 ml. casseroles were not available, 600 ml. beakers were used in the preparation of the sample. After initial period of heating and addition of 100 ml. of hot water, volume was greater than 250 ml.—capacity of centrifuge bottles available. It might be wise to have the volume of water to be added limited in order to keep sample in one centrifuge bottle. 20–25 minutes was required for complete solution of Samples 1 and 3.

It might be well to specify a definite speed and time for centrifuging after precipitation of casein; low speeds and short period of centrifugation would result in more of the liquid containing the gum being occluded in the precipitated casein. At this point I centrifuged at 1800 r.p.m. for 5 minutes.

Under "Separation of Gum" the analyst is directed to hold at 70° until the precipitate coagulates, avoiding prolonged heating. Sample X was analyzed first; sample was held at 70°C. for 5 minutes after addition of trichloracetic acid reagent, and then carried on to the next step. Precipitate did not coagulate well. The supernatant liquid after centrifuging was quite cloudy. Addition of alcohol to the decanted supernatant liquid produced a heavy precipitate; since no gum had been added to this sample, the precipitate must have been casein. The material was centrifuged and supernatant liquid discarded; 35 ml. of hot water was added to the contents of the centrifuge bottle, and the bottle was placed in a bath at 70°C.; the bottle was shaken and swirled until the precipitate was thoroughly dispersed (it was not completely soluble in hot water). An additional 10 ml. of trichloracetic acid reagent was added, and the bottle and contents were held at 70°C. until the precipitate coagulated (about 10 minutes). After centrifugation, the supernatant liquid was clear, and the regular procedure was followed.

TABLE 1.—*Collaborative results*

COLLABORATOR	SAMPLE NO.	ADDED GUMS	NATURE OF SECOND ALCOHOL PPT.	CASEIN TUBE READING	WT. OF ALCOHOL PPT.	BENEDICT TEST	ADDED GUM
		per cent		*ml.*	*gram*		
1	X	none	Flocculent	0.1	0.008	No ppt.	No
2	X	none	Very small & fine	0.1	0.004	Negative	No
3	X	none	Slight, floats	0.3	0.010	Negative	No
1	1	0.1	Floc. to stringy	0.8	0.054	Orange ppt.	Yes
2	1	0.1	Heavy, gelatinous	3.0	0.062	Positive, large red	Yes
3	1	0.1	Floc., settles	1.8	0.071	Orange red	Yes
Assoc. Ref.	1	0.1	Heavy, floc. to stringy	1.6	0.136	Large, red ppt.	Yes
1	2	0.05	Floc. to stringy	0.45	0.040	Orange ppt.	Yes
2	2	0.05	Medium, gelatinous	1.0	0.031	Positive, large, red	Yes
3	2	0.05	Floc., settles	1.3	0.042	Brick red	Yes
Assoc. Ref.	2	0.05	Heavy, floc. to stringy	1.45	0.077	Large, red ppt.	Yes
1	3	none	Flocculent	0.2	0.014	Cloudy	No
2	3	none	Very light, gelatinous	0.4	0.008	Positive, light, red	No
3	3	none	Slight, floats	0.3*	—	Negative*	No
Assoc. Ref.	3	none	Very small	0.3	0.023	Very small, reddish	No

* Centrifuge tube broke during 15-minute centrifuging. Volume of precipitate estimated to be about that of Sample X. Contents of cup evaporated, dissolved in water and Benedict test made.

The same difficulty occurred with Samples 1, 2, and 3, although all were held at 70°C. for 20 minutes. The supernatant liquid after treatment with the trichloracetic acid reagent was cloudy and addition of alcohol gave heavy precipitates (heavier than would be expected from small amounts of gum). All three were treated as Samples X above; that is, a second precipitation with the trichloracetic acid reagent was made. Sample 1 was allowed to stand overnight after initial precipitation of the casein with acetic acid. The supernatant liquid from this sample was the least cloudy of the four after the initial trichloracetic acid precipitation.

I believe it would be well to state in the method that the centrifuged supernatant liquid after the trichloracetic acid precipitation should be practically clear; otherwise incomplete precipitation of casein is indicated; that in case the supernatant liquid is not clear the treatment used above be followed (that is, decant the supernatant liquid into a centrifuge bottle, add 4 volumes of alcohol, allow to stand, centrifuge, and discard the supernatant liquid. Add 35 ml. of water, disperse, and add 10 ml. of the trichloracetic acid reagent, allow to coagulate, etc.).

It is more convenient to decant the supernatant liquid from the trichloracetic acid precipitation directly into a 250 ml. centrifuge bottle than into a 400 ml. beaker and transfer to the bottle later. Not quite four volumes of alcohol can be added.

Hart casein tubes were not available. A 50 ml. tube with a tapered lower portion calibrated at 0.5 ml. intervals was used. Unfortunately, one tube (containing Sample 3) broke during the 15 minute centrifugation.

The Associate Referee also tried out the preceding method upon some old soft curd cheese which he had secured 25⅓ months previously. This cheese had been kept in glass jars in 100-gram portions under refrigeration in the laboratory ever since and had survived many defrostings of the refrigerator. The cheese smelled slightly stale but was not rotten. The gum was added to the cheese just prior to the analysis. An old solution of trichloracetic acid was used, and a very heavy precipitate of protein came down with the gum. The entire method was again applied to the precipitates, but a freshly made solution of trichloracetic acid was used (Reagent c). Very good results were obtained, as shown in Table 2.

TABLE 2.—*Results obtained by Associate Referee*

SAMPLE NO.	ADDED GUMS	NATURE OF SECOND ALCOHOL PPT.	CASEIN TUBE READING	WT. OF ALCOHOL PPT.	BENEDICT TEST
	per cent		*ml.*	*gram*	
4	None	Slight, flocculent	0.1	0.017	Very small, reddish
5	0.05	Heavy, floc. to stringy	2.0	0.041	Large red ppt.
6	0.10	Heavy, floc. to stringy	2.7	0.070	Large red ppt.

DISCUSSION OF RESULTS

The results obtained were considered good. The collaborators apparently had no difficulty in detecting 0.05 per cent added gum, although one collaborator and the Associate Referee had some trouble with some protein precipitating with the gum. This has been corrected by specifying nitrazine indicator paper and editing that part of the method covering the approach to the proper *p*H.

Since more gum is separated by the method submitted than by the tentative method, the Associate Referee cut in half the aliquot used for the Benedict test and still got a strong positive test. In doing this he practically eliminated the slight positive test which heretofore has been shown by cheese with no added gum and which has disturbed collaborators in interpreting their results.

SUMMARY AND CONCLUSIONS

The tentative method (*Methods of Analysis, A.O.A.C.*, 1940, 305, **127–130**) has been revised to secure better separation of the gum from the protein, and a greater amount of gum has been separated than was possible by the present tentative method. In addition, details of the method have been changed so that given operations are stated in more specific terms, which lead to more uniformity of procedure among analysts and hence more uniform results.

The investigation this year was confined to locust bean gum in soft curd cheese. However Hart (*This Journal*, **20**, 532, and **23**, 601) showed that the present tentative method can be applied to detection of karaya, acacia, tragacanth, ghatti, and locust bean gums in soft curd cheese. It is only reasonable to conclude that the revised tentative method will also improve the separation of the other gums mentioned by Hart to the same degree that it did in the case of locust bean gum.

RECOMMENDATIONS*

It is recommended—

(1) That the method discussed in this report be substituted for the present tentative method.*

(2) That the method be further studied with a view to making it quantitative as well as qualitative.

(3) That the method be studied in regard to its application to the detection of algin (sodium alginate) in soft curd cheese.

REPORT ON GUMS IN MAYONNAISE AND FRENCH DRESSING

By SAM D. FINE (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

At the 1941 meeting of the Association, it was recommended (*This Journal*, **25**, 64) that the tentative method for the detection of gums in mayonnaise and French dressing (*Methods of Analysis, A.O.A.C.*, 1940, 477, 55) be studied collaboratively. Some laboratories had reported that certain spices interfered with the application of this method. To carry out this recommendation the following work was undertaken.

* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 60 (1945). The method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

The Minneapolis Station of the Food and Drug Administration, which had reported some difficulty with this method during a survey on mayonnaise made in 1940 for the Food Standards Committee, was contacted. A copy of an unpublished report by S. H. Perlmutter was obtained. In this report it was stated that the tentative method was used on three samples of mayonnaise, and that results were observed that indicated the presence of gums. However, factory inspection did not show the use of gums by the manufacturer. Perlmutter obtained samples of each ingredient used and tested the ingredients by the tentative method. On treating the samples of ground yellow mustard and ground paprika in the prescribed manner, he obtained precipitates on the addition of alcohol to the acetic acid solution of the gum-like residue that seemed to prove that gums were present in these spices. Similar results were obtained with samples of Colman's mustard and Marshall's paprika. The Seliwanoff and Tollens tests of these precipitates were negative, and the Molisch tests were positive, indicating carbohydrates.

The present Associate Referee, while at the St. Louis Station of the Food and Drug Administration, analyzed five samples of mayonnaise in the course of the same survey for the Food Standards Committee. The tentative method was used for the detection of gums. Gums were reported negative in all five samples, and these results were confirmed by factory inspection evidence. However, the original laboratory notes made at the time showed that a slight precipitate occurred in all samples on the addition of alcohol. One sample gave a slight positive Benedict test; the other four results were negative. Four samples gave faint positive Molisch tests; one result was negative. Presumably, Perlmutter got precipitates of the sort observed by the Associate Referee, and instead of ignoring such slight amounts concluded that a small amount of gum was present. His later experimental work convinced him that the precipitates he observed were due to the spices used.

A review was made of the papers concerning the method. In the first report by Hart (*This Journal*, **22**, 605), the samples for collaborative study were prepared from salad oil, egg, and vinegar or lemon juice; to each of which small amounts of gum were added. In the second report by Hart (*Ibid.*, **23**, 597), the samples were prepared from cottonseed oil, egg yolks (10 per cent), apple vinegar, sugar, salt, and pepper; to some samples gums were added. The collaborators reported slight precipitates with alcohol in those samples where no gum was present; this must have been due to the carbohydrates from the pepper.

Commercial mayonnaise usually contains one or more spices—pepper, mustard, paprika, etc. All of these substances that have been tested by the Associate Referee give slight alcohol precipitates when subjected to the method. This would be expected from a consideration of the composition of such spices—high in carbohydrates, starch.

Several samples of mayonnaise from the Cincinnati area were examined by the method. None had gums declared; all gave slight alcohol precipitates. Addition of gum to the amount of 0.10 per cent gave a much larger precipitate.

CONCLUSION

The present tentative method for the detection of gums in mayonnaise and French dressing appears to be satisfactory. However, in order to avoid considering the slight precipitates due to spices as a positive test for gums, it is suggested that the method be amended as follows:

In line 6 of the second paragraph of the tentative method (*Methods of Analysis, A.O.A.C.*, 1940, 477, 55), the section "A precipitate at this point indicates gums. This may be . . . ," is changed to read "The presence of a significant amount of gum will be denoted by a heavy flocculent precipitate at this point. A slight precipitate should not be considered a positive test for gums, as the spices present in most mayonnaise and French dressings will usually give such a precipitate. Confirm presence of gums by the following procedure:

"Add 35 ml. of hot water to precipitate in nursing bottle, transfer to a small beaker, add 5 ml. of HCl, and boil gently 2 minutes to hydrolyze gums to sugars. This solution may now be used for various qualitative tests for monosaccharide sugars, as follows:

(1) *Benedict test.*—Transfer 1 ml. of the hydrolyzed gum solution to test tube; neutralize with approximately 2 *N* HaOH, using litmus paper as indicator; remove litmus paper; add 5 ml. of Benedict's qualitative sugar solution, **XXII, 127,** and boil vigorously 1–2 minutes. Allow to cool spontaneously. A voluminous precipitate, which may be green, yellow, or red, indicates reducing sugars. The slight precipitate due to spices will cause very slight, if any, reduction of Benedict's reagent.

(2) *Molisch test.*—Transfer 5 ml. of the hydrolyzed gum solution to test tube, and add 2 drops of 15% solution of alphanaphthol in alcohol. Incline tube and slowly pour down inner side 3–5 ml. of H_2SO_4 so that the two layers will not mix. A reddish violet zone at point of contact indicates carbohydrates. (A 5% solution of thymol in alcohol may be substituted for alphanaphthol.)"

The last six lines, comprising Tests 3 and 4, are deleted.

RECOMMENDATIONS*

It is recommended—

(1) That the amended method be adopted as tentative.

(2) That studies on the detection of gums in mayonnaise and French dressing be discontinued.

No report on Gums in Frozen Desserts was given by the Associate Referee.

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 59 (1945).

REPORT ON GUMS IN STARCHY FOODS

By SUTTON REDFERN (The Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.), *Associate Referee*

A method has been devised for the detection of gums in salad dressing. The salad dressing is first defatted with petroleum benzine, neutralized with magnesium carbonate, and extracted with calcium chloride solution. The gums and starch are found in the filtrate. The starch is removed by adding iodine solution in excess and filtering off the starch-iodide. The gums are then precipitated in the filtrate by the addition of ethanol. The precipitate is redissolved, filtered, and reprecipitated. An immediate precipitate indicates the presence of gums which is confirmed by a test for pentoses.

This is only a preliminary report and it is recommended* that this method be submitted to collaborators for further study.

REPORT ON MEAT AND MEAT PRODUCTS

By C. H. SWANGER (Meat Inspection Division, Livestock and Meats Branch, Washington, D. C.), *Referee*

No new developments are being reported for the past year. In the determination of crude fat or ether extract, the sample is never dried by Method **6**, p. 354, of *Methods of Analysis, A.O.A.C.*, 1940, and because of this it is recommended that the language of section **2** *Ibid.*, p. 374, be changed to delete the words "following **6** when dried sample is to be used for further determinations."

Section **6** should read "Dry 3–4 g of the sample as directed under **3**. Grind dried sample with asbestos, sand, or similar substance, and proceed with ether extraction as directed under **XXVII, 22**."

It is recommended† that the methods for the detection of soybean flour in meats, first published in *This Journal*, **19**, 409, and adopted as tentative *Ibid.*, **20**, 73, be modified. Method I is not satisfactory for meat products, and it is recommended that it be omitted. The microscopic test (Method II) should also be revised.[1]

It is also recommended that the following changes be made: (a) That the portion of Chapter **XXVIII** pertaining to gelatin be transferred to another chapter; (b) that Sections **59–63**, inclusive, be transferred to a new chapter, "Dessert Preparations and Mixes"; (c) that Sections **65–68**, in-

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* For report of Subcommittee C and action of the Association, see *This Journal*, **28**, 60 (1945).
† For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 60 (1945). These methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.
[1] Winton, "Microscopy of Vegetable Foods," 2nd ed., p. 248; "The Structure of the Soybean," *British Yearbook of Pharmacy*, pp. 467–478 (1913).

clusive, be deleted from Chapter **XXVIII**, as methods for metals are given in Chapter **XXIX**; (d) that Section **64** be transfered to Chapter **XXIX**; and (e) that Sections **69** and **70** be deleted.

Polariscopic constants have been replaced by other methods for gel strength and methods for sulfur dioxide are given in Chapter **XXXII**.

No report on dried skim milk in meat products was given, no associate referee having been appointed.

No report on soybean flour in meat products was given by the Associate Referee.

REPORT ON METALS IN FOODS

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

The problem of an appropriate name for Chapter 29 is again before us with the preparation of the material for the new edition of *Official and Tentative Methods of Analysis, A.O.A.C.* The term metal as part of the title of Chapter 29 has been, and is now more than ever, a misnomer, when methods for the determination of such widely different products as lead, fluorine or selenium, and DDT (dichlorodiphenyltrichlorethane) are under consideration; nor is Chapter 29 concerned with spray residues only. The Referee has tried to devise a name that was sufficiently inclusive and exclusive. The Referee believes the methods that should go into Chapter 29 are those used in determining the normal occurrence of metals or other elements in foods; spray residues including fumigation or dusting residues, either organic or inorganic; contaminations other than filth from containers, equipment, or those due to faulty factory practices; and the poisoning of foods with toxic substances other than those clearly classified as drugs or those due to biological processes. The Referee suggested at the last meeting that the title be changed to "Toxic Substances in Foods" on the theory that this designation would include the determination of organic as well as inorganic products, and would not include non-metals under the term metals. The term toxic did not meet wholehearted acceptance. After considering many names, opinion narrowed down to the expression "Metals, Residues, and Other Elements in Foods." The term "Metals" was retained as a liaison between the old and new chapters; "Residues" could include both organic and inorganic spray and fumigation or dusting residues left on foods by processes designed to prevent insect damage; "Other Elements" provided for certain non-metals or anions whose determination in or on foods was of some importance.

Many, if not all, of the methods in Chapter 29 are micro methods and the Association is now well provided with methods that may well be called "trace" methods.

ARSENIC AND ANTIMONY

No report on Arsenic and Antimony was submitted, but the associate referee nevertheless has two recommendations to make on this subject. The paper on the determination of arsenic, referred to by the Referee last year, has now been published. Certain of its features will have to be investigated as soon as possible. There is no time for this now nor can lengthy parts of the paper be incorporated in the new *Methods of Analysis, A.O.A.C.* But to call attention of analysts to this paper, the Referee recommends that the following be inserted into par. 3 of Chapter 29:

"(f) For ultra-micro quantities of As, very labile forms of As, and for a vacuum accelerated Gutzeit reduction system for mercuric bromide spot filtration, consult Satterlee and Blodgett (*Ind. Eng. Chem., Anal. Ed.*, **16**, 400 (1944)."

Gelatin methods which were deleted from Chapter 28, Meat and Meat Products, are to be included in the methods for the determination of arsenic, copper, and zinc. Chapter 29 already contains copper and zinc methods that will provide for their determination in gelatin. The arsenic method for gelatin contains a special method for arsenic isolation involving the precipitation of magnesium arsenate with excess magnesium phosphate. Instead of inserting this paragraph *in toto* in Chapter 29, the Referee recommends that provision be made for it in paragraph 4 by merely adding the words: "Gelatin may be hydrolyzed with HCl and the As isolated as in 3e."[1] The reference cited contains practically the same directions for isolating the arsenic.

CADMIUM

The Associate Referee on Cadmium reports an investigation on the determination of micro amounts of cadmium in foods and biological material involving dithizone extraction and color estimation. No opportunity for collaborative studies was possible in the time available. A similar paper on biological materials was published during the year by Cholak and Hubbard,[2] who used dithizone as well as the naphthol analogue of dithizone. It is recommended that the determination of cadmium be studied collaboratively next year by one or both of these methods.

COPPER

A micro method for copper is needed for the new *Methods of Analysis, A.O.A.C.* This Association has endeavored for a number of years to perfect a carbamate method for the determination of copper in foods. These efforts failed principally because of lack of blank control. A new method

[1] C. R. Gross, *Ind. Eng. Chem. Anal. Ed.*, **5**, 58 (1933).
[2] *Ind. Eng., Chem., Anal. Ed.*, **16**, 333 (1944).

for the final isolation of the copper just before the final carbamate determination has yielded satisfactory results and it is recommended that this method be adopted tentatively.

DICHLORODIPHENYLTRICHLORETHANE, DDT

The new insecticide DDT (abbreviation of its chemical name) is having a phenomenal military success and his been investigated experimentally this past year as an agricultural insecticide for a wide variety of insect pests. According to reports it has great promise and if the military situation allows, more of it may be available to agriculture in 1945. A report on the determination of DDT is included in this meeting's program under insecticides and fungicides. Pharmacological studies, especially those concerning its chronic effects, are not complete, but enough is known to establish the belief that it is toxic under certain conditions and concentrations. This means that when DDT has wider use we shall have another spray residue problem. Studies on methods for determining this organic insecticide on fruits and vegetables have already begun in various quarters. It is expected that several methods will be available next year. Therefore, the appointment of an associate referee to undertake the collaborative testing of the expected methods is recommended.

FLUORINE

The tentative fluorine method adopted last year for spray residues needs certain changes necessitated by the rescent fluorine hearing and the establishment of a fluorine tolerance expressed in parts per million. Most of these changes can perhaps be classified as editorial, but some changes in reagent concentrations are necessary to cover a greater range. No change in principles will be made. The Referee recommends that these minor changes be authorized. The Associate Referee's report presents the fluorine data obtained by nine laboratories on about twenty different foods collected in the area of each one of the analyzing stations. This gave our fluorine methods a severe test, and the comments of the analysts will be considered in clarifying the language of the method for the new edition of the *Methods of Analysis, A.O.A.C.* The Referee also believes that the results constitute about the best and latest information, for an adequate cross-section, on the fluorine content of the American diet.

LEAD

No report on lead is made by the Associate Referee. He has, however, made a careful revision of the lead methods for the new edition of *Methods of Analysis, A.O.A.C.* These revisions include changes from the English system of grains per pound to parts per million consistent with the fluorine tolerance. Concentrations of reagents and aliquots for the spray residue method have been changed a number of times in the past to comply with changing tolerances. The present tolerance of 0.05 grain of lead per

pound of apples or pears is equivalent to approximately 7 parts per million. The Associate Referee has made changes in the concentration of reagents to fit expression of results in parts per million and a range up to 10 parts per million. The Referee recommends that these editorial and concentration changes be adopted.

MERCURY

The Associate Referee is making no formal report. He has been testing the Laug method[3] for the determination of mercury because he believed it to be simpler than the present tentative method. However, he ran into difficulties that he could not master in time for the meeting. The Association must therefore retain the present tentative method in the new *Methods of Analysis, A.O.A.C.*

SELENIUM

A selenium method was adopted as official (first action) last year. No adverse criticism has come to the Referee's attention during this year; therefore he recommends that this method for the determination of selenium be adopted as official, final action.

ZINC

The present tentative method for the colorimetric determination of zinc in foods has been demonstrated to be faulty.[4] A small part of the zinc exists in the final color estimation as colorless zinc carbamate and the remainder as a red dithizonate. Theoretically, reproducible results are possible if the same partition exists in the production of the standards. But the Association has never looked with favor on methods that contained known sources of error. Since the factor of reproducibility was not overly strong in the collaborations resulting in the adoption of the tentative method, it may be concluded there are other sources of error in it. A substitute method that avoids some of these sources of error has been developed and studied collaboratively with satisfactory results. The Referee therefore recommends that the present tentative colorimetric method be dropped and the method studied by the associate referee be adopted as tentative in its stead on the theory that the results will be closer to the truth because the opportunities for error have been reduced. Continued study is recommended.

RECOMMENDATIONS*

It is recommended—

(1) That the name of Chapter 29 be changed to "Metals, Residues, and Other Elements in Foods."

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[3] *This Journal*, 25, 399 (1942).
[4] Cowling and Miller, *Ind. Eng. Chem., Anal. Ed.*, 13, 145 (1941).
* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 60, 1945.

(2) That a reference to the ultra-micro method of Satterlee and Blodgett for arsenic be inserted into par. **3** as "(f)," and that par. **4** be revised to include a reference to the determination of arsenic in gelatin.

(3) That further studies be undertaken for the determination of cadmium.

(4) That the colorimetric method for copper described by the Associate Referee be adopted tentatively.

(5) That a new associate referee be appointed to conduct collaborative studies on the determination of DDT in foods as soon as methods are available.

(6) That changes necessitated by the adoption of a tolerance for fluorine in parts per million be made in the tentative method for fluorine.

(7) That changes in concentration of reagents necessary for changing the method for the determination of lead on apples and pears to a basis of parts per million and to a maximum amount of 10 p.p.m. be authorized.

(8) That methods for the study of mercury in foods be studied further.

(9) That the method for the determination of selenium be adopted as official, final action.

(10) That the present tentative colorimetric method for zinc be dropped and that the method described by the Associate Referee be adopted as tentative in its stead.

No report on selenium was given by the Associate Referee.

REPORT ON CADMIUM

By A. K. Klein, *Associate Referee*, and H. J. Wichmann
(Food and Drug Administration, Federal Security
Agency, Washington, D. C.)

Food chemists have viewed with some concern the recent, although not general, use of cadmium as a plating metal in food containers. (1) It has been known for some time that lemonade, fruit-flavored ices, punch, gelatin, desserts, and similar edibles dissolve toxic quantities of cadmium from containers coated with the metal. Illness caused by eating or drinking such foodstuffs has also been reported. (*1*) The need of a method for the determination of micro quantities of cadmium in foods and, necessarily, in biological materials is therefore obvious.

Although many spot tests, (*2*) both delicate and discriminating, have been developed for cadmium, few micro quantitative methods for estimating the element have been publishedl. This fact is not surprising for, firstly, cadmium has no variable valence and hence is not amenable to a procedure based on oxidation or reduction titration; secondly, except for

the familiar orange-yellow sulfide, its salts, both organic and inorganic, are usually colorless. Fairhall (*3*) based a cadmium method on the color of sulfide; but the quantities determined with accuracy by this method may far exceed that found in biological and food materials. H. Fischer (*4*), who demonstrated the general usefulness of the organic reagent, diphenylthiocarbazone (dithizone), for the colorimetric analysis of micro amounts of metals also employed the reagent for the determination of cadmium (*5*) in metals. More recently, Sandell, (*6*) utilizing the dithizone technic, measured with satisfactory precision the extremely small quantities of cadmium found naturally in rocks. And just as these studies were being concluded Cholak and Hubbard (*7*) also published a micro cadmium method utilizing dithizone and the betanaphthyl analogue.

The following colorimetric dithizone procedure is submitted for the determination of cadmium in the ranges 0–25 micrograms. It consists in brief of the following steps: (1) extraction of all "dithizone metals" from the sample solution at *p*H 8.5 by dithizone in chloroform; (2) a separation of cadmium, zinc, stannous tin, lead, and bismuth from interfering mercury, copper, cobalt, and nickel with dilute hydrochloric acid; (3) a quantitative separation of cadmium as the dithizonate along with small amounts of zinc from lead, zinc, tin, and bismuth by extraction from 10 per cent sodium hydroxide with dithizone in carbon tetrachloride (4), a repetition of the dilute acid "stripping" mentioned in step (2) whereby small amounts of nickel and cobalt yet remaining are completely removed and (5), the final repression of small quantities of zinc in 5 per cent sodium hydroxide and the quantitative evaluation of cadmium as dithizonate in carbon tetrachloride.

METHOD

REAGENTS

(a) *Nitric acid.*—Preferably redistilled from all-glass Pyrex apparatus.

(b) *Sulfuric acid.*—(1+1) by volume.

(c) *Hydrochloric acid.*—0.2 *N*.

(d) *Ammonium citrate.*—The diammonium salt.

(e) *Ammonium hydroxide.*—Redistilled and stored in bottles coated inside with paraffin.

(f) *Chloroform.*—U.S.P.

(g) *Diphenylthiocarbazone* (*dithizone*) (*8*).—Twice purified. Follow the directions but make only three dilute ammonia extractions of the $CHCl_3$ solution. Carry through, including the water-washing steps and then repeat the purification with three ammonia extractions, precipitation with dilute acid, etc. Instead of drying the final dithizone extract, allow to evaporate spontaneously, and complete the drying under vacuum in a bell-jar overnight.

(h) *Carbon tetrachloride.*—Purified by Biddle process (*9*).

(i) *Dithizone in carbon tetrachloride* (**h**). 20 mg. per liter. Prepare just before using by dilution from 300 mg. per liter concentration in tetrachloride (**h**). The concentrated reagent is preserved under 0.1 *M* SO_2 solution and stored in the refrigerator.

(j) *Dithizone in chloroform.*—1000 mg. per liter, preserved and stored as described under reagent (i).

(k) *Sodium hydroxide solution.*—25% W/V.

(l) *Ammonium oxalate.*—Saturated solution.

(m) *Absorbent cotton.*—Rendered free of traces of metals by digesting for several hours with warm 0.2 *N* HCl, filtering on a Büchner funnel, and finally washing with copious volumes of twice-distilled water to remove the acid completely.

(n) *Standard cadmium solution.*—Prepare stock solution of 1 mg. per ml. by dissolving 1 gram of the pure metal in 20–25 ml. of concentrated HCl with warming. Dilute to 1000 ml. Prepare an intermediate standard solution, 100 micrograms of cadmium per ml., by dilution of the stock solution. A convenient final working standard solution is 2 micrograms of cadmium per ml. Add concentrated HCl in the proportion of 5 ml. per liter before diluting to volume to stabilize the solution.

PREPARATION OF STANDARD REFERENCE CURVE

Prepare in duplicate six cadmium standards containing 0, 5, 10, 15, 20, and 25 micrograms of the element by adding the appropriate volume of the standard solution to 125 ml. Squibb-type separatory funnels and adjust to 40 ml. volume with distilled water. To the latter solutions, which should be only faintly acid, add 10 ml. of 25% NaOH, and then 25 ml. of the standard dithizone reagent in the CCl_4. Shake funnels vigorously for exactly 1 minute and then let stand for exactly 3 minutes. Filter organic layer through a pledget of the absorbent cotton, discard first 5 ml., and then determine the absorption of the solution in 1″ cell at wave length 510 in a neutral wedge photometer (*10*) or similar instrument. If necessary translate photometer readings to density or some simple function thereof and compute linear relationship between these quantities and the corresponding cadmium concentrations by the method of least squares. (If the neutral wedge photometer is used, the wedge readings may be used directly in the computation.) (See Table 1.)

SAMPLE PREPARATION

Use an amount of sample that is roughly equivalent to 5–10 grams of the product, calculated to the dry basis. (The size of sample is of concern only when comparatively large proportions of magnesium and phosphate are present.) Digest with 10 ml. of the H_2SO_4 and HNO_3 as needed. If, during the digestion, the sample tends to char rather than to oxidize evenly, add an additional 5 or 10 ml. of H_2SO_4. (b). Continue the digestion, adding the HNO_3 as required, until the operation is completed, and SO_3 is evolved. Cool, add 15 ml. of reagent (l), and heat to fumes again.

Because of the fat present, biological materials, such as liver and kidney, may bump and froth rather energetically during the digestion. If comparatively large samples of such materials are available, make a preliminary infusion with warm HNO_3 until all but the fat is in solution. Cool, filter free of solid fat, wash residue with distilled water, and make up combined filtrate to suitable volume. Digest appropriate aliquots as outlined above.

ISOLATION AND EVALUATION OF CADMIUM

Dilute the digest with 25 ml. of water and filter free from excessive insoluble matter (sulfates or silica) if present, and transfer to a separatory funnel, marked at 75 ml. volume, using additional 10 ml. portions of water for rinsing and completing the transfer. Add 1–2 grams of the citrate reagent, 5 drops of bromothymol blue indicator, and adjust to *p*H 8.5 by adding the NH_4OH slowly, while cooling intermit-

tently, until the color of the solution changes from yellow to light green. Dilute to 75 ml. mark with water. Add 10 ml. of $CHCl_3$ and then 1 ml. portion of conc. dithizone reagent (j) until, after vigorous shaking, the red color of the metal dithizonates is superseded by the green color of the dithizone reagent. Transfer $CHCl_3$ layer to a second funnel previously wet with several ml. of $CHCl_3$. Repeat the operation until all heavy metals have been quantitatively extracted. Add to the combined dithizone extracts 25 ml. of the dilute HCl, shake vigorously for at least 1 minute, and, after the layers have partitioned, drain off carefully the $CHCl_3$ phase containing any Cu, Ni, Co, and Hg that may be present, and discard. Remove remaining droplets of dithizone by extracting with several small portions of CCl_4. (The draining operation must be conducted carefully so that no acid enters the bore or stem of the funnel. Its presence there would decompose in part the cadmium dithizonate subsequently formed and extracted in the next step.) Adjust the aqueous phase to 10% alkalinity by adding 20 ml. of the NaOH and 5 ml. of water. Extract the cadmium with 15 ml. portions of dithizone (i) shaking vigorously for at least 1 minute, and transfer to a third funnel, a 125 ml. Squibb-type that has been previously wet with several ml. of the dithizone reagent. Repeat the extraction with additional 10 ml. portions of the dithizone reagent until the CCl_4 layer becomes colorless. (Although the extraction of cadmium from 10% alkali by dithizone in tetrachloride is somewhat sluggish, the amounts usually found in foods or biological materials are completely removed by the third extraction; for example, 100 micrograms is thus quantitatively extracted. If, therefore, a pale pink color persists after the third extraction, it is probably due to Zn. This happens only when a rather large quantity of Zn is originally present. To verify this assumption, transfer the questionable extract to a fourth funnel containing 5% NaOH, add several ml. of the dithizone in CCl_4, and shake vigorously. If the CCl_4 layer becomes colorless, the original pink color was due to Zn. If, however, the pink color persists, indicating the presence of cadmium, add the extract to the contents of third funnel, and continue the extraction from the second funnel until complete.) Revert the cadmium and zinc dithizonates in funnel 3 to the chlorides by adding 25 ml. of the acid reagent and shaking vigorously for at least 1 minute. Draw off carefully the CCl_4 layer, which may contain traces of Co and Ni that have escaped removal in step (2), and discard. Rid the aqueous phase of droplets of dithizone by several rinsings with small volumes of CCl_4 and drain off as completely as possible in order not to dilute the cadmium dithizonate subsequently formed. No acid must be permitted to pass the bore of the funnel, however. Adjust the alkalinity to 5% by adding 10 ml. of the NaOH and 15 ml. of water. Rinse funnel stems successively with alcohol and CCl_4 and whisk dry. Evaluate the Cd present by adding 25 ml. of the dithizone reagent, shaking vigorously for exactly 1 minute, permitting the layers to partition for exactly 3 minutes, and continuing from then on as directed under "Preparation of Standard Reference Curve." Calculate the Cd content in micrograms by substituting the working density in the linear equation.

If the photometer measurement indicates that the Cd is in decided excess of 25 micrograms, a first approximation may be obtained by diluting the dithizonate with the CCl_4 and evaluating the density. However, for best results the analysis must be repeated with weights or aliquots of sample that contain no more than 25 micrograms of Cd; 30 micrograms is the upper limit of the solubility of Cd dithizonate in 25 ml. of CCl_4. Amounts larger than 30 micrograms are therefore not completely extracted.

TABLE 1.—*Standard reference curve data*

CADMIUM ADDED MICROGRAMS	PHOTOMETER WEDGE READING	CADMIUM FOUND[a] MICROGRAMS	DEVIATION MICROGRAMS
	mm.		
0	2.6	+.1	+.1
0	2.8	+.2	+.2
0	2.1	.0	.0
0	2.8	+.2	+.2
4	21.7	4.0	.0
5	25.3	4.8	−.2
5	25.9	4.9	−.1
5	25.3	4.8	−.2
10	50.0	9.8	−.2
10	50.0	9.8	−.2
10	50.3	9.9	−.1
12	60.9	12.0	0
14	72.0	14.3	+.3
15	75.6	15.0	0
15	74.5	14.8	−.2
15	76.5	15.2	+.2
18	91.0	18.2	+.2
20	100.8	20.2	+.2
20	99.8	20.0	.0
20	101.5	20.3	+.3
22	111.0	22.2	+.2
25	122.5	24.6	−.4
25	124.0	24.9	−.1
25	125.0	25.1	+.1
		Av. Deviation	±.2

Average straight-line formula: Micrograms Cd = 0.204 × wedge reading (mm.) − .4.
[a] Calculated from the average straight line formula.

TABLE 2.—*Cadmium recovery data from digestion experiment*

CADMIUM ADDED	MATERIAL USED AS BASE	AMOUNT	CADMIUM RECOVERED	CADMIUM NET	DEVIATION
micrograms		*ml.*	*micrograms*	*micrograms*	*micrograms*
0	Canned Grape Fruit Juice	150	2.9	—	—
0	Canned Grape Fruit Juice	50	1.0	—	—
0	Canned Grape Fruit Juice	50	0.9	—	—
6	Canned Grape Fruit Juice	50	6.8	5.9	−.1
9	Canned Grape Fruit Juice	50	10.0	9.1	+.1
20	Canned Grape Fruit Juice	50	20.7	19.8	−.2

TABLE 2.—*Continued*

CADMIUM ADDED	MATERIAL USED AS BASE	AMOUNT	CADMIUM RECOVERED	CADMIUM NET	DEVIATION
micrograms		*ml.*	*micrograms*	*micrograms*	*micrograms*
0	Fresh Milk	10	0.2	—	+.2
5	Fresh Milk	10	4.9	4.9	−.1
10	Fresh Milk	10	9.9	9.9	−.1
15	Fresh Milk	10	14.9	14.9	−.1
20	Fresh Milk	10	19.5	19.5	−.5
0	Urine	50	0.2	—	+.2
5	Urine	50	5.2	5.2	+.2
5	Urine	50	4.8	4.8	−.2
8	Urine	50	7.7	7.7	−.3
8	Urine	50	8.0	8.0	0
11	Urine	50	10.8	10.8	−.2
15	Urine	50	15.0	15.0	0
20	Urine	50	19.6	19.6	−.4
		grams			
0	Maple Syrup	15	0.2	—	.2
0	Maple Syrup	15	0.2	—	.2
10	Maple Syrup	15	9.9	9.9	−.1
15	Maple Syrup	15	14.9	14.9	−.1
0	Fresh Spinach	33	1.3	—	—
0	Fresh Spinach	33	1.4	—	—
7	Fresh Spinach	33	8.1	6.8	−.2
0	Hog Liver[b]	10	0.2	—	+.2
4	Hog Liver	10	4.0	4.0	0
6	Hog Liver	10	5.9	5.9	−.1
10	Hog Liver	10	9.5	9.5	−.5
20	Hog Liver	10	19.8	19.8	−.2
0	Rabbit Blood[b]	10	0.2	—	+.2
2	Rabbit Blood	10	2.0	2.0	0
3	Rabbit Blood	10	2.9	2.9	−.1
4	Rabbit Blood	10	3.8	3.8	−.2
5	Rabbit Blood	10	4.8	4.8	−.2
8	Rabbit Blood	10	8.0	8.0	0
0	Beef Kidney[b]	20	12.9	—	—
0	Beef Kidney	20	13.2	—	—
0	Beef Kidney	10	6.7	—	—
5	Beef Kidney	10	11.5	4.8	−.2
11	Beef Kidney	10	17.5	10.8	−.2
	Canned Oysters[b]	10	12.1	—	—
		5	6.1	—	—
			Av. deviation micrograms		±.2

[b] In these instances an appropriate volume of HNO_3 infusion previously filtered from fat was digested and assayed.

TABLE 3.—*Cadmium recovery from dry ashing experiments*

CADMIUM ADDED	MATERIAL USED AS BASE	AMOUNT	CADMIUM RECOVERED
micrograms		*ml.*	*micrograms*
0	Urine	50	0.2
5	Urine	50	4.6
11	Urine	50	10.0
0	Tomato Juice	25	.4
5	Tomato Juice	25	4.4
11	Tomato Juice	25	10.5
		grams	
0	Canned Peas	25	.2
5	Canned Peas	25	4.2
10	Canned Peas	25	8.4
		ml.	
0	Blood	25	.2
10	Blood	25	8.9
15	Blood	25	14.0
		grams	
0	Dried Skim Milk	5	.5
10	Dried Skim Milk	5	9.5
20	Dried Skim Milk	5	19.5

TABLE 4.—*Cadmium recovery data from digestion of 25 ml. of tomato juice containing 100 micrograms of the following metals: Co, Ni, Cu, Hg, Bi, Pb, and Zn*

CADMIUM ADDED	CADMIUM RECOVERED	DEVIATION
micrograms	*micrograms*	*micrograms*
0	.2	+.2
0	.2	+.2
1	1.3	+.3
5	5.2	+.2
10	9.9	−.1
15	14.9	−.1
25	24.5	−.5
	Av. Dev.	±.2

DISCUSSION

A wet digestion procedure was used for the sample preparation instead of dry ashing at elevated temperatures because slight losses due to volatilization of cadmium salts result at even 500°C. (Table 3). This tendency has been observed by others (*3*) (*7*). A wet digestion is more economical from the standpoint of time, and since it offers no difficulties per se, no experiments were made with dry ashing fixatives.

The proposed method contains no questionable feature except the final step, the estimation of cadmium as the dithizonate in carbon tetrachloride

in the presence of 5 per cent sodium hydroxide. The first operation, the removal from the sample solution of all dithizone metals by extraction with dithizone at *p*H 8.5, is quite common. Chloroform is specified as the solvent because the dithizonates are more soluble in it than in tetrachloride. The *p*H recommended, 8.5, must not be greatly exceeded because too much ammonia is then extracted and the efficiency of the subsequent stripping procedure is decreased. Since cadmium is quantitatively extracted by excess dithizone from *p*H 7.5 upwards, the analyst should err (if at all) on the lower side; the indicator specified, however, changes quite visibly in color at *p*H 8.5, and there should be no difficulty in adjusting the acidity at this level.

The manner in which mercury, copper, nickel, and cobalt are removed by stripping in dilute hydrochloric acid, step 2, is also the conventional one (*5*) (*7*). The results listed in Table 4 demonstrate the efficiency of their removal by this procedure. Step 3, wherein cadmium is extracted quantitatively and almost free from zinc, is based on the observations of H. Fischer (*5*). He noted that cadmium may be separated from relatively large quantities of zinc by extraction with dithizone in tetrachloride from a 5 per cent sodium hydroxide solution (in which zinc dithizonate is very soluble). Chloroform may not be used as the solvent in this separation because it extracts too great an amount of zinc. Some zinc is extracted even with tetrachloride-dithizone when comparatively large quantities are present, say in excess of 2 mg. In the latter instance, Fischer increased the alkalinity to 10–15 per cent sodium hydroxide. For the proposed method the alkalinity has been arbitrarily fixed at 10 per cent because some foods (*11*) (*12*) contain relatively large quantities of zinc. When the sample contains more than 2 mg. of zinc, microgram quantities are extracted by dithizone in carbon tetrachloride even from 10 per cent sodium hydroxide. These amounts, however, do not cause errors because such quantities of zinc are completely repressed by the 5 per cent sodium hydroxide used in the final evaluation of cadmium.

Step 4, in which the last traces of nickel and cobalt are removed, is a repetition of step 2 and requires no comment. The final step, wherein cadmium is converted quantitatively to the dithizonate in carbon tetrachloride contiguous with 5 per cent sodium hydroxide, seems to ignore the observations and warnings of Fischer (*5*). This step, therefore, requires some justification. Fischer states that cadmium dithizonate in carbon tetrachloride, thus developed, is too unstable to permit a direct color measurement and quantitative evaluation. It was, however, observed early in this work that, once removed from the alkaline phase, the tetrachloride solution of cadmium dithizonate seemed as stable as any metal dithizonate. There was no fading or shifting in the absorption peak of the complex during the density measurement, nor did the complex apparently fade more rapidly in direct sunlight than did lead dithizonate. However, cer-

tain brands of carbon tetrachloride even though labeled as reagent grade yielded erratic results when used as the solvent. The cadmium dithizonate developed in these instances was very unstable; the color complex faded rapidly during short intervals of shaking until false blanks were

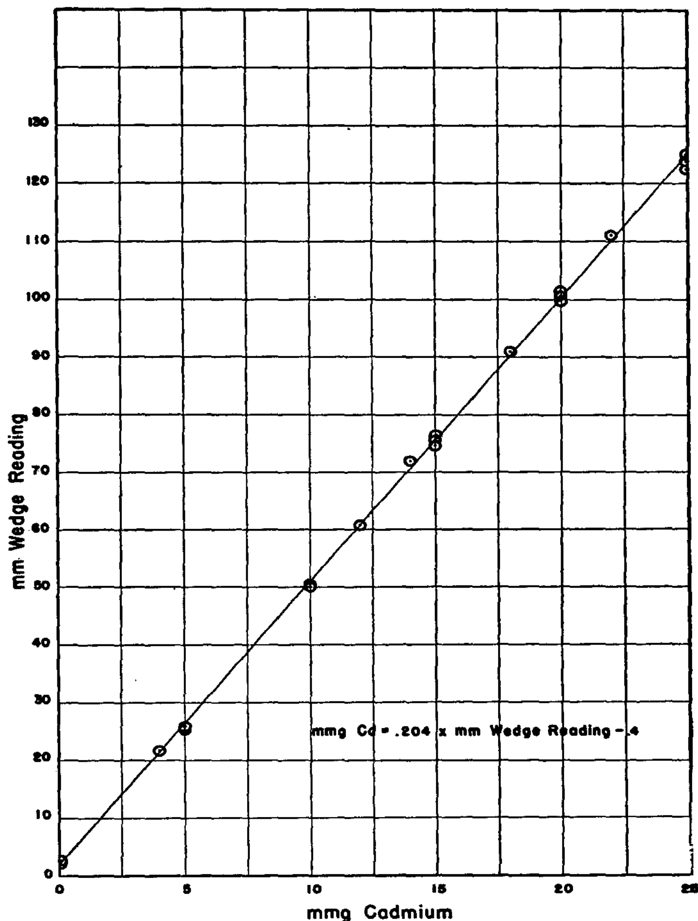

FIG. 1.

obtained regardless of the original concentration of cadmium. This occurred only when the extraction was made from 5 per cent sodium hydroxide; no abnormalities were noted when the color complex was developed at *p*H 9.5 in the ammonia system. However, no brand of analytical grade carbon tetrachloride subsequently used has produced false blanks or

erratic cadmium dithizonate colors if the solvent has been given a previous purification, specifically the one recommended by Biddle (*9*). Attention is called to the standard curve data, Table 1, and to the graph derived therefrom. The calculated amounts of the metal agree quite well with those actually added; the average deviation of 24 measurements is somewhat less than $\pm .2$ micrograms. Two empirical conditions, however, have been arbitrarily imposed, the time of shaking and of standing prior to filtration and density measurement.

It cannot, of course, be guaranteed that each new batch of dithizone, no matter how carefully purified, will yield a reagent capable of producing standard curves exactly superimposable on those previously prepared. In obtaining the results reported in this paper the equation, micrograms cadmium $= .204 \times$ mm. wedge reading $-.4$ has been used. Five months earlier the relationship, micrograms cadmium $= .206 \times$ mm. wedge reading $-.6$, was obtained by using a different batch of dithizone, which, however, had been carefully purified and preserved. The slopes of the two lines agree quite well but the intercepts show a small but somewhat significant difference. Neither can it be postulated with certainty how long a given batch of dithizone reagent will remain stable, even though preserved under sulfur dioxide and stored in the cold. The analyst must satisfy himself in this regard by checking various points on the curve at periodic intervals.

The alternative (*7*) to the dithizonate development in 5 per cent sodium hydroxide is one at a lower alkalinity, e.g. *p*H 8.5, as provided by dilute ammonium hydroxide or the ammonium citrate system. It is true that cadmium dithizonate in excess dithizone is very stable at this acidity. Since, however, such a method would also evaluate zinc quantitatively, a complete removal of zinc from the sample must necessarily be effected prior to the final cadmium evaluation. It also requires a purification of all reagents whenever possible and scrupulous attention to the cleaning of all glassware. Zinc is sometimes leached from Pyrex separatory funnels by weak alkaline solutions even after a most rigorous cleaning. The difficulty of controlling the zinc blank is also reported elsewhere (*14*). Since the method will be applied to samples containing varying quantities of zinc, and because of the danger of zinc contamination from the glassware, it has seemed preferable to use 5 per cent sodium hydroxide in the final step. The results listed in Table 2 indicate that the choice is in the main justified. The method has a tendency in some instances to yield slightly low values, but the average deviation is no greater than that found in the standard curve itself. It is doubtful that a greater uniform accuracy could be obtained in the range 0–25 micrograms by operating at a lower alkalinity, and the analyst need not be fearful of contamination with zinc regardless of its original concentration.

NATURALLY OCCURRING CADMIUM IN FOODS AND BIOLOGICAL MATERIALS

The writers have found cadmium in canned grape fruit juice, fresh spinach, and beef kidney in the following respective amounts: .019, .04, and .66 parts per million (Table 2). Another sample of beef kidney contained .56 p.p.m. and hog kidney purchased from the same store on the same day contained .12 p.p.m. Kidneys from two test rabbits contained .21 p.p.m. No cadmium in significant amounts was found in any liver specimen whether beef, hog, or rabbit. All analyses were made in duplicate, frequently in triplicate, on aliquots of a prepared solution containing the entire biological specimen. The examination therefore involved no sampling error nor was the cadmium found attributable to local or fortuitous contamination. Furthermore, blank determinations made in the conventional manner with the reagents used in the method did not indicate the presence of cadmium in significant amounts. A result listed in Table 2 is 1.21 p.p.m. of cadmium obtained from a sample of canned oysters; the value was checked by a duplicate determination. These oysters contained so large a proportion of zinc (*12*) and other metals that solid dithizone was added in the initial extraction to limit the volume of chloroform. Such a sample provides a rigorous test of the proposed method. The presence of cadmium in the instances just cited was confirmed each time by an adaption of Feigl's test (*13*). The cadmium dithizonate obtained in the last step of the method was decomposed with dilute hydrochloric acid; the latter solution was treated with Feigl's reagent on a glass slide, and the precipitate thus formed was examined under the microscope and compared with mounts prepared from known cadmium solutions.

Since the cadmium found in the biological materials referred to above might come from accidental contamination in storage, or from solder if the product was a canned food, there arose some doubt that cadmium might occur naturally in significant amounts in food or biological materials. As an added precaution, therefore, two beef kidneys were purchased from a local meat distributing firm before they were offered for sale. The kidneys were cut from the calves in cold storage in the senior author's presence. The two organs, totaling 600 grams in weight, were digested several hours with 300 ml. of concentrated nitric acid filtered free of fat, and diluted with distilled water to exactly 900 ml. Aliquots of the final solution were examined for cadmium with the following results: .65, .67, and .66 p.p.m. (Table 2).

As an added check, enough of the kidney infusion was digested, carried through the method, and stripped in dilute hydrochloric acid so that 1 ml. of the acid contained 1 microgram of cadmium. The experiment was repeated with amounts of sample solution such that 1 ml. of acid contained 10 micrograms of cadmium. Standard solutions of cadmium in a like con-

centration of hydrochloric acid containing respectively 1 and 10 micrograms of the metal per ml. of acid were also prepared. These four solutions were then submitted to A. T. Myers, Spectroscopic Laboratory, Bureau of Plant Industry, Beltsville, Maryland.

Myers' report is quoted in part; ". . . Three separate determinations were made on three U. V. sensitized plates, exposing the dried extract by burning in an A. C. arc with a setting of the spectrograph for the far ultraviolet region where several sensitive lines of cadmium occur. A known cadmium standard, a C. P. salt of cadmium, and a reference spectrum were also exposed to these photographic plates. Another spectroscopic determination was made in the near ultraviolet in the same manner to make use of other sensitive lines of cadmium.

"In all cases cadmium was found in the unknown or beef kidney extract, and it was found present in the extract in the same order of concentration as in the artificially prepared standard. This determination demonstrated that at least .1–.05 micrograms of cadmium can be detected with certainty by the spectrographic method.

"The lines of cadmium that were identified are as follows: 2265.017 II, 2288.018 I, and 3261.057 I. All values are taken from the M.I.T. wave length tables."

It seems therefore established that certain biological materials may contain significant quantities of cadmium. No explanation, however, is offered, especially in the case of the kidneys, as to the original source of the cadmium.

CONCLUSION

Cadmium in the range 0–25 micrograms may be determined with dithizone reagent with an accuracy of $\pm .2$ microgram in foods and biological materials.

RECOMMENDATION,* AND ACKNOWLEDGMENT

It is recommended that this method and the one published by Cholak and Hubbard (*7*) be studied collaboratively to determine which one yields the better results.

The writers wish to thank A. T. Myers, Bureau of Plant Industry Beltsville, Maryland, for his careful and helpful spectrographic studies.

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REPORT ON COPPER*

By C. A. GREENLEAF (National Canners Association, Washington, D. C.), *Associate Referee*

Based on work reported in 1941 and 1942,[1] a method was formulated in 1943 and submitted to collaborators. It involved preliminary isolation of copper by dithizone extraction at *p*H 2, removal of bismuth by an acidified potassium iodide wash, stripping of copper from the dithizone solution with dilute acid (decomposing the dithizone with bromine water at the same time), and final colorimetric estimation as diethyl-dithiocarbamate.

Two serious difficulties were encountered in applying this procedure:

1. If a substantial amount of bismuth is present it tends to precipitate out as a basic salt, which apparently occludes copper, giving low recoveries.
2. Some decomposition product or products of dithizone are stripped along with copper. These are extremely resistant to destruction and give erratic false blanks in the final estimation with carbamate.

It was found that the first difficulty could be overcome by adding potassium iodide to the sample solution after adjusting it to *p*H 2. This holds the bismuth in solution and at the same time prevents its extraction with dithizone at that *p*H. However, it diminishes the rate of extraction of copper, which at *p*H 2 is seriously affected by the concentration of electrolytes. In extracting at this low *p*H it is necessary to use a large excess of dithizone which makes it impossible to judge when extraction of copper is complete.

Further investigation showed that by increasing the *p*H to somewhere in the range 3.0–3.3, the rate of extraction of copper was greatly increased, while this *p*H was still low enough to prevent extraction of cobalt and nickel when present. There is a slight extraction of bismuth, though not enough to obscure the end-point of copper extraction. This necessitates washing the extract with dilute potassium iodide solution at *p*H 2 to remove bismuth, but this extra step seems more than offset by the increased ease of extraction of copper.

The overcoming of false blanks has proven the most stubborn difficulty.

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 25, 26, 1944.
[1] *This Journal*, **25**, 389 (1942).

Decomposition of dithizone with bromine was abandoned on this account, and stripping with 6*N* hydrochloric acid substituted. This was some improvement, but not enough. Even when the stripping solution was heated to fumes with sulfuric acid and perchloric acid, false blanks persisted, simulating several times as much copper as could be demonstrated in the reagents.

On the possibility that some decomposition of dithizone was induced by

TABLE 1.—*Collaborative results, using orange and grapefruit as samples*

COLLABORATOR	Cu		RECOVERY	
	ADDED, γ	FOUND, γ	Cu, γ	PER CENT
1	Blank	2.9		
		2.9		
	0[a]	8.2		
		8.0		
	25[a]	33.4	25.2	100.8
	25	33.3		
	27.5[b]	36.8	28.5	103.6
	27.5	36.5		
2	Blank	1.9		
		1.9		
	0[c]	8.3		
		7.9		
	25[c]	34.1	25.3	101.2
		32.7		
	25[d]	33.1	25.0	100.0
		33.0		

[a] 25 gm. orange juice.
[b] 25 gm. orange juice plus 500 γ each bismuth, cobalt, and nickel.
[c] 25 gm. grapefruit juice.
[d] 25 gm. grapefruit juice plus 500 γ each bismuth, cobalt, and nickel.

shaking with 6*N* acid, a slightly acid solution of mercuric chloride was substituted as stripping solution. This proved to be a further improvement, but still gave occasional blanks that were out of reason. It was finally found that the blank could be brought under control by extracting the copper as carbamate from the mercuric chloride solution, evaporating to dryness and heating with sulfuric acid and perchloric acid before final estimation as carbamate.

The method as submitted was subjected to collaborative trial on sam-

ples of orange and grapefruit juice, with the results shown in the accompanying table.

Some attention was also given to development of an all-dithizone method for copper. Such a method should have much greater sensitivity than the combined dithizone-carbamate method, but requires solution of the problem of removing excess dithizone if the full sensitivity is to be realized. Various methods have been proposed, usually involving use of dilute ammonia or alkaline solutions for removing excess dithizone. Preliminary investigations indicate that the following difficulties exist in removal of excess dithizone:

1. Dilute ammonia partially dissociates the copper-dithizone complex.
2. The copper-dithizone complex is partially enolized by alkaline solutions.
3. Any zinc in the alkaline solutions or taken up from glassware appears as copper in the determination.

RECOMMENDATIONS

It is recommended—

(1) That the carbamate method be adopted as tentative;*

(2) That further study be given to simplifying the carbamate method;

(3) That all-dithizone methods be studied.

REPORT ON ZINC

By L. V. Taylor, *Associate Referee*, and O. R. Alexander
(American Can Company Research Department,
Maywood, Ill.)

In a paper by the authors (*This Journal*, **27**, 325) a dithizone procedure for zinc in foods was described; it was designed to overcome certain shortcomings and criticisms of previously published dithizone methods for the determination of zinc in foods.[1,2,3]

This procedure involves the wet oxidation of the sample; elimination of lead, copper, cadmium, bismuth, antimony, tin, mercury, and silver as sulfides with added copper as a scavenger agent; a simultaneous elimination of cobalt and nickel by extracting the metal complexes of a-nitroso-b-naphthol and dimethyl-glyoxime, respectively, with chloroform; extraction of the zinc dithizonate with carbon tetrachloride; transfer of the zinc to dilute hydrochloric acid; and a final extraction of the zinc dithizonate for color measurement.

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* The method will be published in the 6th Edition, *Methods of Analysis, A.O.A.C.*, 1945.

[1] Cholak, Hubbard, and Burkey, *Ind. Eng. Chem., Anal. Ed.*, **15**, 754 (1943).

[2] Cowling and Miller, *Ibid.*, **13**, 145 (1941).

[3] Holland and Ritchie, *This Journal*, **22**, 333 (1939).

In order to corroborate the success of the procedure in the authors' hands it was subjected to collaborative studies during 1944. Samples for the studies were submitted to nine collaborators, and eight complete reports were received. Each collaborator was supplied with a sample of purified sucrose, a solution of zinc of known concentration, a solution of various elements which are potential interferences, and a sample of soybean meal. The sucrose was carefully checked in this laboratory and was found to be essentially free from dithizone reactive metals. The zinc solution was prepared from pure zinc and contained 0.5 mg. per ml. The collaborators were instructed to dilute 10 ml. of the zinc solution to 1000 ml. with 0.04 *N* hydrochloric acid. The solution of interfering elements contained lead, nickel, cadmium, cobalt, and bismuth, all at a concentration of 1.0 mg. per ml. The collaborators were directed to dilute 10 ml. of this solution to 50 ml. The soybean meal was ground in a laboratory Wiley mill and was thoroughly mixed.

Detailed directions were given for the preparation of a series of six samples, and the collaborators were requested to make duplicate determinations on each sample as well as a blank determination including all oxidizing acids and other reagents used in the determination. The preparation of the six samples is outlined in Table 1.

TABLE 1.—*Samples*

SAMPLE NO.	BASE MATERIAL		ZINC SOLN ADDED 1 ML.=5 MMG.	ZINC PRESENT IN 20 ML. ALIQUOT OF FINAL SOLN	IMPURITY SOLN ADDED 1 ML.=200 MMG.	IMPURITY PRESENT PER 20 ML. ALIQUOT
		grams	*ml.*	*mmg.*	*ml.*	*mmg.*
1	Sucrose	10	2	2	None	None
2	Sucrose	10	2	2	2	80
3	Sucrose	10	15	15	None	None
4	Sucrose	10	15	15	2	80
5	Sucrose	10	None	None	None	None
6	Soya meal	2	None	—	None	—
7	Reagent Blank		None	—	None	None

Each of the collaborators was supplied with a copy of the detailed procedure essentially the same as that given in the succeeding section with the request that the procedure be followed as closely as possible.

ZINC IN FOOD PRODUCTS

REAGENTS

(All water must be redistilled from glass. Pyrex glassware should be used exclusively and must be scrupulously cleaned with hot HNO_3.)

(a) *Nitric acid.*—C.P. concentrated (should be redistilled if appreciably contaminated, although not usually necessary).

(b) *Sulfuric acid.*—C.P., concentrated (should be tested if Zn contamination is suspected).

(c) *Ammonium hydroxide.*—C.P., concentrated (should be redistilled if appreciably contaminated).

(d) *Copper sulfate solution.*—Dissolve 8 grams of $CuSO_4 \cdot 5H_2O$ in water and dilute to 1 liter. 1 ml. contains 2 mg. of Cu.

(e) *Ammonium citrate solution.*—Dissolve 225 grams of diammonium citrate in water, make alkaline to phenol red with NH_4OH, i.e. to first distinct color change (pH about 7.4) and add 75 ml. excess. Dilute to 2 liters. Extract this solution immediately before use as follows: Add a slight excess of dithizone and extract with CCl_4 until the solvent layer is a clear bright green. Remove excess dithizone by repeated extraction with $CHCl_3$, and finally extract once more with CCl_4. It is essential that excess dithizone be entirely removed, otherwise Zn will be lost during the elimination of Co and Ni.

(f) *Dimethylglyoxime solution.*—Dissolve 2 grams of the reagent in 10 ml. of NH_4OH and 200–300 ml. of water, filter, and dilute to 1 liter.

(g) *a-Nitroso-b-naphthol solution.*—Dissolve 0.25 gram in $CHCl_3$ and dilute to 500 ml.

(h) *Chloroform.*—Redistilled.

(i) *Diphenylthiocarbazone (dithizone).*—Dissolve 0.050 gram in 2 ml. of NH_4OH and 100 ml. of water, and extract repeatedly with CCl_4 until the solvent layer is a clear, bright green color. Discard the solvent layer and filter the aqueous portion through a washed ashless paper. (This solution is best prepared as needed since it is only moderately stable, even when kept in the dark and under refrigeration.)

(j) *Carbon tetrachloride.*—Redistilled.

(k) *Hydrochloric acid solution.*—0.04 *N*. Dilute the required amount of concentrated C.P. acid with water (redistilled acid may be used although not usually required).

(l) *Standard zinc solution.*—Dissolve 0.500 gram of pure granulated Zn in a slight excess of dilute HCl and dilute to 1000 ml. For use dilute 10 ml. of this stock solution to 1000 ml. with 0.04 *N* HCl. 1 ml. of this working standard contains 5 micrograms of Zn.

DETERMINATION

Preparation of Sample.—Weigh a representative sample not exceeding 25 grams of material, estimated to contain 25–100 micrograms of Zn, into an Erlenmeyer flask of suitable size. If the sample is a liquid, evaporate to a small volume. Add concentrated HNO_3 and heat cautiously until the first vigorous reaction subsides somewhat, then add 2.5 ml. of H_2SO_4. Continue heating, adding more HNO_3 in small increments if necessary to prevent charring, until fumes of SO_3 are evolved and the solution remains clear and almost water white. Add 0.5 ml. of $HClO_4$ and continue heating until the $HClO_4$ has been almost completely removed. Allow to cool and dilute to about 40 ml. If the necessary equipment is available, the wet digestion and subsequent sulfide separation may be advantageously carried out in small Kjeldahl flasks.

Separation of Sulfide Group.—To the H_2SO_4 solution add 2 drops of methyl red indicator and 1 ml. of the $CuSO_4$ solution and neutralize with concentrated NH_4OH. Add sufficient HCl to make the solution approximately 0.15 *N* with respect to this acid (approximately 0.5 ml. excess in 50 ml. of solution is satisfactory). The pH of the solution at this point as measured with a glass electrode is 1.9–2.1. Pass a stream of H_2S into the solution until precipitation is complete. Filter through a fine textured paper (Whatman No. 42 or equivalent, previously fitted to the funnel and washed with 5% HCl, then with redistilled water). Receive the filtrate in a 250 ml. beaker and wash the flask and filter with 3 or 4 small portions of water. Boil het filtrate gently until the odor of H_2S can no longer be detected, then add 5 ml. of

saturated bromine water and continue boiling until the bromine has been expelled. Allow the solution to cool, neutralize to phenol red with NH_4OH, and make slightly acid with HCl (an excess of 0.2 ml. 1+1 HCl). Dilute the resultant solution to volume. For the optimum conditions of measurement the solution should contain 0.2–1.0 mmg. of Zn per ml.

Elimination of Nickel and Cobalt.—Transfer a 20 ml. aliquot of the prepared solution to a 125 ml. separatory funnel; add 5 ml. of the ammonium citrate buffer, 2 ml. of the dimethylglyoxime solution, and 10 ml. of the a-nitroso-b-naphthol solution; and shake for 2 minutes. Discard the solvent layer and extract with 10 ml. of $CHCl_3$ to remove the residual a-nitroso-b-naphthol. Discard the solvent layer.

Isolation and Estimation of Zinc.—To the aqueous phase following the removal of Ni and Co, which at this point has a *p*H of 8.0–8.2, add 2.0 ml. of the dithizone solution and 10 ml. of CCl_4, and shake for 2 minutes. Allow the phases to separate and remove the aqueous layer as completely as possible, withdrawing the liquid by means of a pipet attached to a vacuum line. Wash down the sides of the funnel with approximately 25 ml. of water and without shaking again draw off the aqueous layer. Add 25 ml. of the 0.04 *N* HCl and shake for 1 minute to transfer the zinc to the acid-aqueous layer. Drain off and discard the solvent, being careful to dislodge and remove the drop that usually floats on the surface. To the acid solution add 5.0 ml. of the ammonium citrate solution and 10.0 ml. of CCl_4. (The *p*H of the solution at this point is 8.8–9.0.) Determine the amount of dithizone to be added as follows: To a separatory funnel containing 4.0 ml. of the working Zn standard (20 micrograms) made up to 25 ml. with the 0.04 *N* HCl, 5.0 ml. of the citrate buffer, and 10.0 ml. of CCl_4, add the dithizone reagent in 0.1 ml. increments, shaking briefly after each addition until a faint yellow color in the aqueous phase indicates a bare excess of the reagent. Multiply the volume of dithizone solution required by 1.5 and add this volume (to the nearest 0.05 ml.) to all samples. Shake for 2 minutes. By means of a pipet, transfer exactly 5.0 ml. of the solvent layer to the spectrophotometer cell, dilute with 10.0 ml. of CCl_4, mix, and determine the spectral transmission at 540 mu. (The dilution may be made in a clean, dry test tube if the design of the cell does not permit mixing directly. A Coleman Universal Model 11 spectrophotometer with square test tube cuvettes was used throughout this investigation.)

Preparation of Standard Curves.—Prepare a series of separatory funnels containing 0, 5, 10, 15, and 20 micrograms of Zn and dilute to 25 ml. with the 0.04 *N* HCl, add 5.0 ml. of the citrate buffer, and proceed with the final extraction of Zn as directed in the previous paragraph. Plot the transmittance on a logarithmic scale against concentration and draw a smooth curve through the points. (The intercept of this curve may vary slightly from day to day, depending on the actual concentration of dithizone employed in the final extraction, but the slope should remain essentially the same.)

DISCUSSION OF RESULTS

The results submitted by the different collaborating analysts are reported by code in Table 2. The authors wish to express their appreciation for the gratifying response of the collaborators listed.

The values under the columns marked "A" in Table 2 are the amounts of zinc actually found in the 20 ml. aliquot taken for analysis. The values under the columns marked "B" have been corrected for the zinc present in the blank. The blank for samples 1, 2, 3, and 4 is assumed to be the average of the values reported for Sample 5, which contains any zinc contributed by the 10 grams of sugar as well as by the oxidizing acids.

TABLE 2.—*Results of analyses of collaborative samples*

(A—total zinc present in 20 ml. aliquot; B—corrected for zinc present in blank)

COLLABORATOR NO.	SAMPLE NO. 1 A	1 B	2 A	2 B	3 A	3 B	4 A	4 B	5 A	6 A	6 B	7
1*	2.6	1.9	2.7	2.0	15.2	14.5	15.6	14.9	0.6	19.7	19.1	0.6
	2.8	2.1	2.7	2.0	15.5	14.8	15.5	14.8	0.7	19.7	19.1	0.6
2	5.2	1.8	5.2	1.8	19.0	15.2	17.8	14.4	3.4	23.0	19.8	3.4
			5.4	2.0	18.2	14.8	17.8	14.4		22.8	19.6	3.1
3	2.8	2.0	2.8	2.0	14.8	14.0	14.7	13.9	1.0 (0.9)[a]	22.0 (22.0)[a]	21.2	(1.7)[b]
	3.0	2.2	2.7	1.9	15.0	14.2	15.0	14.2	0.6 (0.8)[a]	17.9	17.1	(1.3)[b]
										19.9	19.1	
										18.9	18.1	
4[e]	8.1		7.5		19.1		18.9		8.7	19.3	(13.4)[e]	6.0
	8.1		7.1		19.1		21.3		6.6	18.7	(12.8)[e]	5.7
5	3.7	2.2	3.9	2.4	15.8	14.3	15.6	14.1	1.5	22.2[d]	21.2	1.0
										(21.2)[c]	(20.4)[c]	
	3.5	2.0	3.7	2.2	16.0	14.5	16.1	14.6	1.5	22.2[d]	21.2	0.9
										(21.2)[c]	(20.4)[c]	
6	2.6	2.5	2.8	2.7	17.2	17.1	18.0	17.9	0.0	19.3	19.3	0.0
	2.7	2.6	2.8	2.7	17.1	17.0	17.6	17.5	0.1	20.3	20.3	0.0
7	4.0	1.9	4.0	1.9	17.0	14.9	16.1	14.0	2.2	20.5	19.0	1.5
	3.9	1.8	4.0	1.9	16.1	14.0	16.1	14.0	2.0	20.0	18.5	1.5
8	3.7	2.0	3.5	1.8	17.7	16.0	17.4	15.7	1.6	21.9	20.8	1.1
	3.6	1.9	3.5	1.8	17.4	15.7	17.8	16.1	1.7	21.8	20.7	1.1
Av. and Av. dev. from the av.		2.07±0.18		2.08±0.25		15.1±0.8		15.0±0.8			19.6±0.9	

[a] Repeat determinations on an aliquot of the same solution.
[b] Ashed blanks were run later—the blank was assumed to be 0.8 mmg.
[c] Determination on 15 ml. aliquot of the same solution.
[d] Read on an extrapolation of the standard curve.
[e] Omitted from average.
* Associate Referee's laboratory.

The blank for Sample 6 is assumed to be the average of the values reported for the reagent blank, Sample 7. In some cases the values reported for Sample 5 are higher than the reagent blank. Whether this reflects the presence of a trace of zinc in the sugar or the use of varying amounts of slightly contaminated nitric acid in the wet digestion is unknown. The authors were unable to detect any significant quantities of any of the dithizone reactive metals in the sugar, and this is further substantiated by the results of collaborators No. 2, 3, and 6.

The collaborators were in general agreement that the procedure under discussion is straightforward although somewhat lengthy, and that the separation and determination of zinc even in the presence of relatively large quantities of interfering elements is quite satisfactory. No essential difference is noted between the recoveries reported for Samples 1, 2, 3, and 4, indicating that the interfering elements are completely eliminated. Furthermore, the amount of zinc recovered is generally in good agreement with the actual amount added in the preparation of the samples.

The major difficulty appears to be due to the ever-present danger of contamination by zinc from the glassware, or from reagents. The effect of the difficulty in controlling the blanks is well illustrated in the data of collaborator No. 3. This analyst reports a value of 0.8 micrograms of zinc in the blank, but on the reagent blank containing only the oxidizing acids, run 4 days later, this same analyst reports a value of 1.5 micrograms. This variability in the blank can obviously become quite serious as is shown by the data of collaborator No. 4. The blanks in this case are quite high, making the determination of small amounts somewhat uncertain. To judge from the results as a whole, it should be possible to hold the blank at a sufficiently low level to avoid serious error. If precautions are observed in the cleaning of glassware and in the selection and purification of reagents, the blank will in most cases amount to less than one microgram.

The sample of soybean meal was included merely to determine the variation in results that might be expected in applying the method to a natural product. The only criterion to follow in judging these results is the degree of variability, and it is evident that with the exception of the low results of collaborator No. 4, the results are uniformly good.

A number of collaborators expressed the belief that the substitution of small Kjeldahl flasks for the Erlenmeyers used in the wet digestion would tend to eliminate the slight loss of material due to spray accompanying the rather vigorous oxidation. The authors concur in this opinion and would recommend that this suggestion be followed where the equipment is available.

SUMMARY AND CONCLUSIONS

The dithizone procedure for the determination of zinc in foods has been subjected to what is considered a critical collaborative study. The

determination of small amounts of zinc in the presence of relatively gross amounts of potential interfering elements has been shown to be satisfactory when due consideration is given to the very important problem of avoidance of contamination.

It is recommended* that the method as presented be adopted as tentative and published in the forthcoming edition of the methods of the Association.

COLLABORATORS

(1) E. M. Godar, American Can Company, Maywood, Ill.
(2) W. Rankin and N. J. Linde, American Can Company, San Francisco, Calif.
(3) A. K. Klein, Food and Drug Administration, Washington, D. C.
(4) C. A. Greenleaf, National Canners Association, Washington, D. C.
(5) G. Kirsten, Food and Drug Administration, New York, N. Y.
(6) Jonas Carol, Food and Drug Administration, Chicago, Ill.
(7) H. S. Mitchell, Swift & Company, Chicago, Ill.
(8) Craig Townsend, Food and Drug Administration, San Francisco, Calif.

REPORT ON FLUORINE

By Paul A. Clifford (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

In the course of a survey conducted during May and June of 1944, various foods were analyzed for fluorine by nine field stations of the Food and Drug Administration. The stations are located, 3 each, in the eastern, central, and western sections of the country. The foods analyzed included a number of those that comprise a principal part of the American dietary; also certain others of known high fluorine content such as fish, teas, etc. Analyses were made by the A.O.A.C. tentative method discussed last year (*This Journal*, **27**, 90). The results are considered worthy of publication because they represent recent analyses by an accepted method; it has been felt that many of the older data represent ordinary foods as containing altogether too much fluorine.

Results on various foods and the methods of sample preparation are given in Table 1. The analysts were: J. P. Alden, H. M. Bollinger, J. W. Cook, L. W. Ferris, H. W. Gerritz, A. W. Hanson, J. L. Hogan, L. C. Jones, G. Kirsten, P. A. Mills, H. O. Moraw, S. H. Perlmutter, C. D. Schiffman, R. D. Stanley, C. A. Townsend, J. F. Weeks, Jr., J. T. Welch, and W. C. Woodfin.

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* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 60 (1945).

TABLE 1.—*Fluorine in various foods*

FOODS ANALYZED	PREPARATION FOR ANALYSIS	PARTS PER MILLION FLUORINE									
		CHICAGO STATION	MINNE-APOLIS STATION	NEW ORLEANS STATION	SEATTLE STATION	SAN FRANCISCO STATION	LOS ANGELES STATION	NEW YORK STATION	ATLANTA STATION	BUFFALO STATION	AVERAGE
APPLES (Peel)[1]	Apples thinly peeled, peels ground	.13	.17	.20	[22].13			[46].60	.14	.15	(7) .22
(Flesh)	Apples peeled, cored, ground, and mixed	.09	.03	.10	.03			.05	[55].17	.02	(7) .07
BEANS (Dry)	Washed under tap, ground	2.10	.66	.30	1.38	[30].06	[38]2.40	[47]1.00		.40	(8) 1.04
(String)	Snipped, ground, and mixed	.04	.15	.05	.08	.38	.29-.18	None	.10	.12	(10) .14
BEEF (Fresh)	Freed from bone and gristle, ground	.45	.44	.07	.09	.11	.03	.06	.78	.55	(9) .29
BEER	As purchased	.18	.17	.30	.09	.07	.18	.07	.10	.67	(9) .20
BONE MEAL (Tablets)	Ground and mixed[6]	[7]236.0	[11]19.2	[17]188.0	[23]141.0	[31]132.0	[39]317.0	[48]170.0			
BREAD (White)	Dried, ground, result figured to fresh basis	3.10	.24	.50	.26	.15	.31	None	.30	None	(9) .54
CABBAGE (Fresh)	Undesirable outer leaves removed, ground, and mixed	.02	.13	.05	.11	.51	.11	.10	.07	.05	(9) .13
CELERY (Fresh)	Undesirable portions removed, ground and mixed	None	.08	.06	.31	.28	.13		.11	.16	(8) .14
CHEESE[2]	Rind removed, ground	.49	.88	.80	.22	None	[40].13	None	.45	.40	(9) .37
COCOA	As purchased	4.40	1.21	.60	None	.36	.73	.10	.49	.37	(9) .92
CORN MEAL	As purchased	.26	[12].33	.15	[24].19	[32].02	[41].45	[49].12	.23	[60].27	(9) .22
EGGS	1 dozen broken out and mixed	None	.37	.07	.11	.09	.16	None	None	.28	(9) .12
FISH (Fillets)	As purchased, ground, and mixed	[8]1.54	[13]1.96	[18].20	[25].40	[33].74	[42].21	[50].70	[56]1.09	[61]5.53	(9) 1.49
FLOUR (Self-rising)	As purchased	.70	.36	.50	.80	.24	.19	None	.83	.40	(9) .45
FRUIT JUICE (Fresh or canned)	As purchased	[9].10	[14].07	[19].03	[26].00	[34]Trace	[43].03	[51]None	[57].08	[62].02	(9) .04
MILK (Fresh)	One quart, mixed	.18	[15].18	[20].14	[27].02	[35].09	[44].04	[52]None	[58].16	[63].08	(9) .09
MACKEREL (Canned)[3]	Entire can ground up fine as possible to distribute bone evenly	15.4	15.6	13.0	8.92	6.90	13.0	[53]1.50	17.25	17.40	(9) 12.1
PEANUTS IN SHELL	Shelled, hulled, and ground	4.80	2.16-1.89	.60		.24	[45].27	.10	.21-1.65	.50	(10) 1.24
PEARS (Peel)	See apples, above	.32		.20				None			(3) .17
(Flesh)		.10		.02				None			(3) .04
PHOSPHATE (Di-calcium)	As purchased	[10]12.2	[16]20.2	[21]8.50	[28]451.0	[36]6.93	15.7	[54]10.2	[59]6.40	[64]22.7	
(Mono-calcium)	As purchased	3.70	2.53	3.50	271.0	7.20	8.20	4.30	6.11	2.00	
PORK (Fresh)	Freed from bone and gristle, ground	.74	1.15	.30	.22	.36	None	None	.17	.10	(9) .34
POTATOES (White)	Unpeeled, scrubbed, ground	.53	.12	.24	.05	.08	.15	.06	.11-.21	.10	(10) .16
POULTRY (Canned boned chicken)	Ground and mixed	.80	.60	.90	.09	[37].31		1.88	.45	None	(8) .63
PRUNES (Dried)[4]	Washed, pitted, ground	.20	.24	.70	[29].68	.30	.10	None	.21	.03	(9) .27
SALMON (Canned)	(See Mackerel	2.20	5.44	6.40	2.04	2.95		3.50	5.70	5.05	(8) 4.16
SHRIMP (Edible portion)	Peeled, ground and mixed	1.00	1.30	.30	.75	.55	1.16	.85	1.29	1.15	(9) .93
SWEET POTATOES	Unpeeled, scrubbed, ground	.03	.31	.10	.31	.01	.19	None	.13	.10	(9) .13
TEA (Infusion)[5]	15 grams to 1 liter boiling water 10 min. steep, strain, analyse infusion	1.01	.94-1.90	1.17	.97	1.03	.74	1.62	1.25	1.23	(10) 1.19

Other foods analyzed included fresh oysters, molasses, canned asparagus; dehydrated mustard greens, spinach, turnip greens, and celery powder; black-eye peas, fresh cocoanut, port wine, canned tuna fish flakes, sardines in olive oil, chick peas, mate (infusion), soy beans, soy flour (low and full fat), fresh spinach, fresh kale, wheat (Deaf Smith Co., Texas, 2), wheat flour (Deaf Smith Co., Texas). Preparation consisted of the usual processes of grinding, mixing, and washing and draining when necessary. The results were as follows: fresh oysters, 0.65; molasses, none; canned asparagus, none; dehydrated mustard greens, spinach, turnip greens and celery powder, 1.96, 2.78, 1.36, 1.29, respectively;* black-eye peas, 0.23; fresh cocoanut, none; port wine, 0.10; canned tuna fish flakes 0.10; sardines in olive oil, 16.1; chick peas, 0.14; maté (infusion), 0.05; soy beans, 1.33; soy flour (low and full fat respectively), 1.45 and .52; fresh spinach, 0.36; fresh kale, 0.16; wheat (Deaf Smith Co., Texas) 1.15 and 0.76; wheat flour, 0.50.

* (If converted to fresh basis by factor of 1/13.4, results become .15, .21, .10, and .10).

[1] Average ca 13% peel. [2] All cheddars except Los Angeles. [3] Practically all samples California mackerel. [4] Practically all California prunes. [5] Infusions made in the proportion of about one TSP per 4.5 oz. teacup. If it is assumed that 80% of the fluorine in these teas goes into the strained infusion, F content of the original dry teas are (p.p.m.) about: 81, 94, 84, 135, 66 (actual test), 158, 85, 104, 102, 62; Av_{10}=97 p.p.m. [6] Recent analyses of bone meals used for feeding purposes average 451 (p.p.m.) (14 samples; high=635; low=201). [7] With viosterol. [8] Ocean perch. [9] Canned orange. [10] Tablets. [11] "Bone flour" and D.C.P. milk solids, iron, vit. D etc., tablets. [12] Yellow. [13] Canadian white fish. [14] Grapefruit. [15] Carton. [16] Tablets. [17] Same as Seattle. [18] Red fish. [19] Canned grapefruit. [20] Bottle. [21] Tablets [22] Two samples of entire apples ground=.09 and .08 p.p.m. [23] 50% bone phosphate, 50% D. C. phos. [24] Yellow. [25] Sole. [26] Canned grapefruit. [27] Bottle. [28] Tablets. [29] Oregon. [30] Large white. [31] Same as Se and N.O. [32] Yellow. [33] Codfish [34] Canned grapefruit. [35] Bottle. [36] D. C. P. with vit. B and D, skim milk, dextrose. (tablets). [37] Boned turkey. [38] Kidney beans. [39] Bulk D. C. P. from bone meal. [40] Mexican "Jack" cheese. [41] Yellow. [42] Cabrilla. [43] Fresh orange. [44] Carton. [45] F in peanut *hulls*=1.91 p.p.m. [46] Slight pos. F. res. test. [47] Black-eye. [48] Same as Seattle and New Orleans. [49] Yellow. [50] Red perch. [51] Canned grapefruit. [52] Carton. [53] "Grated"—packed in cotton seed oil. [54] Tablets. [55] Small, green. [56] Mullet. [57] Canned orange. [58] Bottle. [59] Capsules and Ca gluconate. [60] Yellow. [61] Lake pike. [62] Fresh orange. [63] Bottle. [64] Tablets.

The survey not only furnished valuable data on the natural fluorine content of many foods, but it also subjected the general method to rigorous test. The analysts indicated several points in the procedure where the directions needed slight amplification or revision. These points are enumerated briefly as follows:

(1) The natural fluorine content of most foods is quite low, and this fact taxes the sensitivity of the general method. If 50 grams of a food containing 0.1 p.p.m. of flourine is taken for analysis, only 5 micrograms of food fluorine will appear in the total final distillate. Accordingly, the sample analyzed should be as large as can conveniently be handled, and the aliquot titrated should be as large as possible. Where low-fluorine foods are analyzed, the use of large (100 ml.) Nessler tubes should be urged.

(2) For precise results the distillation blank must be minimal and constant. This necessitates clean "well-tempered" stills, low acid distillates, distillation at constant temperature and uniform rate, and good reagents.

The proposed tolerance for fluorine on fruits defines the permissible amount of residue in terms of p.p.m. of fluorine. In the interest of simplicity, this necessitates certain changes (mostly editorial) in the rapid method restricted to fluoride residues on apples and pears (*Ibid.*, 98), and slight changes in reagent strengths calculated to allow the method to cover a greater fluorine range.

It is recommended* that the directions for the general method be amplified and certain sources of error stressed, and that the rapid method restricted to fluoride residues on apples and pears be modified as indicated above.

No report on preparation of large sized samples was given by the Associate Referee.

No report on mercury was given by the Associate Referee.

No report was given on microbiological methods by the Referee, and with the exception of the report of the Associate Referee on Eggs and Egg Products, no reports were given by the associate referees under this general subject.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 28, 60 (1945). The method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

REPORT ON MICROBIOLOGICAL METHODS IN EGGS AND EGG PRODUCTS

By M. T. BARTRAM (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

The microbiological methods for the examination of frozen eggs was adopted as official, first action, in 1941, and the subject was extended to include eggs and egg products. Since that time no further collaborative study of the methods for frozen eggs has been undertaken, owing to the inherent difficulties in distributing frozen products to collaborators, which at this time is greatly increased by slow transportation.

These methods have now been extended to include liquid and dried eggs in addition to frozen eggs. It should be emphasized that no essential changes in the methods, as applied to frozen eggs, have been made and that the only additions are the inclusion of methods of sampling and the preparation of samples for the liquid and dried product. These methods have been applied to both liquid and dried eggs for a number of years by those engaged in production and analysis of these products.

Collaborative studies on the methods embracing viable bacteria in liquid eggs appear at this time impossible owing to the changes that occur in this product unless held within more narrow temperature ranges than are possible during transportation. Methods of preserving liquid and frozen eggs for microscopic bacterial counts are at present being investigated.

In a contributed papter published in *This Journal,* **26**, 172, it was pointed out that in dried eggs there was a decrease in viable bacteria on storage and that this fact, in addition to the decrease which occurs during the drying process, serves to make this examination of minor value as compared to the microscopic bacterial count on this product.

The microscopic bacterial method presented in the proposed revised chapter on eggs and egg products has been subjected to study by the writer and five collaborators. In all, 37 separate samples were examined, although in the majority of instances only 3 collaborators examined any one sample. The results of these examinations are reported in the accompanying table and in all but one instance the agreement is within the limits of error inherent in the method.

RECOMMENDATIONS*

It is recommended—

(1) That the revised methods submitted herewith, including liquid, dried, and frozen eggs, be adopted as tentative. The method will be published in *Methods of Analysis*, 6th edition, 1945.

(2) That methods for the preservation of liquid and frozen eggs for microscopic counts be studied.

* For report of Subcommittee C and action by the association, see *This Journal*, **28**, 61 (1945).

Microscopic bacterial examination of dried eggs

SAMPLE	COLLABORATOR A	B	C	D	E	F
1	31*	17	75	36	15	
2	374	414	690	480	360	
3	1,320	1,237	1,728	1,406	1,320	
4		1.4			2.0	
5		93			82	
6		533			722	
7		1.4			2.0	
8		9,350			8,840	
9		1,400			1,500	
10		3,000			2,720	
11		185			209	
12		5,500			5,600	
13		0.5			0.7	
12		8.4			10	
15		1,950			1,720	
16		376			370	680
17		415			762	950
18		271			943	800
19		487			873	880
20		2.8			3.2	9.6
21		2.1			2.1	7.2
22		4.2			4.0	5.3
23		0.7			3.5	3.6
24			1,280	1,630	1,380	
25			2,880	3,928	2,920	
26			7,300	8,624	8,300	
27			2,240	1,720	2,940	
28			4,800	3,952	4,000	
29			960	2,526	900	
30			6,400	6,212	6,800	
31			7,360	8,720	7,200	
32			3,200	2,046	3,400	
33			4,160	7,400	3,380	
34			640	958	872	
35			5,440	2,756	3,060	
36			2,240	3,800	1,860	
37			3,840	2,680	3,730	

* Microscopic bacterial count in millions per gram of dried egg.

Credit is extended to the following collaborators who participated in these studies:

A. H. Robertson, Director State Food Laboratory, Albany, N. Y.
C. E. Safford, State Food Laboratory, Albany, N. Y.
E. W. Coulter, U. S. Food & Drug Adm., Chicago, Ill.
A. Chuckrow, U. S. Food & Drug Adm., New York, N. Y.
W. R. North, U. S. Food & Drug Adm., Washington, D. C.

No report on michrochemical methods was given, no associate referee having been appointed.

REPORT ON OILS, FATS, AND WAXES

By J. FITELSON (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Referee*

Reports have been received from the Associate Referees on Unsaponifiable Matter and on Peanut Oil. No report on refractometric methods for oil in seeds will be made this year.

UNSAPONIFIABLE MATTER

The Referee concurs in the recommendation that the S.P.A. method for unsaponifiable matter be adopted as a tentative method. In view of the forthcoming revision of *Methods of Analysis, A.O.A.C.*, it would be desirable to have only one method for unsaponifiable matter. However, although this year's collaborative results definitely demonstrate the superiority of the S.P.A. method, further studies should be made on this important subject, particularly since the present official F.A.C. method has such widespread acceptance.

DETECTION OF PEANUT OIL

The Bellier test for peanut oil, as modified by Evers,[1] has been used in this laboratory for several years as a rapid sorting test. The test has been applied successfully to several hundred samples of oil, and positive reactions for peanut oil have been confirmed by the more time-consuming official method. It has been shown that 10 per cent or more of peanut oil, in mixtures also containing olive, corn, cottonseed or soybean oils, can be definitely detected by this rapid test. The Referee agrees that this method should be adopted as a tentative qualitative test, to be used for sorting purposes, and that, after accumulation of additional authentic data, collaborative studies should be conducted.

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[1] *Analyst*, 62, 96 (1937).

DETECTION OF OLIVE OIL IN EDIBLE OIL MIXTURES

The Referee has conducted collaborative studies on the recently published method for the determination of squalene in oils (*This Journal,* **26,** 499). Since olive oil contains considerably more squalene than the other common edible oils, this method can be used for the detection of olive oil in edible oil mixtures. Four samples of oils were submitted to eight collaborators, and their results are recorded in Table 1.

TABLE 1.—*Collaborative results on the determination of squalene* (mg. per 100 grams of oil)

COLLABORATOR	LOCATION*	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D MIXTURE
		CORN OIL	OLIVE OIL	SOYBEAN OIL	40% A 40% C 20% B
A. G. Buell	San Francisco, Calif.	36.8 36.1 Av. 36	414.9 413.4 Av. 414	17.7 16.6 Av. 17	106.5 104.1 Av. 105
H. V. Claborn	Washington, D. C.	36 37 Av. 37	386 378 Av. 382	14 15 Av. 15	99 92 Av. 96
M. M. Jackson	Philadelphia, Pa.	37 37 36 Av. 37	378 387 Av. 383	18 16 Av. 17	101 102 Av. 102
C. S. Purcell	Boston, Mass.	32 32 Av. 32	398 402 Av. 400	9 9 Av. 9	98 97 Av. 98
J. Carol	Chicago, Ill.	40 40 Av. 40	412 414 Av. 413	16 17 Av. 17	104 108 Av. 106
R. S. Georgiade	New York, N. Y.	34 34 Av. 34	404 408 Av. 406	9 12 Av. 11	96 96 Av. 96
G. Kirsten	New York, N. Y.	33 33 Av. 03	398 399 Av. 399	11 11 Av. 11	97 97 Av. 97
J. Fitelson	New York,	34	406	14	99
Mean		35	400	14	100
Mean absolute dev.		2.1	9.4	2.5	3.4
Maximum dev.		+5	−18	−5	+6

* Food and Drug Administration, Federal Security Agency.

The collaborative results for the olive oil, Sample B, showed somewhat greater deviations from the mean than had been anticipated, and it was suspected that this sample, made from several batches of olive oil, had not been mixed sufficiently before bottling. Accordingly, each of two subdivisions was analyzed successively by two collaborators, who reported 378 and 383 mg. of squalene per 100 grams of oil in one subdivision and 406 and 398 mg. in the other. These results indicate that the collaborative deviations in Sample B were in part caused by the lack of homogeneity of the oil.

The results on the other three samples are within the limits of precision expected for this method, with a mean deviation equivalent to less than 0.1 ml. of the 0.05 *N* sodium thiosulfate used in the final titration. The collaborators reported no difficulties in the operation of the method. Several helpful suggestions were offered, including vigorous shaking of the initial refluxing mixture in order to insure complete saponification.

Since publication of the method, additional authentic oils have been examined for squalene content. The summary (Table 2) includes the data originally published.

TABLE 2.—*Squalene content of edible vegetable oils*

OIL	OLIVE	COTTON-SEED	PEANUT	CORN	SOY-BEAN	SUN-FLOWER	TEA SEED	SESAME	RAPE
No. of oils examined	103	41	37	31	48	5	3	3	2
Squalene (mg./100 grams of oil)									
(Max.)	708	15	49	42	22	19	16	9	28
(Min.)	136	3	8	16	5	8	8	3	24
(Ave.)	383	8	27	28	12	12	12	5	26

Certain minor precautions and additions to the method as published in *This Journal,* **26,** 502, should be inserted in order to clarify some of the details. It is recommended* that the revised method be adopted as tentative.

Page 503, (f), *line 2.*—Change end of 1st sentence to read "1% of isoamyl alcohol, dilute to 1 liter, mix, and filter."

"Apparatus," *line 1.*—After word "prepare" insert "a fresh column for each determination."

"Determination," *1st paragraph, line 3.*—After "30 minutes" add "shaking the flask occasionally"; *1st paragraph, line 20.*—After "CO_2" add "or other inert gas"; *2nd paragraph, line 6.*—Change "rise" to "rinse"; *2nd paragraph, lines 6 & 7.*—After word "portions" add "that have been used to rinse the beakers"; and *2nd paragraph, line 10.*—After "CO_2" add "or other inert gas."

Page 504, *line 3.*—After "add" insert "quickly."

* For report of Subcommittee C and action of the Association, see *This Journal,* **28,** 61 (1945).

DETECTION OF MINERAL OIL IN FATS

Mineral oil has been used occasionally to replace some of the normal fatty ingredients in such products as candy, mayonnaise, cheese, and butter. The rapid turbidimetric test of Bornmann (*This Journal*, **22,** 194) has been applied to the detection of this adulterant in many fats. Substantially the same test is given in *Methods of Analysis, A.O.A.C.*, 1940, 95, **9,** and it is also known as Holde's test.[1] Polenske's method for the quantitative determination of paraffin wax in unsaponifiable matter[2] has also been successfully used on fats containing mineral oil. In this method the normal unsaponifiable constituents are dissolved in hot concentrated sulfuric acid, leaving the inert mineral oil in a condition suitable for isolation and measurement.

During the past few years, these methods have been used extensively in the Food and Drug Administration laboratories, particularly for the detection of small quantities of mineral oil. The Referee has used and checked these methods and found them to be reliable. When very small quantities of mineral oil are present, it is necessary to handle large volumes of oil in order to obtain sufficient unsaponifiable matter for quantitative manipulation. A simple procedure for handling such large volumes has been adopted from accepted quantitative methods and has been used satisfactorily by the Referee and his associates. Since the proposed methods have been known for a great many years and have been extensively studied and used within the past few years, it is recommended that they be adopted as official (final action) without additional collaborative studies.

IODINE ABSORPTION NUMBER

Several chemists have recently informed the Referee that the procedure for the standardization of the sodium thiosulfate solution used in the Hanus method (*Methods of Analysis, A.O.A.C.*, 1940, 429, **18 c**) yields low, erroneous factors. These findings have been confirmed by work done in this laboratory. The error is probably introduced by air oxidation of the potassium iodide at the high acidity used in the standardization.[3] Comparison of the A.O.A.C. method with five other published methods using dichromate as the primary standard showed the highest initial acidity in the former method. The following tabulation lists the values obtained by several methods of standardization, including the A.O.A.C. procedure modified by decreasing the initial acidity to a level comparable with that used in other dichromate methods.

Potassium dichromate usually conforms to a very high degree of purity, but it may contain a little water and oxidizable organic matter. The re-

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[1] Jamieson, "Vegetable Fats and Oils," p. 412. Reinhold Publishing Co., New York (1943).
[2] Lewkowitsch, vol. 1, p. 605. Macmillan & Co., London (1921).
[3] Kolthoff and Furman, "Volumetric Analysis, vol. II, p. 369. John Wiley & Sons, New York (1929).

TABLE 3.—*Standardization of $Na_2S_2O_3$ solution by various methods*

METHOD OF STANDARDIZATION	INITIAL ACIDITY (ML. CONC. HCl USED/100 ML.)	PRIMARY STANDARD USED	NORMALITY FOUND
Methods of Analysis, A.O.A.C. 1940, 429, **18(c)**	14.7	$K_2Cr_2O_7$	0.1011 0.1011 Av. 0.1011
Methods of Analysis, A.O.A.C. 1940, 429, **18(c)** (modified to reduce acidity)	7.7	$K_2Cr_2O_7$	0.1014
This Journal, **25**, 660 (1942)	2.0	$K_2Cr_2O_7$	0.1014
Fales & Kenny, "Inorganic Qualitative Analysis," p. 461. Appleton-Century Co., New York (1939)	—	Resublimed I	0.1014 0.1015 Av. 0.1015
Ibid., p. 460	—	KIO_3	0.1016

crystallized and dried salt can be used directly as a primary standard and need not be standardized against pure iron. It is therefore recommended that the following changes be made in the method as published (**XXXI, 18**):

Page 429.—Delete **18(b)** and **18(c)**.

Page 430, **19**, *line 8*.—After "0.1 *N* $Na_2S_2O_3$" insert a numerical reference to the method for preparation and standardization of this reagent as recommended for official adoption in the 1944 report of the Associate Referee on Sodium Thiosulfate Solution.

20, *line 7*.—Delete sentence "The other reagents and solutions used are described under **18**."

21, *line 8*.—After "0.1 *N* $Na_2S_2O_3$ solution" insert reference to section on standard solutions as noted above.

THIOCYANOGEN NUMBER OF FATS AND OILS

The constants used in the calculations involved in the thiocyanogen number method have been under consideration for some time, and several investigators have shown conclusively that the constants now used are incorrect for the conditions specified in the determination. All these investigators have recommended revised constants that are in reasonable agreement. These constants were found to depend largely on the concentration and excess of reagent, temperature, and time of reaction. Both the A.O.A.C. tentative method (*Methods of Analysis, A.O.A.C.*, 1940, 431, **22**) and the A.O.C.S. method (Committee on Analysis of Commercial Fats and Oils, *Ind. Eng. Chem., Anal. Ed.*, **8**, 233) were used in these investigations. The essential difference between these two methods is in the concentration of the thiocyanogen reagent, the former method re-

quiring 0.1 *N* solution while 0.2 *N* is specified in the A.O.C.S. method. Most of the workers preferred the more concentrated reagent since smaller volumes were required in the analyses. Several investigators report satisfactory use of the A.O.A.C. method modified by increasing the thiocyanogen concentration to 0.2 *N*.

The A.C.S.—A.O.C.S. Committee on the Analysis of Commercial Oils and Fats has recently recommended the adoption of the averages of the new constants reported by the various investigators, based on the use of 150–200 per cent excess of 0.2 *N* thiocyanogen reagent. Their report (*Chem. Eng. News*, p. 606, April 25, 1944) also contains a tabulation of the individual proposed constants, with references. It is recommended that these new average constants be adopted in place of the present values and that the tentative method be further modified to permit use of 0.2 *N* thiocyanogen reagent. The Referee has prepared this stronger reagent and found no difficulties in the preparation or standardization made by the proposed modification. The recommended changes in the method **(XXXI, 22)** are as follows:

Page 431, **22(b)**, *line 1*.—Insert "0.2 *N*" after "Thiocyanogen soln"; *line 5*.—Change "4.2 g" to "8.4 g"; *line 7*.—Change "12.5 g" to "25 g"; *line 15*.—Change "24–26 ml" to "48–52 ml"; **23**, *line 2*.—Change "100–150%" to "150–200%"; *line 4*.—Change "20–24" to "24" and "10 ml of 10% KI" to "10 ml of 20% KI";*line 13*.—Delete section containing the calculations beginning "Using the thiocyanogen number" to page 432, line 11. Substitute the following:

"When the iodine and thiocyanogen values are determined on the oil, calculate the percentages of hypothetically pure triglycerides by the following formulas:
When no linolenin is present:

Y = 1.246 I.V. − 1.253 T.V.; Z = 2.525 T.V. − 1.348 I.V.; and S = 100 − (Y + Z).
When linolenin is present:

X = 1.6610 T.V. − 0.1332 I.V. + 1.3056 S − 130.56; Y = 1.4137 I.V. − 3.3449 T.V. − 1.6441 S + 164.41; and Z = 1.6839 T.V. − 1.2805 I.V. − 0.6615 S + 66.15; where X = % linolenic acid glycerides; Y = % linoleic acid glycerides; Z = % oleic acid glycerides; and S = % saturated acid glycerides and unsaponifiable matter.

When iodine and thiocyanogen values are determined on fatty acids, calculate as follows:
When no linolenic acid is present:

S = 100 − (Y + Z); Y = 1.194 I.V. − 1.202 T.V.; and Z = 2.421 T.V. − 1.293 I.V.
When linolenic acid is present:

X = 1.5902 T.V. − 0.1290 I.V. + 1.3040 S − 130.40; Y = 1.3565 I.V. − 3.2048 T.V. − 1.6423 S + 164.23; and Z = 1.6146 T.V. − 1.2275 I.V. − 0.6617 S + 66.17; where X = % linolenic acid; Y = % linoleic acid; Z = % oleic acid; and S = % saturated acid and unsaponifiable matter.

SPECIFIC GRAVITY

The factor 0.0007 used in the temperature correction equation for specfiic gravity of oils (*Methods of Analysis, A.O.A.C.*, 1940, 423, **5**) is not correct. The correct factor 0.00064 was used in Bull. 107 (rev.) p. 129. The correction equation requires use of the change in specific gravity per

degree (0.00064), whereas the factor 0.0007 was developed by Wright[1] for use in a general equation expressing volume relations of oils with temperature. Wright's factor is equivalent to the change in specific gravity per degree divided by the specific gravity. It is recommended that the following changes be made in **XXXI, 5**:

On Page 423, 5, line 3, and page 424, line 3.—Change 0.0007 to 0.00064.

TITER TEST

The glycerol-potassium hydroxide method for the isolation of the fatty acids used in the titer test (*This Journal*, **25**, 95) is customarily used in this country and the Referee believes that the alternative official aqueous or alcoholic sodium hydroxide method (*Methods of Analysis, A.O.A.C.*, 1940, 428, 16) is superfluous and unnecessary. Since the recommendations of Subcommittee C (*This Journal*, **25**, 65) do not definitely specify any action on this alternative method, the following changes are proposed for official (final) action:

Pages 427, 428, and 429 (**XXXI**).—Delete sections **15**, **16**, and **17**, and replace with the method and specifications published in *This Journal*, **25**, 95.

SESAME OIL

Positive results for sesame oil are occasionally encountered in pure olive oil examined by the Baudouin test (*Methods of Analysis, A.O.A.C.*, 1940, 441, **45**). In such cases, re-examination by the Villavecchia test (*Ibid.*, 441, **46**) is directed. Since both tests depend on the same reaction and yield similar colors, the Referee recommends deletion (official, final action) of the less satisfactory Baudouin test **(XXXI, 45)**.

RECOMMENDATIONS*

It is recommended—

(1) That the S.P.A. method for determination of unsaponifiable matter as presented by the associate referee be adopted as a tentative method and that further collaborative studies be made.

(2) That the modified Bellier test for the detection of peanut oil, used as a sorting test, be adopted as a tentative method and that collaborative studies on this method be conducted.

(3) That the method for the determination of squalene (*This Journal*, **26**, 499), with the changes proposed by the associate referee, be adopted as a tentative method under the heading "Squalene," and that further collaborative studies be made.

(4) That the methods for the detection of mineral oil in fats discussed by the referee in this report be adopted as official, final action under suspension of the rules.

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[1] *J. Soc. Chem. Ind.*, **26**, 513 (1907).

* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 61 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

(5) That the referee consult with the Referee on Standard Solutions regarding the proposed change in the method for standard sodium thiosulfate.

(6) That the tentative method for thiocyanogen number (**22**, p. 431) be modified as suggested by the referee and that the revised constants and equations used with this modified method be accepted.

(7) That the factor 0.0007 used in the correction equation for specific gravity (**5**, p. 423) be changed to 0.00064.

(8) That the official aqueous or alcoholic NaOH method for the preparation of the fatty acids used in the titer test (**16**, p. 428) be deleted (official, final action).

(9) That the official titer test (**15**, **16**, and **17**, p. 427) be replaced by the modified method published in *This Journal*, **25**, 95), (official, final action).

(10) That the official Baudouin test for sesame oil (**45**, p. 441) be deleted (official, final action).

(11) That the official methods for cottonseed (**50–62** inc., p. 443) be brought up to date in accordance with current regulations of the United States Department of Agriculture.

(12) That the studies on refractometric methods for the determination of oil in seeds be discontinued temporarily.

(13) That studies on methods of determining the stability of fats be initiated.

(14) That studies on the improvement of the official method for peanut oil (**42**, p. 439) or other quantitative methods for the examination of peanut oil be initiated.

No report on the refractometric determination of oil in seeds was given by the Associate Referee.

REPORT ON UNSAPONIFIABLE MATTER

By Gardner Kirsten (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

In accordance with the recommendations of the A.O.A.C. (*This Journal*, **25**, 65) the study of methods for the determination of unsaponifiable matter was continued, and the method that appeared most suitable was subjected to collaborative investigation.

Previous work (*Ibid.*, **26**, 728) demonstrated that higher recoveries of unsaponifiable matter are obtained with the S.P.A. method[1] than with the F.A.C.[2] or Kerr-Sorber (*This Journal*, **8**, 439) methods and that the

[1] *Analyst*, **58**, 203 (1933).
[2] *Methods of Analysis*, *A.O.A.C.*, 1940, 432.

F.A.C., Kerr-Sorber, and the continuous extraction method of Wood and Roschen[3] give comparable results. Wood and Roschen also showed that the F.A.C. and continuous extraction methods give results which are in good agreement.

Since the S.P.A. method appeared to give more complete extraction of the unsaponifiable matter and since it seemed adapted to wide application, additional studies were confined to this method and to comparing it with the F.A.C. method, which is the present official method of the A.O.A.C.

The S.P.A. method as published requires the drying of the unsaponifiable matter at 80°C. while the other methods specify drying at 100° C. Since drying at 100° is more widely used, tests were made to determine whether any significant differences in results would be obtained between the two temperatures.

A sample of shark liver oil and a sample of corn oil were used for this experiment. Ten gram samples were saponified and extracted according to the S.P.A. method, and the final washed ether solution was adjusted to 110 ml. Four 25 ml. aliquots were taken and after being evaporated just to dryness on the stream bath, two were dried to constant weight at 80° and two at 100°C. The results are given in Table 1.

TABLE 1.—*Results showing results of varying periods of drying*

	% UNSAPONIFIABLE MATTER	
	DRIED AT 80°C.	DRIED AT 100°C.
	per cent	*per cent*
Shark liver oil	2.71	2.69
	2.71	2.69
Corn oil	1.21	1.18
	1.19	1.19

While drying at 80°C does appear to give higher results, the difference is not appreciable, and in all further determinations the drying was conducted at 100°C.

To further check the efficiency of the S.P.A. method, a sample of whale oil and a sample of shark liver oil were saponified and extracted according to this method. Two additional extractions were then made and 0.02 and 0.01 percent of additional unsaponifiable matter was recovered, showing that the extractions had been virtually complete. To check on the possibility that unsaponifiable matter might be lost during the washing process, the first four washings were re-extracted. The amount recovered was negligible.

It was found convenient to use 1.5 ml. of 50 percent aqueous potassium hydroxide and 25 ml. of 95 percent alcohol in place of the 0.5 *N* alcoholic potassium hydroxide specified in the method. No difference was found in the results when the same oil was saponified by the different reagents.

[3] *Oil and Soap*, 15, 287 (1938).

Three oils were submitted to collaborators for the determination of unsaponifiable matter. Collaborative results are reported in Tables 2 and 3. Collaborators were instructed to determine unsaponifiable matter by the method submitted and by the official method and to report: (1) Total percent unsaponifiable matter weighed by both methods; (2) titrable acidity on unsaponifiable residues obtained by both methods, calculated as oleic acid; (3) percent of unsaponifiable matter after correcting for titrable acidity; (4) blank determinations on reagents (both methods); (5) whether or not the third washing in the official method was alkaline to phenolphthalein; and (6) any difficulties encountered and any comments on the methods.

TABLE 2.—*Collaborative determination of unsaponifiable matter*

	UNSAPONIFIABLE MATTER—CORRECTED FOR TITRABLE ACIDITY					
	OFFICIAL METHOD			MOD. S.P.A. METHOD		
SAMPLE	1	2	3	1	2	3
Collaborator		*per cent*			*per cent*	
1	0.99	0.46	3.04	1.09	0.53	3.77
	0.97	0.40	3.00	1.10	0.51	3.76
2	0.99	0.40	2.84	1.09	0.57	4.00
	0.96	0.39	2.91	1.10	0.51	4.17
	0.97					
3	1.09	0.49	2.75	1.18	0.53	4.10
	0.95	0.49	2.78	1.16	0.53	4.19
	0.98		2.82	1.17	0.53	
				1.17		
4	1.01	0.41	3.24	1.00	0.41	3.74
	1.01	0.45	3.19	1.04	0.46	3.69
5	0.97	0.52	3.06	1.19	0.52	4.24
	1.02	0.47	3.09	1.19	0.49	4.11
6	0.95	0.41	2.92	1.17	0.51	4.15
	0.93	0.42	2.92	1.18	0.52	4.13
7	0.98	0.41	2.53*	1.04	0.48	4.05
	0.93	0.42	2.52*	1.09	0.43	4.03
8	1.01	0.46	3.06	1.20	0.55	4.15
	0.98	0.48	3.13	1.17	0.56	4.19
Average	0.98	0.45	3.00	1.13	0.51	4.03

* Not included in average. Collaborator reported that unsaponifiable matter was not entirely soluble in petroleum benzin at room temperature. The insoluble residues, which amounted to 0.61% and 0.52%, were filtered off and not included in results reported.

The following members of the Food and Drug Administration participated in the collaborative work: A. Alter, Baltimore, Md.; H. W. Haynes, Boston, Mass.; Twyman M. Klaydor, Buffalo, N. Y.; S. M. Stark, Jr., New Orleans, La.; Mary A. McEniry, St. Louis, Mo.; and Ruth S. Georgiade, F. B. Jones, and G. Kirsten, New York, N. Y.

TABLE 3.—*Free fatty acid in unsaponifiable residues*

	TITRABLE ACIDITY—CALC. AS OLEIC ACID					
	OFFICIAL METHOD			S.P.A. METHOD		
SAMPLE	1	2	3	1	2	3
Collaborator		*per cent*			*percent*	
1	0.16	0.21	0.22	0.05	0.03	0.20
	0.14	0.16	0.18	0.05	0.04	0.24
2	0.08	0.06	0.19	0.07	0.03	0.09
	0.12	0.07	0.18	0.06	0.03	0.08
	0.15					
3	0.08	0.10	0.12	0.03	0.04	0.15
	0.02	0.09	0.09	0.03	0.05	0.11
	0.02		0.09	0.04	0.05	
				0.05		
4	0.22	0.14	0.23	0.11	0.10	0.15
	0.17	0.08	0.12	0.10	0.11	0.16
5	0.04	0.10	0.04	0.08	0.14	0.16
	0.08	0.18	0.04	0.18	0.14	0.13
6	0.07	0.06	0.12	0.01	0.03	0.08
	0.11	0.08	0.14	0.01	0.03	0.08
7	0.19	0.20	0.09	0.03	0.04	0.09
	0.23	0.20	0.07	0.03	0.05	0.05
8	0.07	0.05	0.11	0.02	0.02	0.08
	0.08	0.08	0.10	0.02	0.02	0.07
Average	0.11	0.12	0.13	0.06	0.06	0.12

Sample 1 consisted of corn oil, Sample 2 of peanut oil, and Sample 3 of a mixture of fish liver oils. The collaborative results show good agreement for each method on Samples 1 and 2; with the S.P.A. method giving consistently higher figures. With Sample 3, which contained a larger amount of unsaponifiable matter, the agreement between collaborators for the same method is not so good. The difference in results on this sample by the two methods is striking and shows that a much more complete recovery of the unsaponifiable matter is obtained with the S.P.A. method.

The procedure for washing in the S.P.A. method was devised to eliminate the need for a correction for free fatty acids and the previous experience of the Associate Referee had been that only in very rare instances had more than 0.10 ml. of 0.1 *N* alkali been required to neutralize the unsaponifiable residue obtained. The collaborative results show that the correction for fatty acids is often too large to be disregarded. This is es-

pecially true in Sample 3, although in Samples 1 and 2 the correction falls within the limit of 0.10 ml. of 0.1 *N* alkali (equivalent to 0.11 per cent on a 2.5 gram sample) for all but one of the collaborators. The official method gives a higher percentage of fatty acids and no provision is made for any correction.

It had been the Associate Referee's experience that the third washing of the petroleum benzin extract in the official method was usually still alkaline to phenolphthalein. This was corroborated by the collaborators, all but one reporting that the third washing was alkaline in the majority of instances. This would indicate that three washings are insufficient to remove all the soaps in this method.

Of the collaborators who commented, the majority expressed a preference for the S.P.A. method. Two reported trouble with emulsions, and further instructions have now been included in the method in an effort to minimize difficulties from this source.

SUMMARY

The S.P.A. method for the determination of unsaponifiable matter has been further studied. It was concluded that there was no advantage in drying at 80°C. over drying at 100°C. Further experiments showed that extraction of the unsaponifiable matter is virtually complete under conditions specified by the method and that no appreciable loss occurs during the washing process.

The S.P.A. method and the official F.A.C. methods were subjected to collaborative study. The agreement between collaborators was about equal for each method. Consistently higher results were obtained by the S.P.A. method. This difference was especially marked in the case of the sample of fish liver oil.

RECOMMENDATIONS*

It is recommended—

(1) That the S.P.A. method for the determination of unsaponifiable matter in fats and oils discussed in this report be made tentative.

(2) That the collaborative study of the determination of unsaponifiable matter be continued.

REPORT ON PEANUT OIL

By T. H. RIGGS† and GARDNER KIRSTEN (Food and Drug Administration, Federal Security Agency, New York, N. Y.)

In accordance with the recommendations of the A.O.A.C. that studies on a rapid qualitative test for peanut oils in admixture with other oils be

* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 61 (1945). The methods will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

† Associate Referee on Peanut Oil.

initiated (*This Journal*, **25**, 65) a survey of the literature was made, and the modified Bellier test as proposed by Evers[1] was selected for study as being the most promising. The original Bellier test[2] consisted of saponifying the oil with alcoholic potassium hydroxide and adding sufficient acetic acid to neutralize the potassium hydroxide followed by 50 ml. of 70 per cent alcohol containing 1 per cent of hydrochloric acid. The resulting solution was cooled to 17–19°C. and the presence of 10 per cent or more of peanut oil was indicated by the formation of a precipitate. This test was modified by several workers including Franz and Adler,[3] who omitted the hydrochloric acid and also pointed out that the temperature at which the precipitate formed was an index of the amount of peanut oil present. Evers[4] reported good results with the original and the modified test. In 1937, Evers proposed another modification[1] in which the use of acetic acid is omitted and sufficient hydrochloric acid is added to liberate all the fatty acids. The resulting solution is cooled and the turbidity temperature noted. In the case of olive oil, if no turbidity was observed by the time the temperature reached 9°C. peanut oil was regarded as absent. This test has been used successfully as a sorting method in the New York laboratory of the U. S. Food and Drug Administration for several years and has been found to give good results when peanut oil is mixed with cottonseed, corn, and soybean oils as well as with olive oil. The British Pharmacopoeia[5] uses a modification of the Bellier test as a sorting test for the absence of peanut oil.

Evers tested 26 olive oils by his procedure and obtained turbidity temperatures from 4° to 10°C., and only two of them were above 8.5°C. He also examined 13 samples of peanut oil of various origins and grades, all of which gave turbidity temperatures of 39°–40°C. It will be noted that somewhat lower results were obtained in this laboratory for peanut oils. Evers states that when peanut oil is present in olive oil in amounts over 10 per cent the turbidity temperature of the olive oil used does not exert any appreciable influence on the result. He also shows that the turbidity temperature is a reliable quantitative measure of the amount of peanut oil present in mixtures of peanut and olive oil. Experience with this test indicates that this behavior is also characteristic of mixtures of peanut oil with other oils, and further investigation will be made to determine to what extent this is the case.

According to Evers, 5 per cent of peanut oil can be detected in mixtures of peanut and olive oils. In the case of the other oils mentioned, the test will definitely detect 10 per cent of peanut oil and will give positive indications with less than 10 per cent.

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[1] *Analyst*, **62**, 96 (1942).
[2] *Ann. Chem. Anal.*, **4**, 4 (1899).
[3] Z. Unters. Nahr-Genussm., **24**, 676 (1912).
[4] *Analyst*, **37**, 487 (1912).
[5] British Pharmacopoeia," 1932, p. 576. Constable & Co., Ltd., London.

The oils examined and the ranges of their turbidity temperatures are shown in Table 1 and Table 2.

TABLE 1.—*Turbidity temperatures of oils examined*

KIND OF OIL	NO. OF SAMPLES	MAXIMUM	MINIMUM	AVERAGE
		°C.	°C.	°C.
Peanut Oil	29	38.0	34.5	36.1
Olive Oil	25	9.0	3.5	6.2
Corn Oil	9	13.0	8.5	10.4
Soybean Oil	13	12.5	11.0	11.8
Cottonseed Oil	5	13.0	9.5	11.4
Tea Seed Oil	3	2.5	2.0	2.2
Sesame Seed Oil	2	11.0	11.0	11.0
Sunflower Seed Oil	2	18.5	17.0	17.7
Rape Seed Oil	1	—	—	14.5

TABLE 2.—*Turbidity temperatures of mixtures containing 10% peanut oil (turbidity temperature of peanut oil = 36.5°C.)*

OTHER OIL IN MIXTURE	TURBIDITY TEMP. OF PREDOMINANT OIL	TURBIDITY TEMP. OF MIXTURE
	°C.	°C.
Corn Oil	9.5	16.5
Soybean Oil	11.5	17.5
Cottonseed Oil	11.5	15.5
Olive Oil	5.5	15.5

The method will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the detection of peanut oil in the presence of olive, cottonseed, corn, or soybean oils be made tentative.

(2) That the study of the detection of peanut oil in admixture with other oils be continued and that the method be subjected to collaborative investigation.

No report on olive oil was given by the Associate Referee.

* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 61 (1945).

REPORT ON PRESERVATIVES AND ARTIFICIAL SWEETENERS

By MARGARETHE OAKLEY (State of Maryland Department of Health, Baltimore 18, Md.), *Referee*

SULFUR DIOXIDE

Associate Referee.—C. E. Hynds, State Department of Agriculture and Markets, Albany, N. Y.

The Associate Referee was unable to do any work on the subject during the past year and submitted no report.

The Referee recommends that work be continued.

MONOCHLORACETIC ACID

Associate Referee.—J. B. Wilson, Food and Drug Administration, Washington, D. C.

A verbal progress report will be given.

The Referee recommends that work on this subject be continued.

BENZOATE AND ESTERS OF BENZOIC ACID

Associate Referee.—E. B. Boyce, Massachusetts State Health Department, Westfield, Mass.

The Associate Referee made some exploratory determinations of pure parahydroxy benzoic acid, using a bromide-bromate titration method with very good results.

The Referee recommends continuation of this work.

SACCHARIN

Associate Referee.—Margarethe Oakley,

The Associate Referee submitted a report on collaborative work done on pure saccharin; benzoic acid and saccharin; and saccharin in ice cream.

The Referee recommends that work on this subject be continued.

FORMALDEHYDE

Although the subject "Tests for Formaldehyde" was not assigned to an associate referee during the past year, the Referee received correspondence relative to the inclusion of further tests for this substance and was able to make a preliminary survey of the subject.

David Horn of Bryn Mawr, Penn., suggested that the Fulton test[1] for formaldehyde be included in *Methods of Analysis, A.O.A.C.*, and H. M. Bailey suggested that if revision of the tests for formaldehyde were considered the Shrewsbury test[2] should also be included in the group, as it had been found satisfactory in his laboratory and could be made roughly quantitative for low concentrations. The Referee surveyed the seven

[1] *Ind. Eng. Chem., Anal. Ed.*, 3, 199 (1931).
[2] *Analyst*, 32, 5 (1907).

TABLE 1.—*Comparison of qualitative tests for formaldehyde*

NAME OF TEST	ABSTRACT	BLANK	2 P.P.M.	4 P.P.M.	6 P.P.M.	8 P.P.M.	10 P.P.M.	50 P.P.M.	100 P.P.M.	200 P.P.M.	300 P.P.M.	500 P.P.M.	700 P.P.M.	REMARKS
(1) Shrewsbury Analyst **32**, 5	10 ml. milk+7 ml. reagent estimations after 30 min. (reagent=H_2SO_4 +$FeCl_3$)	golden yellow	mauve	lavender	purple	purple	purple	purple	purple	purple	purple	purple	purple	
(2) Fulton Ind. Eng. Chem., Anal. Ed., **3**, 199	4 ml. (5+8) H_2SO_4 +1 ml. milk, cool, +.5 ml. (H_2SO_4 +Sat. Br_2) (1+1), shake	white	pink	lavender	lavender	lavender	violet	purple	purple	red purple, yellow before reagent added	reddish lavender, yellow before reagent added	mauve, yellow before reagent added	mauve, yellow before reagent added	Must have few gtt. milk in sample—alcohol makes no difference
(3) Hehner A.O.A.C.	5 ml. sample, underlay with commercial H_2SO_4	yellow to brown ring	violet ring	violet ring	violet ring	violet ring	violet ring	violet ring	violet ring	violet ring	violet ring	violet ring	violet ring	Milk or other protein needed
(4) $C_6H_5NHNH_2$ · HCl–Nitroprusside A.O.A.C.	$C_6H_5NHNH_2$ · HCl +3 gtt Na Nitroprusside (10%)+10 gtt NaOH (10%)	yellow	yellow green	green fleeting	green fleeting	blue green fleeting	blue green fleeting	blue green	deep blue green	deep blue green	blue black	blue black	blue black	May be masked by other aldehydes
(5) Leach A.O.A.C.	10 ml. sample+10 ml. HCl+$FeCl_3$ Δ80°C.	darkened somewhat blue gray on standing	pale gray	lavender	violet	violet	violet	violet	blue black	blue black	blue black	blue black	blue black	Milk needed. In about 20 min. all samples same blue black
(6) Phloroglucinol A.O.A.C.	underlay sample with reagent	—*	—	—	—	—	faint pink ring	decided pink ring	pink ring	orange red ring	entirely red	entirely red	entirely red	
(7) $C_6H_5NHNH_2$ · HCl+$K_3Fe(CN)_6$ A.O.A.C.	Same as 4, substituting $K_3Fe(CN)_6$ for Na Nitroprusside	—	—	—	—	—	—	—	—	pink	pink	orange red	red red	Not applicable in presence of blood
(8) $C_6H_5NHNH_2$ · HCl+$FeCl_3$ A.O.A.C.	15 ml. sample+1 ml. (1%) $C_6H_5NHNH_2$ · HCl+few gtt $FeCl_3$ (1%)+HCl	—	—	—	—	faint pink after 10 min.	faint pink after 5 min.	pink after 5 min.	pink after 5 min.	pink after 5 min.	pink after 5 min. faded at 15 min.	pink after 5 min. faded at 15 min.	never became pink only yellow	Acetaldehyde or benzaldehyde does not interfere
(9) $C_6H_5NHNH_2$ · HCl+$FeCl_3$ A.O.A.C.	Sample+alcohol (1+1) filter	with milk	—	—	—	—	—	—	—	—	—	—	—	Unsatisfactory for milk
	5 ml. filtrate –$C_6H_5NHNH_2$ · HCl +$FeCl_3$+H_2SO_4	without milk	—	—	—	—	faint pink	bright pink	light red	red on first addition of H_2SO_4	red fading to orange on addition of more than 8 gtt. H_2SO_4	red fading on addition of xs H_2SO_4	red fading on addition of xs H_2SO_4	Regardless of whether more than ¼ ml. H_2SO_4 added or not, more than 200 p.p.m. turned orange in 5 min. Neither acetaldehyde nor benzaldehyde interferes

* Dash—=no reaction, no change.

qualitative tests now official and these other two as suggested, with the results shown in the accompanying table.

In reviewing the references it was found that extensive work was done on formaldehyde in 1907,[3] and in the same bulletin the tests were included in practically the same form as they are today, having been carried through the years from 1907 to 1944 unchanged, and probably unreviewed, as there are no further references to this subject in *The Journal* of the A.O.A.C. during those years.

The Referee considers that it might be wise to replace some of the present official tests, such as the Phenylhydrazin Hydrochloride-Ferric Chloride No. VI, which is practically a duplicate of the Phenylhydrazin Hydrochloride Test No. 1 without the benefit of its adaptation to various types of products, and to include the Fulton test. This test is more sensitive in the lower ranges than any of the present methods, and it would be particularly valuable for samples which were several days old and had lost the greater part of the added formaldehyde, as the sensitiveness of most of the official methods does not reach into the lower ranges. Another advantage of the Fulton test is the clear, sharp, and distinct blank. There is no doubt as to when a sample is negative, and as little as 2 p.p.m. gives a definite positive test.

It is recommended[4] that this subject be reopened, that these nine tests be submitted to collaborative study, and that an entire revision of the qualitative tests for formaldehyde be made, based upon the comments and opinions of the collaborators.

REPORT ON SACCHARIN

By MARGARETHE OAKLEY (State of Maryland Department of Health, Baltimore 18, Md.), *Associate Referee*

For the background of the work on saccharin during the past few years together with a bibliography, see *This Journal*, **25**, 369. The work for this year was directed along three channels, the first and most important being the securing of collaborative work on the sublimation of saccharin, since the foundation for preliminary collaborative work had been laid by the former referee. Two members of the Food and Drug Administration consented to try the sublimation procedure on pure saccharin. The following results were obtained.

The titration of the sublimate does not appear to be sufficiently accurate to be used as a criterion of the purity of the sublimate.

The directions submitted to the collaborators follow:

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[3] *U. S. Bur. Chem. Bull.*, **107**, 121, 183.
[4] For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 62 (1945).

(1) Weigh accurately ca 30 mg. of insoluble saccharin in a sublimator dish containing a little ignited sand.

(2) Moisten with ether so that the saccharin is spread on the sand in a finely divided state rather than left in lumps. Allow ether to evaporate.

(3) Sublime at a pressure of 1–2 mm., raise the temperature of the sublimator slowly so that 135°C. is reached in about 30 minutes, and hold at 140°–160°C. for 60 minutes under the 1–2 mm. pressure. (This may not be long enough for solid saccharin; another hour under same conditions may serve as check for complete sublimation.)

(4) Wash saccharin from bulb into weighed beaker with hot neutral alcohol. Evaporate, and weigh saccharin.

(5) Titrate this saccharin after dissolving in a little alcohol (1 ml. of 0.05 *N* NaOH = 9.15 mg. of saccharin).

(6) Weigh sublimator dish containing the sand.

ANALYST	WT. OF SAMPLE	LOSS IN WT. OF DISH	WT. OF SUBLIMATE		SACCHARIN BY TITRATION	
	mg.	*mg.*	*mg.*	*per cent*	*mg.*	*per cent*
C. A. Wood	30.3	30.3	29.7	98.0	28.1	94.4
	29.4	29.4	29.6	100.7	28.3	95.6
	31.9	31.7	31.7	99.4	29.9	94.3
	39.2				37.9	96.6
C. F. Bruening	34.0		34.0	100.0	29.1	85.6
	31.4		31.7	100.9	26.9	84.9
Referee	31.0	30.5	30.5	98.4	30.0	96.8
	35.0	34.6	34.5	98.6	34.7	99.2
	58.3	57.5	56.9	97.6	54.7	93.8
	25.2				24.8	98.5

These directions were based upon the premise that the Hortvet sublimator would be used, inasmuch as it was employed in the development of the method. However, the type used by the collaborators was a macro sublimator with standard-type joints similar in design to the Werner Klein microapparatus. At the present time at least three of the larger scientific equipment houses list this instrument and not one catalogue offers the Hortvet type. The glassware of the Hortvet sublimator costs about $22.00, and that of the other, $3.50. Each one has certain advantages in ease of handling. Both have been proved to be efficient.

BENZOIC ACID AND SACCHARIN

As one collaborator suggested that the presence of benzoic acid in a sample would interfere, the Associate Referee investigated this problem further. The former associate referee had found that with fractional sublimation, benzoic acid came over at 45°–55°C. and saccharin at 145°–155°C., effecting an efficient separation. The Associate Referee confirmed these findings with solid benzoic acid and saccharin, and with mixtures of the two in various types of soft drinks and obtained the following results:

Saccharin	Benzoic Acid	3 hr. 45°–60°C. pressure 1–2 mm.		3 hr. 145–155°C. pressure 1–2 mm.	
mg.	*mg.*	*mg.* loss in wt.	*mg.* wt. sublimate	*mg.* loss in wt.	*mg.* wt. sublimate
30.6	85.5	85.5	79.5	30.9	30.9

Grape flavored soft drink with saccharin and benzoic acid added

No.	Saccharin	Benzoic Acid	45°–55°C. 1–2 mm.		145°–160°C. 1–2 mm.	
	mg.	*mg.*	*mg.* loss in wt.	*mg.* sublimate	*mg.* loss in wt.	*mg.* sublimate
1	40	10	9.1	6.9	38.7	38.4
2	40	10	11.4	7.1*	36.8	36.5
3	40	200	112	108.4	35.4	35.3
4	40	100	100.8	86.5*	34.7	34.6†
5	40	100		92.9*		39.5†

* Sweet taste.
† Benzoate positive. Hortvet sublimator used except on No. 5.

This problem of a combination of saccharin and benzoic acid, which would be likely in liquid or semi-solid food products, was also treated in another way. To a sample of 200 ml. of cherry soda, 40 mg. of saccharin and 10 mg. of benzoic acid were added. After being acidified with 15 ml. of hydrochloric acid the material was extracted with three 80 ml. portions of ether. The 240 ml. of ether was washed with 50 ml. of 1 per cent sodium hydroxide and discarded. The sodium hydroxide solution was acidified and extracted with two 30 ml. portions of carbon tetrachloride in one case and petroleum benzine in the other. After this the ether extraction was repeated with three 80 ml. portions.

The following results were obtained when to 200 ml. of a soft drink 40 mg. of saccharin and 10 mg. of benzoic acid were added:

CCl_4 extract	11.1 mg.	Ether extract	38.1 mg.
Pet. benzine extract	10.7 mg.	Ether extract	38.4 mg.

For practical purposes fractional sublimation is fairly effective; however, one primary extraction of a sample known to contain both substances with either carbon tetrachloride or petroleum benzine frees the saccharin from benzoic acid and grease so that the ether extract is in a purer state for sublimation.

SACCHARIN IN ICE CREAM

Some work was also done this year on saccharin in ice cream. A Roese Gottlieb type of extraction was used, and a recovery of 66.5 per cent was

obtained. A Soxhlet extraction was tried on ice cream dried on sand. It was planned to extract the fat with petroleum benzine and the saccharin with ethyl ether. Since at least a 50 gram sample is required, the large quantity of sand necessary for this type of determination made the method unwieldy. The recovery here did not justify further work in this direction. There was then used a lead acetate precipitation followed by an ether extraction, as directed for the preparation of the sample for the present official method, with 4 ml. of concentrated ammonium hydroxide substituted for the 5 ml. of acetic acid. By keeping the sample alkaline it was thought the saccharin would remain in a soluble form and appear in the filtrate; however, although the filtrate was clear and the separation of the ether clean and free from emulsion, the recovery was not as good as that by the official method. The lead acetate precipitation with 5 ml. of acetic acid present was then used. Emulsions that formed during the ether extraction were broken by centrifuging. The recovery was not so good as hoped for, probably owing to the inclusion of saccharin with the heavy precipitate of lead soaps from the fat of the ice cream; nevertheless the use of the sublimator method of quantitative estimation gave results that are as good as could be secured by the present official ether-soluble sulfur method. The results are shown in the table.

Saccharin in ice cream

	Saccharin	Sublimate	Recovery of Saccharin
		per cent	*per cent*
HAc	30	29.1	97.0
HAc	24	21.5	89.6
HAc	24	19.3	80.4
NH_4OH	24	17.7	73.8
NH_4OH	24	17.0	70.8

RECOMMENDATION*

In the opinion of the Referee the results of the collaborators warrant the continuation of the study of the sublimation method for the determination of saccharin.

REPORT ON BENZOATE OF SODA AND ESTERS OF BENZOIC ACID

By E. B. Boyce (Massachusetts State Health Department, Westfield, Mass.), *Associate Referee*

Following the lead of an article by W. Diemer and others,[1] the Associate Referee attempted to find out whether benzoic acid and p-OH benzoic acid could be separated with immiscible solvents from a neutral

* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 62 (1945).
[1] *Microchemie*, **25**, 347–355 (1938); *C. A.*, **33**, 822 (1939).

aqueous solution that had been made slightly alkaline by the addition of sodium carbonate. The following results were obtained.

Vol. aqueous solution	Vol. ethyl ether	$NaHCO_3$ added	Benzoic acid present	Benzoic acid extracted
ml.	*ml.*	*ml.*	*mg.*	*mg.*
13	13	3 ml. of 5% soln.	10	2.5
20	20	10 ml. of 5% soln.	10	0.4
20	20	1 gram	10	0.1
			p-OH benzoic acid present	
10	20	1 gram	20	less than 0.14

Apparently the addition of sufficient sodium carbonate to hold the benzoic acid in the aqueous phase will also hold the p-acid.

F. Reimers[2] recommended the determination of the p-acid by a bromide-bromate titration quite similar to the U.S.P. method for the determination of phenol. This method was tried on a sample of p-OH acid obtained from Eastman Kodak Co. (No. 1520) after a preliminary vacuum drying for 4 hours at 45°C. The melting point of this product was 112°–112.5°C. uncorrected.

The following results were obtained:

Amount p-OH acid taken	Amount determined	Purity or recovery
mg.	*mg.*	*per cent*
57.0	56.8	99.6
33.3	32.9	98.8

These results show that there is promise of this method being satisfactory for the determination of the p-acid in the absence of salicylic acid of other phenolic substances. A benzoic acid solution containing 82.7 mg. gave no precipitate with the reagent, and the blank was not affected. It might be well to hydrolyze esters of the p-acid and determine the p-acid.

No report on sulfur dioxide in meats was given by the Associate Referee. See report of the Referee.

REPORT ON MONOCHLORACETIC ACID

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

During the past year the methods for determination of monochloracetic acid have been studied further. Since the monochloracetic acid content of commercial preservatives varies widely (6–60 grams of the acid per 100 ml. has been found) it was considered to be advisable to make a prelimi-

[2] *Z. anal Chem.*, **122**, 404–418 (1941); *C. A.*, **36**, 4058 (1941).

nary determination as a guide to the quantity of sample best suited to the actual analysis.

The preliminary determination should be made as directed below.

DETERMINATION OF MONOCHLORACETIC ACID IN COMMERCIAL PRESERVATIVES

Determine chloride volumetrically in 1 ml. of sample. (Silver monochloracetate precipitates in neutral or alkaline solution but if free HNO_3 acid is present, the chloride may be determined in the usual manner.)

Place 1 ml. of sample in a 250 ml. flask and add 25 ml. of 1 *N* NaOH. Heat mixture on the steam bath for 2 hours or boil under a reflux for 30 minutes. Cool, add 50 ml. of water and 15 ml. of HNO_3. Have two burets containing the standard $AgNO_3$ solution and the NH_4CNS solution, respectively. Add 5 ml. of the $AgNO_3$ solution and 1 ml. of ferric alum indicator. Now add a few drops of the NH_4CNS solution. (If an excess of $AgNO_3$ was present, there will be no change in color; if there is insufficient Ag solution to combine with the chloride, the solution will turn pink.) Add more $AgNO_3$ solution with occasional additions of NH_4CNS if the color fades out, until an excess of $AgNO_3$ has been added. Now titrate the excess of $AgNO_3$ with NH_4CNS solution. Correct the titration for the quantity of chloride found above and calculate the approximate quantity of $CH_2ClCOOH$ in the sample.

Prepare a dilution of the sample that will permit the measurement of a convenient aliquot containing 50–100 mg. of monochloracetic acid and determine as directed in Method I (*This Journal*, **27**, 199).

In conducting the qualitative test upon commercial preservatives, it has been found possible to shorten somewhat the time required, since sodium chloride is usually the only other crystalline substance present and it does not interfere with the crystallization of the barium monochloracetate. The procedure is described below.

MONOCHLORACETIC ACID IN COMMERCIAL PRESERVATIVES AS BARIUM MONOCHLORACETATE

Pipet 1 ml. of a saturated solution of $Ba(OH)_2$ into a 50 ml. beaker and add 1 or 2 drops of phenolphthalein indicator. Now add the sample dropwise from a pipet until the solution is colorless. (This requires 2–3 drops of a highly concentrated preservative and only 0.6–0.7 ml. of the weakest preservative found so far.) Allow the mixture to evaporate spontaneously and identify the crystals microscopically as directed in *This Journal*, **27**, 446.

If the laboratory is not equipped for examination of the crystals by the immersion method, or it is desired to check the results by a chemical method, apply the indigo test as described below.

Indigo Test

REAGENTS

(a) *Anthranilic acid.*—Dissolve 1 gram of anthranilic acid in 40 ml. of water, add 0.3 gram of NaOH, and make up to 50 ml.

(b) *Caustic soda solution.*—Dissolve 10 grams of NaOH in 10 ml. of water and filter if necessary.

PROCEDURE

Dissolve 0.17 gram of the barium salt in 5 ml. of water in a 10 ml. graduate, add 1.05 ml. of 1 *N* H_2SO_4, make up to 10 ml. and mix. Allow mixture to stand until the precipitate settles, or filter if preferred. Pipet 3 ml. of the clear liquid into a small beaker, and add 2 ml. of anthranilic acid reagent and 30 mg. of Na_2CO_3 (weighed). Test with litmus paper. If acid, add an additional 30 mg. of Na_2CO_3. Pour into a test tube and heat in a water bath for 30 minutes. Place the test tube

in an oven at 125° ± 5°C. until only a moist residue remains. Remove from the oven and add 2 drops of caustic soda solution directly upon the residue. (If the residue is entirely dry, add 1–2 drops of water and let stand until water is absorbed by the residue before adding the strong NaOH.) Return to the oven until completely dry (at least 1 hour). Remove from the oven and heat the test tube at 310°–320°C. until the contents assume an orange color. (This requires 15 seconds to 2 minutes, but the tube must be carefully watched and removed from the heat as soon as the reaction is complete.) Cool slightly add 5–7 ml. water from a wash bottle, splashing the water to incorporate air into it. Warm over a flame and blow air through the solution 1–2 minutes, using a pipet or glass tube. Heat to boiling over the flame and again blow air through the solution. (As the oxidation progresses, the solution turns red if monochloracetic acid is present, then green or blue or a combination of the two, and finally solid particles of indigo separate out. They have a tendency to rise to the surface at first.) Let the mixture stand ca. 10 minutes, then acidify slightly with HCl (1+1). After allowing to stand for an additional 30 minutes filter and wash the precipitated indigo with water to remove acid. Allow the paper to dry in the air and preserve as an exhibit.

During the past year a number of samples of preservatives have been analyzed by two chemists with very good agreement. The method for determination of monochloracetic acid in beverages has also been applied with equally good results to beverage bases, India relish and salad dressings. In the last instance it was found advisable to mix 20 ml. of H_2SO_4 (1+5) with 50–100 grams of salad dressing before placing it in the extractor and make the volume to approximately 150 ml. with water.

It is recommended that study of methods for the determination of monochloracetic acid be continued.

REPORT ON SPICES AND OTHER CONDIMENTS

By SAMUEL ALFEND (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Referee*

Associate Referee Henry submitted recommendations concerning changes in the vinegar methods in anticipation of the publication of the new edition of *Methods of Analysis*. Some of these were editorial in nature and were embodied in the Referee's communication to the revision Committee.

Henry recommends combining paragraphs **56** and **87**, which relate to the organoleptic examination of vinegar. Since one heading is official, and the other tentative, the Referee recommends that the combined method be made official.

Henry's recommendation that the method for specific gravity be dropped is predicated on the assumption that the purpose of the determination was to enable the analyst to calculate the percentage of the various constituents from the weight per given volume. The Referee is not aware of any practical use for the specific gravity figure, and therefore concurs in the recommendation to drop the method.

The method for formic acid was adopted many years ago for the detection of the presence of commercial acetic acid, which was then prepared by destructive distillation of wood. Acetic acid prepared by present methods apparently does not contain formic acid, so there appears to be no use for the determination. The permanganate oxidation number is the value now used in detecting addition of commercial acetic acid. The Referee agrees that the formic acid method should be dropped.

As a result of critical consideration of the methods for "Free Mineral Acid," Henry suggested changes in the tentative "Logwood Method," and has proposed that the "Quantitative Method" be studied further. It is obvious that the latter method is not accurate, since it takes no account of the organic alkali salts in the vinegar. This error is negligible in distilled vinegar, but not so in cider vinegar, where the error might be 5 per cent of the total acidity. The estimated amount of free acid will be low by an amount equivalent to the alkalinity of the ash. It would be well to investigate the substitution of *p*H value for the color and titration tests for free mineral acids. This would entail a determination of the normal *p*H range of different types of vinegar, and the effect of addition of mineral acids.

Henry has commented on the brevity of directions in the qualitative test for dextrin. The Referee sent a more detailed procedure to the Revision Committee, which is recommended for adoption as tentative. The details will be published in the 6th edition of *Methods of Analysis, A.O.A.C.*, 1945.

MUSTARD

Associate Referee Garfield submitted a progress report on starch in mustard. His results are promising enough to warrant further study. No work was done on ash or volatile oil in prepared mustard. The present method for ash does not work, because a carbon-free ash cannot be obtained at 525°C., and because some salt is lost during the operation. The method should be dropped, and the subject studied further.

MAYONNAISE AND SALAD DRESSINGS

Associate Referee S. D. Fine, who is now in the Navy, did not submit a report. An associate referee should be appointed to carry on the work.

In a personal communication F. L. Hart has pointed out the omission, in the method for reducing sugars after inversion in mayonnaise, **XXIII, 47,** of directions for neutralizing the acid solution before reduction of copper. This omission has been corrected in a note to the Revision Committee.

VOLATILE OIL IN SPICES

No report was submitted by the Associate Referee on Volatile Oil in Spices.

The Referee's report for 1939 discussed the shortcomings of the method,

particularly as applied to spices rich in volatile oil. It is now recommended that the parenthetical statement "(Not suitable for spices high in volatile oil, such as cloves") be inserted after the heading.

RECOMMENDATIONS*

It is recommended—

(1) That the tentative method for the detection of caramel in vinegar (*This Journal*, **26**, 234) be further studied collaboratively with a view to its adoption as official.

(2) That the tentative permanganate oxidation number (*This Journal*, **27**, 101) be studied collaboratively.

(3) That the official method for determination of specific gravity in vinegar (**58**, p. 478) be dropped (final action under suspension of the rules).

(4) That the official method for formic acid in vinegar (**79**, 482) be dropped (final action under suspension of the rules).

(5) That the official method for physical examination and the tentative method for spices and added pungent materials (**56**, **87**, pp. 478, 483) be combined under one heading "Organoleptic Examination" and that the combined method as described in the associate referee's report be made official (final action under suspension of the rules).

(6) That the tentative logwood method for determination of free mineral acids in vinegar (**82**, 482) be modified as recommended in the associate referee's report, and that further consideration and study be given to the methods for free mineral acids.

(7) That the tentative qualitative test for dextrin in vinegar (**86**, 483) be modified as described in the report of the referee.

(8) That the official qualitative test for tartrates (**80**, 482) be further studied.

(9) That studies be continued on methods for the determination of starch in salad dressing.

(10) That studies be continued on methods for the determination of starch in prepared mustard and mustard flour.

(11) That the official method for ash in prepared mustard be dropped (final action under suspension of the rules).

(12) That studies be made of a suitable method for the determination of ash in prepared mustard.

(13) That the official method for the determination of volatile and non-volatile ether extract in spices be modified by the insertion of the following caution immediately under the heading: "(Not suitable for spices high in volatile oil, such as cloves)."

(14) That the tentative method for determination of total ash in spices (*This Journal*, **24**, 83) be made official (final action under suspension of the rules).

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* For report of Subcommittee C and action by the Association, see *This Journal*, **28**, 62 (1945).

REPORT ON VINEGAR

By A. M. HENRY (Food and Drug Administration, Atlanta, Ga.),
Associate Referee

The Associate Referee on Vinegar recommends*—

(1) That paragraphs 56 and 87 be combined and the wording be changed to read as follows:

ORGANOLEPTIC EXAMINATION—OFFICIAL

(Applicable to vinegars and spices, and added pungent materials.)

Note appearance, color, odor, and taste. Neutralize portion of sample with NaOH solution and note odor and taste. Extract neutralized vinegar with ether, evaporate ether extract, and note odor and taste of residue. Detect spices and pungent materials by their characteristic odors and tastes. Evaporate portion of sample on water bath. Odor of material as last of volatile matter evaporates and appearance and taste of residue give information as to source and character of vinegar.

(2) That paragraph 57 be changed to read as follows:

PREPARATION OF SAMPLE—OFFICIAL

Mix thoroughly and filter through rapidly acting paper before proceeding with analysis.

(3) That paragraph 58, "Specific Gravity—Official," be deleted as results are now expressed as "grams per 100 ml," and it has little, if any, use.

(4) That paragraph 78 be amended by deleting in the first line the words "the absolutely clear." This is taken care of in preparation of sample.

(5) That paragraph 79, "Formic Acid—Official," be deleted, as this method has little value since adoption of the permanganate oxidation number.

(6) That paragraph 82, 1. Logwood Method—Tentative, be changed to read:

FREE MINERAL ACIDS

Logwood Method

Prepare extract of logwood as follows: Pour 100 ml. of water upon 2 grams of unused logwood chips, allow infusion to stand for several hours, and filter. Place 3–4 drops of liquid in depression of porcelain spot plate and dry on water bath. Add to residue 1–2 drops of sample and allow to stand 2–3 minutes. Do not allow complete evaporation. Yellow tint remains if free mineral acids are absent, red tint if they are present. Test is improved if sample is first decolorized by treatment with Nuchar or similar vegetable carbon and filtration.

(7) That paragraph 83, II.—Methyl Violet Method—Tentative, be amended by changing in line 2 "Methyl Violet" to "Methyl Violet C. I. 680 or S. & J. 451."

(8) That the caramel method be put under coloring matters.

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* For report of Subcommittee C and action by the Association, see *This Journal*, 28, 62 (1945).

(9) That the permanganate oxidation number be studied collaboratively.

(10) That the qualitative test for tartaric acid and tartrates be further studied.

(11) That the Quantitative Method—Tentative, under Free Mineral Acids be further studied.

(12) That color in vinegar be further studied.

No report on salad dressings was given by the Associate Referee.

No report on volatile oil in spices was given by the Associate Referee.

No report on moisture and ash in spices was given by the Associate Referee.

REPORT ON STARCH IN MUSTARD

By FREDERICK M. GARFIELD (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

It was recommended by the Association that the tentative method for the determination of starch in prepared mustard (*This Journal*, **24**, 700, and **25**, 97, 705) be further studied in its application to mustard flour, and that collaborative work on both prepared mustard and mustard flour be undertaken.

During a routine check on starch methods, as a prelude to this work, two reports were found which seemed desirable to review: (1) Fine's modification of the tentative method for starch in mustard, as applied to mayonnaise and salad dressing (*Ibid.*, **27**, 260); and (2) the Whale volumetric iodide method (*Analyst*, **63**, 328, 441 and **64**, 588).

The mayonnaise method differs essentially from that for mustard in that it provides for neutralization of any acidity in the sample before solution of the starch content in a boiling calcium chloride solution. This modification should prevent possible hydrolysis and subsequent loss of starch during this step. Other modifications are manipulative changes in the procedure.

The Whale procedure treats the sample with 8 per cent alcoholic potassium hydroxide to remove interfering substances (such as fat and protein). The starch is then taken into solution in a 0.7 per cent aqueous potassium hydroxide solution and recovered and purified by a double precipitation with iodine. The starch is determined indirectly by titration, with thiosulfate, of the iodine in the starch-iodine complex. The isolation and purification procedures appear to have two advantages over the two A.O.A.C. methods: (1) No correction for moisture in the sample is necessary (this would be important if the sample were a prepared mustard and the starch content comparatively high); and (2) no correction for acidity

need be made. On the other hand the actual titration is dependent on a number of variables, such as quantity of starch, excess iodine, etc., which makes this portion of the procedure of questionable value.

Several recovery experiments were made by the Whale isolation and purification procedures. (Only single precipitations were made with iodine, although two are called for by the method.) The starch-iodine complex was not titrated with thiosulfate but decomposed with an alkaline alcoholic solution (70 ml. alcohol plus 25 ml. 0.1 *N* NaOH). The starch was hydrolyzed, and dextrose was determined by the Munson-Walker method. Two determinations and two blanks were made by the mayonnaise method. The results are given in the table.

Results by modified Whale method

	DRY MUSTARD USED	STARCH USED (100%)	STARCH RECOVERED	BLANK	STARCH RECOVERED CORR. FOR BLANK	RECOVERY
	gram	*gram*	*gram*	*gram*	*gram*	*per cent*
(1)	0	0.2094	0.2038	—	0.2038	97.3
(2)	0	0.2094	0.2063	—	0.2063	98.5
(3)	0.7500	0.2094	0.2149	0.0058	0.2091	99.9
(4)	0.7500	0.2094	0.2149	0.0058	0.2091	99.9
(5)	2.0000	0.2094	0.2308	0.0256	0.2052	98.0
(6)	2.0000	0.2094	0.2326	0.0256	0.2070	98.9
			Mayonnaise method			
(1)	0.7500	0.2094	0.2088	Negligible	0.2088	99.7
(2)	0.7500	0.2094	0.2052	Negligible	0.2052	98.0

The recoveries by both methods are good. The blank on dry mustard by the Whale method appears to be quite high. A lower blank might be obtained if the double iodine precipitation as called for in the original method were used.

No work was done on prepared mustard.

No work was done on volatile oils in mustard seed.

It is recommended* that studies on the method for starch in prepared mustard and mustard flour be continued.

APPOINTMENTS

E. W. Meyers, Hershey Chocolate Company, Hershey, Penn., Associate Referee on Maltose in Cacao Products.

E. F. Steagall, Food and Drug Administration, Washington, D. C., Associate Referee on Stability of Fats.

R. H. Carter, Bureau of Entomology and Plant Quarantine, Beltsville, Md., Associate Referee on DDT in Foods.

Urban Oakdale, Food and Drug Administration, Chicago, Ill., Associate Referee on Phenothiazine, and on Phosphorus, Calcium, and Iron in Vitamin Preparations.

Iman Schurman, Food and Drug Administration, Chicago, Ill., Referee on Miscellaneous Drugs.

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* For report of subcommittee C and action by the Association, see *This Journal*, 28, 63 (1945).

CONTRIBUTED PAPERS

TITRATIVE DETERMINATION OF THE NEUTRALIZATION VALUE OF CALCIUM SILICATE SLAGS*

By W. M. SHAW (The University of Tennessee Agricultural Experiment Station, Knoxville)

Investigations at the Ohio (*1, 11*) and Pennsylvania (*12*) agricultural experiment stations demonstrated that blast furnace slag is an effective liming material. The merits of the distinctive quenched calcium silicate from the phosphate-reduction furnace have been demonstrated recently through studies conducted cooperatively by the University of Tennessee Agricultural Experiment Station and the Tennessee Valley Authority (*4, 5*). This slag serves as an effective liming material and as a carrier of P_2O_5 (*6*).

The war-time urge for increased crop production has resulted in a marked increase in the use of these two types of liming materials. Unfortunately, there has been no direct procedure for the evaluation of the neutralization value of slags. Although appropriate for other liming materials, the A.O.A.C. procedure (*2, 13*) proved inapplicable to slags and registered deviations as high as 35 percent of the values computed from determined Ca+Mg content. To ascertain precisely the neutralization value of silicate slags, it has been necessary to determine calcium, magnesium, P_2O_5, and fluorine content and correct for calcium attributable to phosphatic and fluoride combinations (*6, 13*). Since these determinations require more time than is admissible in the control laboratory, it seemed imperative to evolve a direct titrative procedure for the determination of the neutralization value of slags. The Associate Referee on Liming Materials, therefore, undertook a study of the factors that affect the accuracy of the titrative determination of the neutralization value for silicate slags of both types.

FACTORS THAT AFFECT THE ACCURACY OF DIRECT TITRATION OF SLAGS

One factor is that of completeness of dissolution of the analytical charge by the titratant acid within the period prescribed by the A.O.A.C. procedure for liming materials. Another factor is the acid-base balance of incidental components at the titration end point. For example, when the slag solution is titrated to the methyl orange end point, one-third of the phosphate ion will be neutralized, and two-thirds of the original calcium phosphate will be registered as basic. But when the solution is titrated to the phenolphthalein end point, approximately two-thirds of the phosphate radical will be neutralized and only one-third of the original phosphate

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C. October 25 and 26, 1944.

will be accorded basic value. Findings in the research laboratories of the National Slag Association led to the suggestion that the substitution of methyl orange for phenolphthalein would give a more exact evaluation in the titration of blast furnace slags (*13*).

Another vitiative factor is the evolution of hydrogen sulfide during the analytical digestion of the slag. The sulfide sulfur content of the blast furnace slags may reach the equivalence of 5 percent of calcium carbonate. The factors considered in the investigation of titration procedure for calcium slags were, therefore: (a) Completeness of the dissolution of analytical charge, (b) the end point that registers the neutralization equivalence of effective base content, (c) disturbance in neutralization equivalence due to incidental components, and (d) volatilization of sulfide sulfur.

MATERIALS USED

The quenched glassy material from the rock phosphate reduction furnaces at Wilson Dam (*4*) and analyzed blast furnace samples supplied by the National Slag Association Research Laboratories were used in this

TABLE 1.—*Partial analysis of calcium silicate slags used in the present study*
(Results expressed as per cent)

SLAG TYPE	SLAG NO.	SiO_2	Al_2O_3	Fe_2O_3	MnO	CaO	MgO	S	F	P_2O_5	$CaCO_3$≈ or Ca+Mg
Blast furnace[a]	1	34.16	13.28	0.50	2.11	46.14	1.84	1.67			86.69
	2	33.68	16.03	.75	1.44	42.19	3.64	1.76			84.13
	3	35.78	11.17	.37	1.56	43.68	4.63	1.14			89.23
	4	34.98	13.41	.75	.92	41.76	5.64	1.61	Traces	Traces	88.32
	5	35.24	15.24	.62	1.80	41.04	4.10	1.35			83.22
	6	33.92	21.01	.65	1.58	37.62	3.68	1.54			76.09
	7	35.88	12.23	.75	1.38	42.01	5.24	1.24			87.78
	8	37.32	10.17	.75	1.62	43.24	4.26	0.76			87.53
Phosphate furnace[b]	349	35.8				54.3			3.17	1.38	85.6[c]
	693	39.9				52.1			2.30	1.30	84.2
	795	34.9				52.7			3.20	1.25	83.0
	920	37.9	8.0			52.8		Not determined		0.20	93.8
	921	36.6	8.1			51.7			2.39	0.20	85.5
	1053	36.7				50.8			2.25	0.93	82.8
	1058	36.9				52.2			2.31	2.30	82.2
	1083	39.2	6.6			50.4			1.87	1.40	82.1
	1084	38.7	7.0			50.2			1.92	1.30	81.8
Fused $CaSiO_3$[d]		48.4	0.60			50.6					90.3

[a] Data for Nos. 1 to 8 were supplied by H. T. Williams, National Slag Association Research Laboratory. These data were modified by analytical values submitted after the completion of the present text. The corrected analyses were more in accord with the analytical values obtained in the present study.
[b] Data for rock phosphate furnace slags were taken mostly from Bulletin 184, University of Tennessee Agricultural Experiment Station, 1943. The $CaCO^3$-equivalences were recalculated from those data.
[c] Corrected for F_2 and PO_4 equivalences.
[d] From a mixture of marble and quartz.

study. The inclusion of both types of slags was fortunate, since findings from one type would not be applicable to the other. Fusions of calcium silicate slag were used as controls. Partial analyses of the slags are given in Table 1. The phosphate furnace slag is characterized by P_2O_5 and fluorine content, whereas the blast furnace slags carry appreciable quantities of magnesium, manganese, and sulfur.

DISSOLUTION OF BLAST FURNACE SLAGS BY TITRATANT ACID

The A.O.A.C. method prescribes a 5-minute boiling of a 1-gram charge of limestone in 50 ml. of 0.5 *N* nitric acid. In this laboratory, however, it has been customary to digest a 0.5-gram charge with 25 ml. of 0.5 *N* acid, and this technic was applied to eight blast furnace slags with 25 ml. and with 35 ml. of 0.5 *N* hydrochloric acid in 250 ml. Erlenmeyers and back titration with 0.5 *N* sodium hydroxide to *p*H 6.5 against bromothymol blue. The flasks were provided with short reflux funnels and also with reflux condensers, which permitted vigorous boiling. It should be stressed that *it is essential to keep the charge in continuous suspension after the addition of the acid, to prevent the cementation of the slag particles*, particularly during the heating period.

TABLE 2.—*Effect of quantity of titratant acid and of digestion technic upon the dissolution of blast furnace slags in terms of* $CaCO_3$*
(Results expressed as percent)

SLAG NO.	IN 25 ML. OF 0.5 *N* ACID			IN 35 ML. OF 0.5 *N* ACID		
	WITH REFLUX CONDENSER	WITH FUNNEL	DIFFERENCE	WITH REFLUX CONDENSER	WITH FUNNEL	DIFFERENCE
1	83.8	80.3	3.5	86.8	86.2	.6
2	80.8	75.4	5.4	82.6	82.6	.0
3	89.0	85.2	3.8	88.8	87.5	1.3
4	84.5	80.5	4.0	83.5	83.1	.4
5	81.5	80.1	1.4	82.9	81.8	1.1
6	83.8	80.6	3.2	84.8	85.2	−.4
7	87.5	85.2	2.3	87.0	86.4	.6
8	85.0	84.0	1.0	83.8	83.5	.3
Average	84.5	81.4	3.1	85.0	84.5	.5

* Titrated to *p*H 6.5 against bromothymol blue indicator.

The data of Table 2 show that a 5-minute gentle boiling in 25 ml. of 0.5 *N* acid failed to effect complete dissolution of the 0.5-gram charge of slag, although the acid was 30 percent in excess of the base equivalence. Near-complete dissolution was attained, however, by the more vigorous 5-minute reflux digestion. When 35 ml. of the acid was used, concordant values were attained by reflux digestion, and by gentle boiling under a

funnel guard. Five minutes of gentle boiling and the conventional 25 ml. of acid proved inadequate. It is evident, therefore, that the conditions appropriate for other liming materials are not adequate for a corresponding charge of silicate slag.

The values in Table 2, obtained by titrations to *p*H 6.5, are merely indicative of the degrees of dissolution effected by the digestions, rather than of the ultimate values. It is apparent, however, that with few exceptions, the values obtained by titration to *p*H 6.5 were in accord with the analytical values given for blast furnace slags in Table 1.

POTENTIOMETRIC TITRATION OF HYDROCHLORIC ACID SOLUTIONS OF SLAGS

Since the acidic solutions of slags constitute complex systems, it was not expected that distinct transition points would be established by the plotting of the *p*H values against titrative increments of sodium hydroxide. It was hoped, however, that an integration of other analytical data with the assemblage of potentiometric titration curves would reveal whether a specific *p*H would register the titration equivalence point for slag solutions in general.

Solutions of eight blast furnace slags, and five of phosphate furnace slags, were obtained by the dissolution of 0.5 gram per 35 ml. of 0.5 *N* hydrochloric acid. These solutions were diluted to 80 ml. and back titrated with 0.5 *N* sodium hydroxide. Titration curves representative of the two types of slag are given in Figures 1 and 2, in which ml. of 0.5 *N* sodium hydroxide of the back titrations are given as the abscissae. At a given point on the curve, the acid neutralized by the charge is registered by the difference between 35 ml. of acid and the ml. of sodium hydroxide of the back titration. Multiplied by five, this difference expresses the neutralization value of the sample, in terms of percentage calcium carbonate equivalence, for any particular *p*H on the curve.

Blast furnace slags.—The 10 ml. sodium hydroxide back titration was taken as the first point on the curves of Figures 1 and 2. This is of particular importance, since that point should connote the specific *p*H value, had the charge been dissolved in 25 ml. of 0.5 *N* acid, rather than in the 35 ml. actually used. The *p*H value at this point was found to range between 2.0 and 3.3, which corresponds to an H-ion concentration range of 0.01 *N* to 0.0005 *N*. The respective acid equivalences of these H-ion concentrations in 100 ml. of solution correspond, respectively, to 2.0 ml. and 0.1 ml. of 0.5 *N* acid, whereas the joint calcium and magnesium content indicates that the excess should have been about 7 ml. of 0.5 *N* acid. The acid in the 25 ml. of 0.5 *N*, beyond that used in the neutralization of the calcium and magnesium content of the charge, was immobilized in the formation of chlorides of aluminum, manganese, and iron. The overall equivalences of these chlorides may be as much as 40 percent of the joint

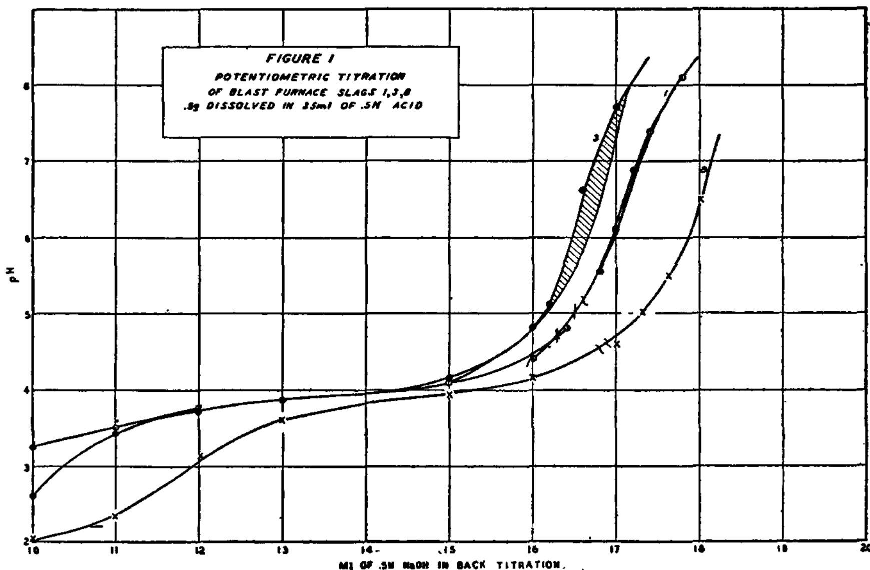


FIGURE 1
POTENTIOMETRIC TITRATION
OF BLAST FURNACE SLAGS 1,3,8
.5g DISSOLVED IN 35ml OF .5N ACID
pH
Ml OF .5N NaOH IN BACK TITRATION.

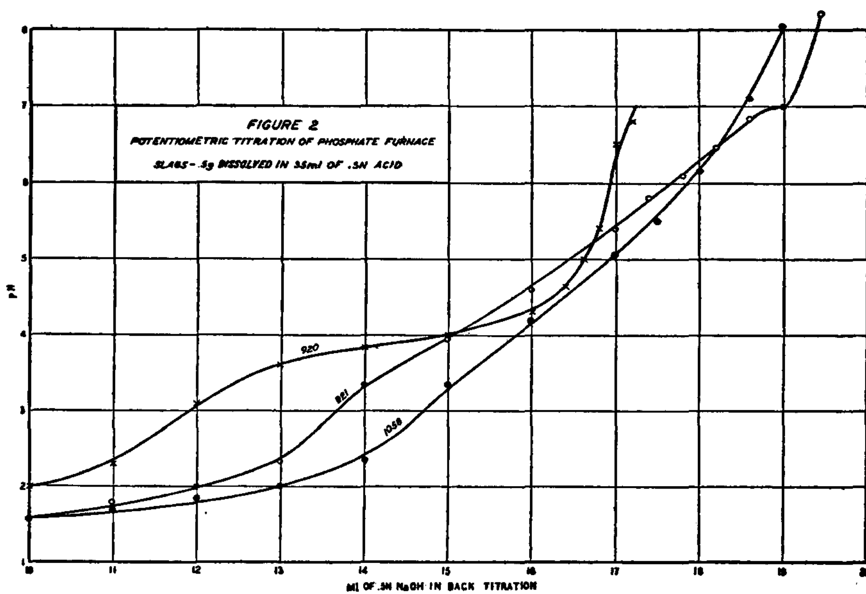


FIGURE 2
POTENTIOMETRIC TITRATION OF PHOSPHATE FURNACE
SLAGS - .5g DISSOLVED IN 35ml OF .5N ACID
920
981
1058
pH
Ml OF .5N NaOH IN BACK TITRATION

equivalence of the calcium and magnesium content of the slag. This explains why 25 ml. of 0.5 *N* acid proved inadequate to effect speedy and complete dissolution of the 0.5-gram charges of the slags.

With an appreciable excess of acid, as in curve 8 of Figure 1, there is a clear indication of an inflexion point between the neutralization of the initial acid and that derived from the hydrolization of solute aluminum chloride, which proceeds slowly and uniformly in the region between *p*H 3.6 and 4.2. The length of the straight line that represents the highly-buffered zone of the curve is directly proportional to the sesquioxide content of the charge. A 5-percent variation in neutralization within this region produces a change of only 0.1–0.2 *p*H in the system. When the titrated system goes from a *p*H of 4.2 to *p*H 8.0, the curve assumes a sigmoid contour with a well-defined transition point between pH 6 and 7. The inflexion point at *p*H 6.5 could be assumed to register complete hydrolization of the aluminum chloride, and taken as the equivalence point of the sytem. The previously mentioned titrations to *p*H 6.5 against bromothymol blue were based on indications given by the exploratory potentiometric titration curves of some of these slags. The significance of this inflexion point in relation to the true neutralization value of blast furnace slags will be discussed further.

Certain pertinent observations should be made to assure reproducibility of results by the proposed procedure. The *p*H values attained by titrations of slag solutions with sodium hydroxide were variant in their constancy along different regions on the curve. The readings are most constant in the acid range up to about *p*H 4.6, beyond which instability becomes more noticeable. The *p*H drift is involved, and is the resultant of two divergent trends. Upon the addition of each sodium hydroxide increment to the system, the inherent trend is from the new high *p*H to a progressively lower *p*H attendant upon gradual attainment of equilibrium between liquid and solid phases. Initially, this tendency is counteracted by the lag in the change recorded by the glass electrode. Consequently, the readings show an early drift towards a higher *p*H and a subsequent reversal towards lower values. The recorded readings were made one minute after *p*H maxima had been observed. Variance to be expected between *p*H values, as obtained by the above procedure and as obtained by 5 minute delay in readings, is indicated by the shaded portion of curve 3, Figure 1.

Phosphate furnace slags.—The titration curve for slag No. 1058 in Figure 2 is characteristic for the type of slag from phosphate reduction furnaces. The other two curves are representative of prepared slags: slag No. 920 contained neither fluorides nor phosphates, whereas slag No. 921 contained fluorides but no phosphate. It is obvious that incidence of fluorides exerted a decisive effect upon the configuration of the titration curves. The curve for the fluorine-free slag, No. 920, is virtually identical to the curves shown for the blast furnace slag in Figure 1, whereas the

one for the fluoride-bearing slag, No. 921, is virtually devoid of the characteristic transition points. The slope of the curve above *p*H 7 changes suddenly toward the horizontal, and then becomes almost vertical. The curve of the phosphate furnace slag, No. 1058, is similar to that of No. 921, except that the upward sweep between *p*H 3.4 and *p*H 8.0 is slightly concave. The titration curves for phosphate furnace slags, Nos. 795 and 1084, have shown characteristics identical to those for slag No. 1058 of like origin.

It is evident that the potentiometric titration curves of slags that contain fluorides, alone or with phosphate, register no characteristic inflexion points within the *p*H range of 3 to 8, and such titration will given no indication of the neutralization capacity of such slags.

POTENTIOMETRIC TITRATIONS OF SLAG COMPONENT COMBINATIONS

To obtain a better insight as to the effect of slag composition upon the titration curve and upon equivalence *p*H, titrations were made on simple systems, combinations of such systems, and systems altered by additions of minor components of possible influence upon the titrations. The simplest system was a combination of lime and silica only and was obtained by a 1600°C. fusion of C.P. lime and pure quartz. The melt was quenched and ground to pass a 100-mesh sieve. A 0.5 *N* solution of aluminum chloride was used as a source of aluminum in acidic Ca-Al-Si systems. A 0.5 percent solution of sodium fluoride was used to afford variant concentrations of fluoride, and the phosphate was provided through highly purified calcium phosphate.

The curves for the separate titrations of calcium silicate, aluminum chloride, and of hydrochloric acid are given in Figure 3. The effects that variant ratios of silica and alumina exert upon the titration curves, and upon the equivalence end point, are shown in Figure 4, and the influence of the variant additions of fluoride to calcium-alumino silicate mixtures are graphed in Figure 5.

Titration of calcium silicate solutions.—The data plotted to curve *A* of Figure 3 represent the 0.5 *N* sodium hydroxide back titration of the solution of 0.5 gram of calcium silicate in 25 ml. of 0.5 *N* hydrochloric acid. At the neutralization point, the system contains calcium chloride, sodium chloride, and silicic acid, part of which is solvated and part is in suspension. Apparently, the incidence of silicic acid exerted no detrimental effect upon the sharpness of the titration end point. The inflexion of the curve appears to be at *p*H 5.5, which corresponds to a 90 percent neutralization value for the silicate. A deviation of 0.5 *p*H at this point would induce a variation of only 0.1–0.2 percent in terms of calcium carbonate. The sensitiveness of the titration end point admits of the accurate determination of the neutralization value of calcium silicates, when incidence of sesquioxides is only a fraction of a percent.

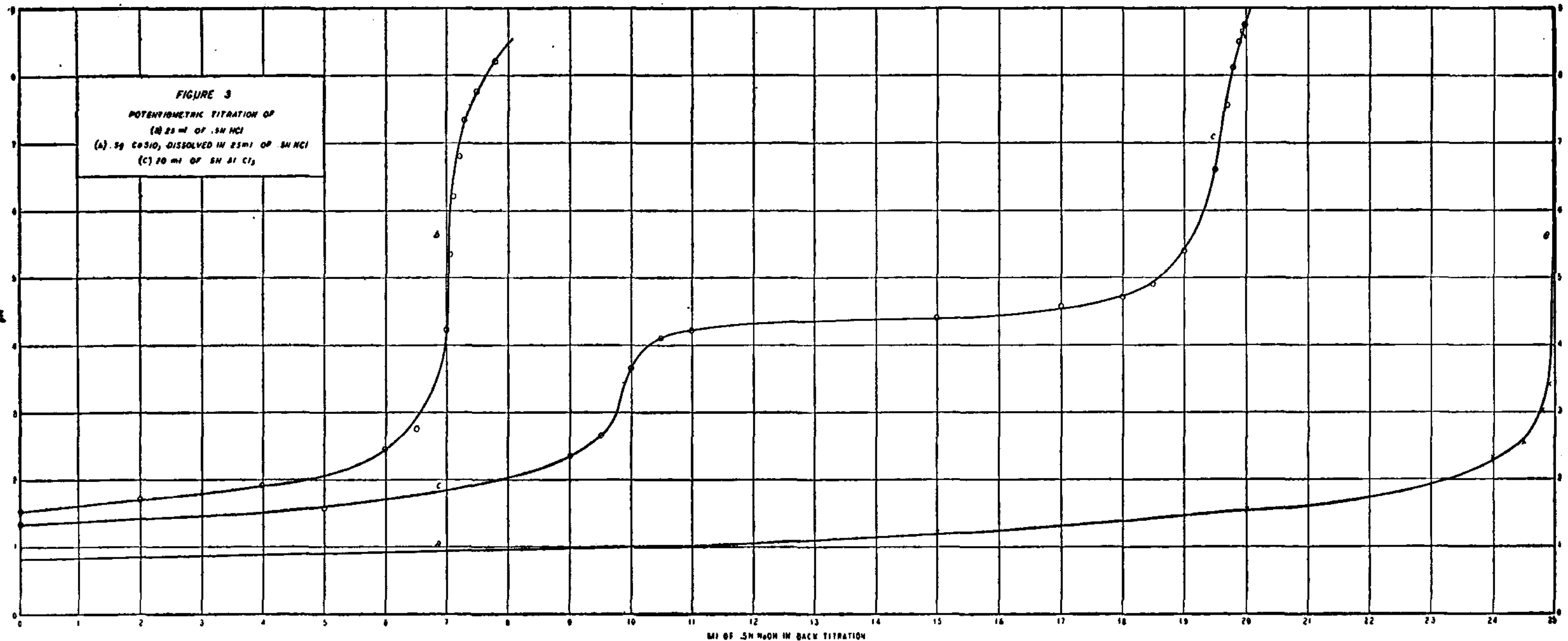


FIGURE 3

POTENTIOMETRIC TITRATION OF

(a) 25 ml OF .5N HCl

(b) .5g $CaSiO_3$ DISSOLVED IN 25ml OF .5N HCl

(c) 20 ml OF 5N $AlCl_3$

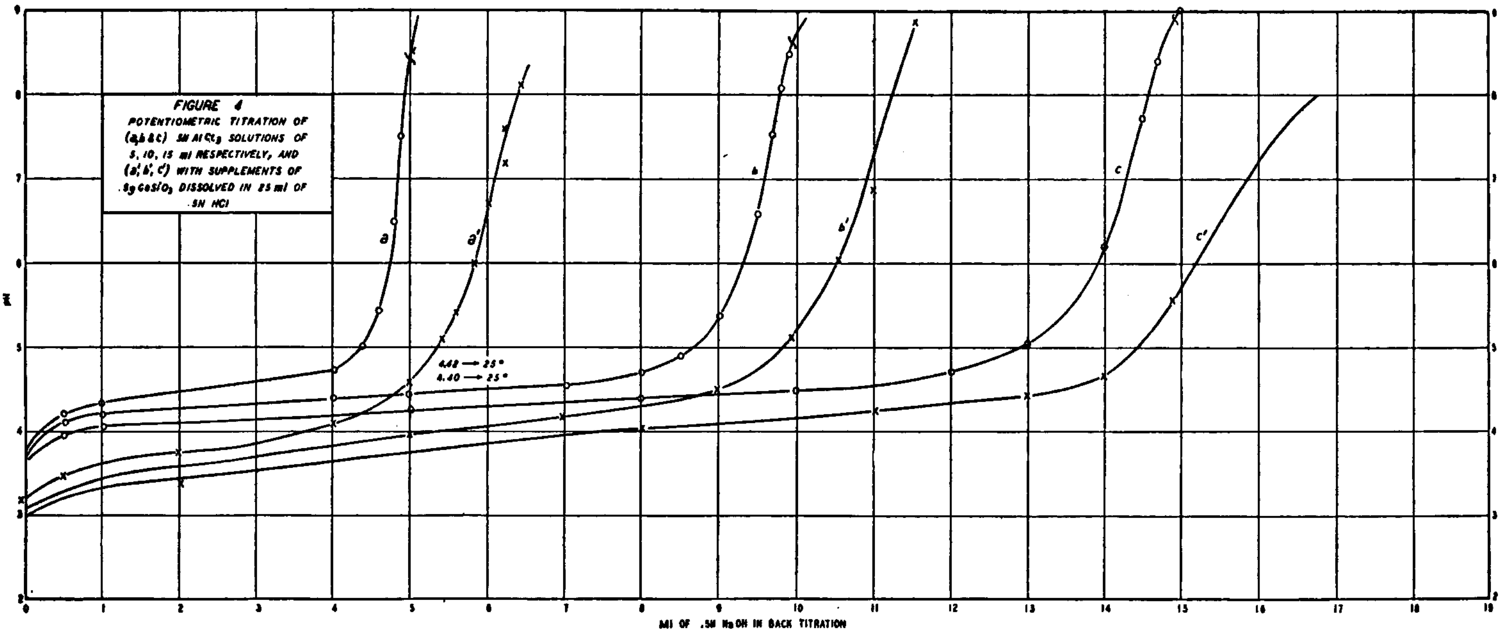


FIGURE 4
POTENTIOMETRIC TITRATION OF (a,b & c) .5M AlCl3 SOLUTIONS OF 5, 10, 15 ml RESPECTIVELY, AND (a', b', c') WITH SUPPLEMENTS OF .8g CaSiO3 DISSOLVED IN 25 ml OF .5N HCl
4.48 → 25°
4.40 → 25°
a
a'
b
b'
c
c'
pH
Ml OF .5M NaOH IN BACK TITRATION

Titration of aluminum chloride solutions.—In the titration of aluminum chloride, alone and as a component of an acidic solution of calcium silicate, the objective was the *complete neutralization* of the hydrochloric acid resultant from the hydrolysis of solute aluminum chloride. The equivalence hydrolization of the aluminum chloride is a governing factor in the subsequent determination of the equivalence *p*H of standard solu-

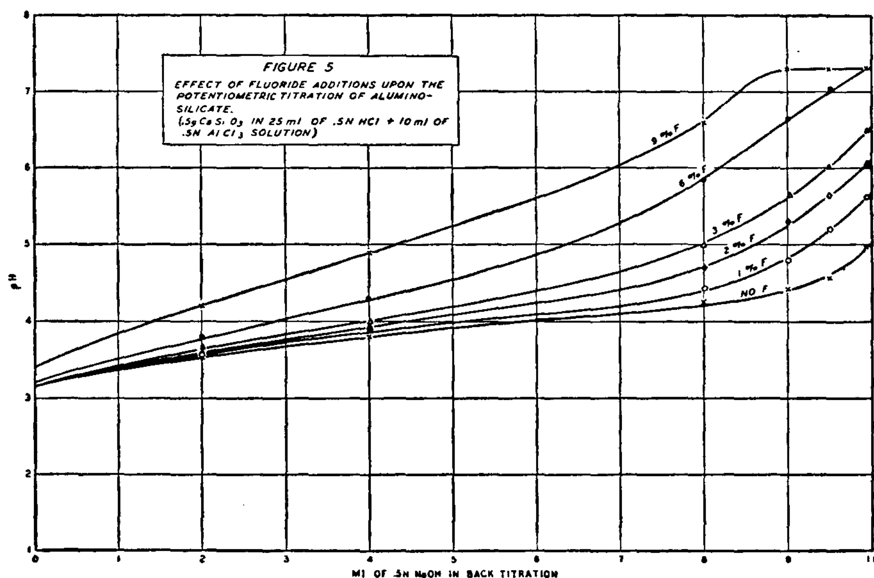


FIGURE 5
EFFECT OF FLUORIDE ADDITIONS UPON THE POTENTIOMETRIC TITRATION OF ALUMINO-SILICATE.
(.5g $CaSiO_3$ IN 25 ml OF .5N HCl + 10 ml OF .5N $AlCl_3$ SOLUTION)

tions of calcium silicate and aluminum chloride. That the equivalence *p*H is distinct from *p*H values that connote the complete precipitation of alumina, may be deduced from the following observations. According to Blum (*3*), the neutralization of a solution of aluminum chloride to *p*H 6.5 ensures complete precipitation of alumina. In a study of the composition of the precipitates formed by titrations of aluminum sulfate with sodium hydroxide to various *p*H values, Miller (*10*) found that only 90 percent of the alkali equivalence was required to attain minimal solubility of alumina, and that the precipitate occluded appreciable quantities of SO_4. According to Mattson (*8*) the isoelectric precipitation of alumina from aluminum chloride solutions requires titration to *p*H 8.1, and the negligibly small quantity of chloride found by him in such precipitates indicates virtually equivalent hydrolization of the aluminum chloride. At this *p*H, however, appreciable quantities of solute sodium aluminate occur. According to Magistad (*7*), the solution of alumina at *p*H 8.35 is 8.4 p.p.m. It is obvious, therefore, that the point of complete precipitation

is not the same as the point of complete hydrolization and neutralization of aluminum chloride solution. The location of the equivalence point in the titration curves was established by the gravimetric analysis of the chloride content of the aluminum chloride solution, by which a normality of 0.497 was found for a formula weight solution of 0.5 normality.

The curve *C* in Figure 3 gives the *p*H changes induced when an 80 ml. solution that contained 10 ml. of the aluminum chloride solution and 10 ml. of 0.5 *N* hydrochloric acid was titrated with 0.5 *N* sodium hydroxide. Two inflexion points appear on this curve. One point lies between *p*H 3.3 and 3.8 and represents the proximate neutralization of the free hydrochloric acid, whereas the other lies between *p*H 7 and 8 and identifies the region of complete precipitation of the alumina. At the point of equivalence titration, i.e., at the point of 19.94 ml. of 0.5 *N* sodium hydroxide addition, the *p*H was 8.6. This equivalence *p*H for the 0.05 *N* aluminum chloride was 0.5 *p*H above that given by Mattson (*8*) for the isoelectric point for the precipitation of alumina from a 0.006 *N* solution. The difference between the inflexion point at *p*H 7, or point of minimal alumina solubility, and the equivalence point represents a difference of about 3.5 percent of calcium carbonate in the neutralization value. The region between *p*H 4.2 and 4.6 is characterized by a slowly rising horizontal line that extends to a point that corresponds to approximately 75 percent of the aluminum chloride neutralization. From that region the *p*H rises sharply until the second inflexion point appears.

TITRATIONS OF SOLUTIONS OF PREPARED CALCIUM SILICATE AND ALUMINUM CHLORIDE

The neutralization curves, *a*, *b*, *c*, of Figure 4, are for the several concentrations of aluminum chloride alone. Curves a^1, b^1, and c^1 represent systems composed of a 0.5 gram charge of calcium silicate dissolved in 25 ml. of 0.5 *N* hydrochloric acid and carrying the indicated additions of aluminum chloride, in parallel with those made to the chloride-only systems of curves *a*, *b*, and *c*. The objective was to establish the equivalence point *p*H, or $pH_{e.p.}$, of such systems in relation to the variable ratio of $SiO_2:Al_2O_3$, since it was shown by Mattson (*8*) that incidence of silicate ions effects displacement of the isoelectric point to the acid side proportionately to the $SiO_2:Al_2O_3$ ratio.

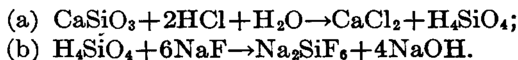
These systems may be regarded as being neutralized in two stages. One stage represents neutralization of the hydrochloric acid not utilized in the dissolution of the calcium silicate, whereas the other involves the complete neutralization of the hydrochloric acid generated by the hydrolysis of solute aluminum chloride. Since the $pH_{e.p.}$ lies in the region of the second stage, the known requirement of 7.05 ml. of 0.5 *N* sodium hydroxide for neutralization of the free acid was not indicated in graphs a^1, b^1, and c^1. The zero point on the sodium hydroxide line of these graphs is, therefore,

the point of neutralization of the free acid. The equivalence *p*H of every alumino-silicate mixture can be located at the point of the sodium hydroxide addition requisite for neutralization of the quantity of aluminum chloride. These quantities are 4.97 ml., 9.94 ml., and 14.91 ml., respectively. The *p*H values that correspond to the equivalent neutralization of these systems were found to be 4.55, 5.10 ,and 5.50. It is therefore obvious that *there can be no* pH *constant for equivalence neutralization of slag solution having indeterminate* $SiO_2:Al_2O_3$ *ratios,* even when other interfering components are absent. The feasibility of a proximate titration end point of solutions of such slags will be considered.

Effect of fluorides upon titration equivalence.—To a solution of 0.5 gram of calcium silicate in 25 ml. of 0.5 *N* hydrochloric acid and 10 ml. of the 0.5 *N* solution of aluminum chloride, additions of sodium fluoride were made to simulate solutions of slag carrying 1, 2, 3, 6, and 9 percent of fluorine. The titration curves of Figure 5 show *p*H changes beyond those induced by the 7.05 ml. sodium hydroxide requirement for the free acid in such systems, and reflect the different stages of aluminum chloride neutralization.

At the equivalence neutralization of these systems, i.e., at the point of 9.95 ml. back titration, there is a distinct rise in *p*H for each increment of fluoride to the otherwise constant mixtures of silica and alumina. The 2 percent addition of fluorine induced an equivalence *p*H of 6.05 against the 4.95 of the fluorine-free system. Larger additions caused greater shifts in the equivalence *p*H, with attendant indication of a chemical change in the system that received the 9 percent addition, as shown by a flattening of the curve above *p*H 7.

Based upon the titration of the alumino-silicate systems carried to *p*H 4.95 in the absence of solute fluorides, the positive error induced by a 2 percent fluorine content would amount to nearly 7 percent in the reading of calcium silicate-equivalence on the curve of Figure 5. This marked effect of fluorine content is the more striking when it is remembered that the additive fluorine was in the form of the *neutral salt,* NaF. The quantitative relationship between the smaller addition of sodium fluoride and the titration error suggests the formation of compounds in stoichiometric proportions. One possibility is the formation of fluosilicates:



(a) $CaSiO_3+2HCl+H_2O \rightarrow CaCl_2+H_4SiO_4$;
(b) $H_4SiO_4+6NaF \rightarrow Na_2SiF_6+4NaOH$.

According to equation (b), four equivalences of sodium hydroxide are released for neutralization of the system when 6 moles of sodium fluoride react with silicic acid. The maximal effect of such reaction would be the alkalization of $\frac{2}{3}$ of the sodium fluoride. Since a fluorine content of one percent is equivalent to a calcium carbonate content of 2.634 percent, $\frac{2}{3}$ of that equivalence would represent only 1.756 percent of calcium carbo-

nate. As indicated in Figure 5, however, the effects of the 1, 2, and 3 percent fluorine additions are twice those computed from the above equations. A further effect of the fluorine ion may be ascribed to the formation of additional fluoride complexes. The formation of alumino-silico fluoride, $2AlF_3 \cdot 3SiF_4$ (*9*) may be responsible for the observed increases in the alkalinity of the systems, as indicated by the equation

(c) $3H_4SiO_4+2Al(OH)_3+18NaF \rightarrow 2AlF_3 \cdot 3SiF_4+18NaOH$.

This postulated reaction would account for the full sodium equivalence of the solute sodium fluoride in the neutralization of the systems. The observed effect of the fluoride on the titration, however, seems to be even greater than the effect attributable to complete stoichiometric alkalization. The effect of the 9 percent fluorine addition in the flattening of the curve above 7 *p*H may be due to the reverse reactions in equations (b) and (c).

The adoption of a titration end point of higher *p*H value for systems that contain fluorides would be admissible only when applied to slags of constant $SiO_2:Al_2O_3$ ratios and of known fluorine content. Furthermore, titrations of alumino-silicate systems to near neutrality is difficult, because of *p*H instability resultant from the continuous cation absorption in the *p*H region above 7.

Effect of phosphates upon titration equivalence.—The phosphate furnace slag carries about 1.4 percent P_2O_5 in tertiary combination and this is equivalent to a calcium carbonate value of about 3 percent. When the acidic solution is back titrated to *p*H 5, only one-third of the H_3PO_4 will be neutralized, and the remaining two-thirds will affect the neutralization value of the slag as though this fraction of the calcium phosphate were of basic value. The dissolution of tricalcium phosphate in the solution of calcium silicate and aluminum chloride did not alter the configuration of the curves shown for the Ca-Al-Si solution. The plus error in the determination of the neutralization value is in accord with the above computations.

CONCLUSIONS AS TO TITRATION END POINT OF CALCIUM-ALUMINO SILICATE SYSTEMS

The data from titrations of solutions simulant to those of calcium silicate slags seem to warrant the following conclusions:

(1) The equivalence point of solutions of pure calcium silicates lies at *p*H 5.5 and can be attained with moderate precision.

(2) The equivalence hydrolization *p*H of an aluminum chloride solution is governed by concentration and lies in the region between *p*H 8 and 9. This is above the inflexion point, *p*H 6.5–7.0, on the titration curve and also above the region in which alumina solubility is minimal. Hence, the curve that reflects *p*H changes during the sodium hydroxide titration is of little help in the locating of the equivalence end point.

(3) The equivalence point for solutions containing silica and alumina is governed by the $SiO_2:Al_2O_3$ ratio and lies well below that for alumina alone and usually below that of the silica alone. The inflexion point lies in the region of *p*H 7 and considerably beyond the equivalence point established for such systems, and the potentiometric titration curve of alumino-silicates, therefore, does not give an exact indication of equivalence point. Nevertheless, the equivalence end point derived from systems of known alumina and silica content affords a simple titration procedure for slags which naturally possess a fairly uniform $SiO_2:Al_2O_3$ ratio.

(4) The equivalence *p*H of aqueous alumino-silicate systems containing fluorides rises in proportion to fluorine content. In a titration of an alumino-silicate system to proper *p*H, the positive error attributable to fluorine content up to 3 per cent amounts to 3.5 per cent of calcium carbonate-equivalence per percentage unit of fluorine.

(5) Although incidence of phosphates in alumino-silicate solutions does not distort the titration curve, phosphate content induces a measurable positive error at the *p*H to which the systems usually are titrated. Since most silicate slags carry only small amounts of phosphates, no serious error should develop from this source.

TITRATION OF SOLUTIONS OF BLAST FURNACE SLAGS TO DEFINITE *p*H

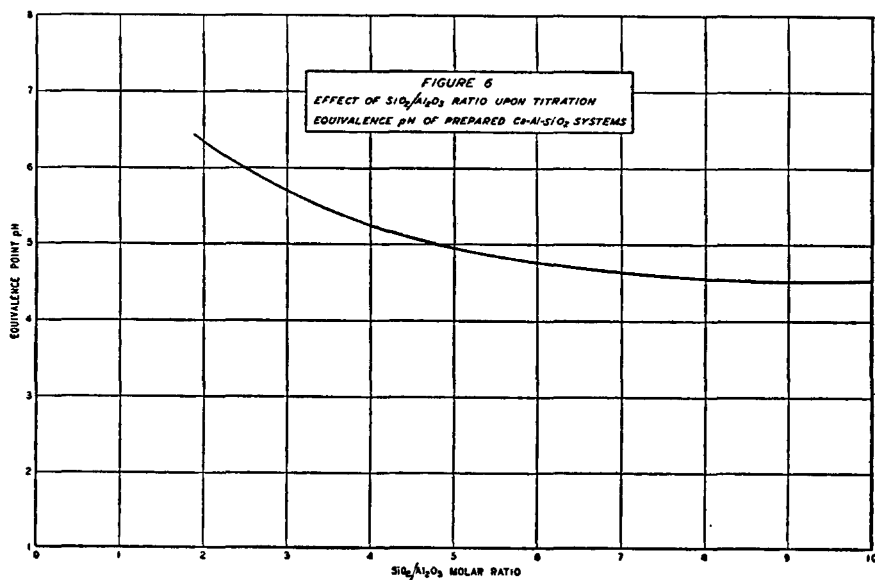
Adoption of a standard *p*H value for the titration end point of solutions of blast furnace slags hinges upon the uniformity in their $SiO_2:Al_2O_3$ ratios, which may be established only upon the analysis of a large number of representative slags. To consider this aspect in the light of the present data, the curve of Figure 6 was set up to show the relationship between the $SiO_2:Al_2O_3$ ratio and the equivalence *p*H as derived from the titration of standardized solutions of aluminum chloride and calcium silicate. The silica and alumina data of Table 1 then were applied in the computation of $SiO_2:Al_2O_3$ molar ratios for the eight slags that were devoid of fluorides and phosphates. From these ratios the corresponding $pH_{e.p.}$ values were read from the graph in Figure 6. The neutralization values of the slags then were obtained from the potentiometric titration curves of Figure 1, in relation to their respective $pH_{e.p.}$, as given in the fourth column of Table 3.

In routine determinations of the neutralization values of slags, however, the $SiO_2:Al_2O_3$ ratios would not be known. To determine the optimal *p*H for the titration of slags of unknown $SiO_2:Al_2O_3$ ratio, the neutralization values of several slags of known composition were read at several assumed titration end points on the potentiometric titration curves. The deviations between such values at *p*H 5 and those obtained at $pH_{e.p.}$, recorded in the last column of Table 3, indicate that a titration end point of *p*H 5.0 gives satisfactory results for most of the slags. The error for calcium carbonate-

TABLE 3.—*Neutralization values of blast furnace slags, by titration to various pH values*
(Results expressed as per cent)

SLAG NO.	SiO_2/Al_2O_3 MOLAR RATIO	$pH_{e.p.}$ BY CURVE, FIG. 6	$CaCO_3$-EQUIVALENCE						
			AT $pH_{e.p.}$	APPARENT VALUES AT VARIOUS pH ON THE CURVES					DEVIATION AT pH 5 FROM $pH_{e.p.}$
				4.6	4.8	5.0	5.2	5.4	
1	4.4	5.10	92.0	94.0	93.0	92.5	91.8	91.3	0.5
2	3.5	5.40	88.0	91.5	90.0	89.3	88.5	88.0	1.3
3	5.5	4.85	94.5	95.5	94.8	94.0	93.4	93.0	−.5
4	4.5	5.10	89.0	91.2	90.0	89.3	88.5	88.0	.3
5	4.0	5.20	87.5	89.8	89.0	88.0	87.5	87.0	.5
6	4.4	5.10	90.8	93.0	91.8	91.2	90.5	89.8	.4
7	5.0	4.95	90.8	92.3	91.3	90.5	90.0	89.5	−.3
8	6.1	4.75	89.3	90.0	89.0	88.5	87.5	87.0	−.8
Average	4.7	5.00							.57

equivalence would be less than ±0.5 per cent in six cases and ±1.0 per cent in two. These findings indicate that potentiometric titration to definite pH is a feasible and adequate procedure for the determination of the neutralization effectiveness of blast furnace slags. It would be advantageous, however, to utilize a larger number of analyzed blast furnace slags in a comparison, as to accuracy, rapidity, and reproducibility, between results attained by the proposed procedure and by other methods.



COMPARISON OF POTENTIOMETRIC TITRATION VALUES WITH COMPUTED VALUES FOR BLAST FURNACE SLAGS

The potentiometrically determined neutralization values of blast furnace slags were based upon the equivalence titration of prepared solutions of silica and alumina in Figure 5. It is recognized that slag components other than those included in the prepared mixtures may affect the titration equivalence. Therefore, it seemed essential to correlate the potentiometrically determined neutralizing values with those computed from the determined contents of calcium and magnesium.

In this correlation, the calcium+magnesium values were those obtained by analyses of solutions obtained in a similar manner to those used in the potentiometric titrations of the slags. The solutions of 0.5 gram charges were freed of silica through dehydration with perchloric acid, and aluminum and iron were eliminated by two ammoniacal precipitations. Calcium was determined by two oxalate precipitations and titration with potassium permanganate, and magnesium was precipitated as magnesium ammonium phosphate and the magnesium pyrophosphate incinerate was corrected for manganese pyrophosphate content.

TABLE 4.—*Comparison between potentiometrically determined neutralization values of blast furnace slags and those computed from Ca+Mg dissolved by dilute HCl digestion*

(Results expressed as per cent)

SLAG NO.	$CaCO_3$-EQUIVALENCE						
	POTENTIOMETRICALLY AT		DISSOLVED BY 0.5 N HCl			DEVIATION OF POTENTIOMETRIC FROM Ca+Mg	
	$pH_{e.p.}$	pH 5.0	Ca	Mg	Ca+Mg	$pH_{e.p.}$	pH 5
1	92.0	92.5	85.8	7.8	93.6	−1.6	−1.1
2	88.0	89.3	76.3	13.1	89.4	−1.4	−0.1
3	94.5	94.0	77.5	19.0	96.5	−2.0	−2.5
4	89.0	89.3	74.7	16.5	91.2	−2.2	−1.9
5	87.5	88.0	74.2	15.1	89.3	−1.8	−1.3
6	90.8	91.2	67.4	25.7	93.1	−2.3	−0.9
7	90.8	90.5	75.0	18.3	93.3	−2.5	−2.8
8	89.3	88.5	77.8	13.3	91.1	−1.8	−2.6
Average						−2.0	−1.7

The correlated data of Table 4 show that the potentiometrically determined neutralization values at $pH_{e.p.}$ run about two per cent below those computed from calcium+magnesium solutes. This relationship is fairly constant, with variance of not more than ±0.5 per cent. The uniformity of the difference between dissolved calcium+magnesium and the neutralization value determined at $pH_{e.p.}$ suggests that the specific end point arrived at by titration of the prepared standards is a correct ap-

proach to the titration of slag solutions devoid of fluorides. The greater individual variations in the values at *p*H 5 were expected, since these are only proximate to the more correct, although variable, $pH_{e.p.}$ that is governed by the $SiO_2:Al_2O_3$ ratio. It will be shown that incidence of solute SO_4 in the slag solutions may account, in part or entirely, for the two per cent higher value arrived at by computation from solute calcium +magnesium.

UTILITY OF INDICATORS IN THE TITRATION OF SOLUTIONS OF BLAST FURNACE SLAGS

In the preceding discussion, it was concluded that the potentiometric titration of blast furnace slag solutions to *p*H 5 affords an admissible index of their neutralization value. The determination would be simplified, however, by the use of indicators in lieu of a potentiometer. As noted, it was suggested that substitution of methyl orange for phenolphthalein (*13*) might allow titration of slag solutions as in the A.O.A.C. procedure for liming materials. The suitability of these two indicators may be judged by the positions of their end points on the potentiometric titration curves in relation to the equivalence *p*H. To facilitate this comparison, the trial neutralization values of the slags are located at several points on the curves within the utility ranges of the two indicators. The data in the last two columns of Table 5 show that a plus error of 6 to 24 percent was encountered when the slag solutions were titrated against methyl orange, whereas a fairly uniform minus error of 6 percent was encountered in titrations to *p*H against phenolphthalein. The buffered nature of the slag solutions in the methyl orange range is reflected by the large change in titration value induced by a change of 0.2 *p*H between *p*H 3.8 and *p*H 4. Obviously, neither methyl orange nor phenolphthalein registers the correct titration end point of blast furnace slag solutions. The data also warrant the conclusion that cited errors of 35 to 40 percent in titrations with phenolphthalein are attributable to factors other than the incorrect end point.

In titrations carried to *p*H 5, by methyl red and by bromocresol green, "off-color" effects were observed. Even when the solutions of blast furnace slags had been freed of suspended matter, the potentiometrically titrated solutions developed the brownish coloration of colloidal manganese oxide upon approach of end point. This colloidal suspension interfered with the proper color development of either of the two indicators utilized. It seemed inescapable that mere titration against conventional indicators cannot be expected to give correct titration of slag solutions. Nevertheless, when a *p*H meter is not at hand, values within 2 percent of true results can be obtained by titration of *p*H 5.2 against bromocresol green.

TABLE 5.—*Neutralization values of blast furnace calcium silicate slags indicated by potentiometric titration to various pH values, in terms of $CaCO_3$*

(Results expressed as per cent)

SLAG NO.	BY TITRATION TO pH VALUES OF:												BY ANALYSIS		DEVIATION FROM ANALYSIS, WHEN TITRATED TO—	
	3.8	4.0	4.2	4.6	5.0	5.2	5.4	5.8	6.2	6.6	7.0	8.0	Ca+Mg	CORRES. pH	pH 4 (M.O.)	pH 8 (PH. PHT.)
1	115.0	104.5	97.5	94.0	92.5	91.8	91.3	90.5	90.0	89.5	88.8	85.5 86.3	91.8	5.2	12.7	−5.5
2	115.0	101.0	95.0	91.5	89.3	88.5	88.0	87.0	86.0	85.5	84.5	82.5	88.2	5.3	23.8	−5.7
3	110.0	103.0	98.5	95.5	94.0	93.5	93.0	92.0	91.5	91.2	90.8	87.5 88.8	95.4	4.6	7.6	−6.6
4	110.0	100.0	95.0	91.0	89.3	88.5	88.0	87.3	86.5	86.0	85.0	83.5	90.2	4.8	9.8	−6.7
5	108.0	100.0	95.0	90.0	88.0	87.5	87.0	86.0	85.5	84.5	—	82.0	88.0	5.0	12.0	−6.0
6	115.0	105.0	97.0	93.0	91.0	90.5	90.0	89.0	88.0	87.5	86.8	83.5 85.0	91.3	5.0	13.7	−6.3
7	110.0	98.0	95.0	92.5	90.5	90.0	89.5	88.8	88.0	87.7	87.3	86.5 85.8	92.5	4.6	5.5	−6.7
8	105.0	99.0	94.0	90.0	88.5	87.5	87.0	86.3	85.5	84.7	—	83.5	89.3	4.8	9.7	−5.8

PROPOSED PROCEDURES FOR THE INDIRECT TITRATION OF SOLUTIONS OF SLAGS

Two simplified analytical methods have been developed to circumvent the vitiations encountered in the direct titration of slags, and more particularly the interference from component fluorides.

Procedure I.—The slag is dissolved in acetic acid (1+4). Silica is dehydrated and sesquioxides are precipitated by ammonia and filtered jointly. The filtrate is evaporated to dryness and the residue of acetates is ignited, dissolved in standard acid, and back titrated.

Procedure II.—The slag is disssolved in hydrochloric acid (1+9). Silica is dehydrated and sesquioxides are removed by ammonia, as in procedure I. The calcium and magnesium are precipitated as oxalates, ignited, dissolved in standard acid, and back titrated.

SOLUTION OF SLAGS IN ACETIC ACID

Acetic acid offered an obvious advantage over hydrocloric or nitric acid. Its ammonium salt can be volatilized by evaporation after the removal or iron and alumina, whereas either ignition or metathesis by nitric acid is necessary for such elimination when the mineral acids are used. Moreover, the acetates of calcium and magnesium can be converted to carbonates by gentle ignition, whereas the chlorides or nitrates would require oxalate precipitation and calcination. According to R. M. Wilson (*14*), acetic acid is an effective dehydrant for silica in cement-clinker analysis. In his use of hydrocloric acid before separation of silica, however, the advantage gained by the use of acetic acid alone is nullified.

The chief objection to the utilization of acetic acid is the slowness of its dissolution of unquenched slag. Complete dissolution of 0.5-gram charges of the quenched glassy slags from phosphate furnaces was effected within 2–3 minutes by agitated digestion with 50 ml. of acetic acid (1+4) at room temperature. In contrast, unquenched crystalline slags were not dissolved completely in 20 minutes at room temperature and boiling for 5 minutes additional was necessary to effect complete dissolution. Quenched *blast* furnace slags underwent complete dissolution during 10–20 minute agitated digestions at room temperature, whereas the same slags, unquenched, required prolonged hot digestion with frequent stirring. An acetic acid concentration of 1+1 proved no more expeditious than the 1+4 acid in the dissolution of *unquenched* blast furnace slags.

ACETIC ACID PROCEDURE

Weigh a 0.5-gram charge into a 250 ml. beaker; moisten and introduce 35 ml. of acetic acid (1+4) and stir the suspension continuously 2–3 minutes. Place the beaker in a boiling water bath—improvised by use of a 400 ml. beaker containing 50 ml. of water—and evaporate to a gel, stirring frequently. Add 25 ml. of acetic acid (1+4) and dilute to 125 ml.; heat to boiling and neutralize with NH_4OH (1+1) to the yellow color of methyl red. Digest on hot plate 5 minutes and filter on 9 cm. open-textured paper into 100×50 mm. Pryex, lipped crystallizing dishes; rinse

beaker three times and wash the filter five times with hot neutral 0.5 *N* ammonium acetate. Evaporate on hot plate, regulating heat to assure rapid evaporation without spattering of the sirupy residue during final stages. When the residue becomes gummy, take up with 15 ml. of water and again evaporate. Repeat this treatment until the residue attains the powdery condition and is devoid of acetic acid odor. Heat 10 minutes at maximal heat on hot plate and ignite in the muffle 10 minutes at 550°C.; allow to cool, and wet with 10 ml. of water; introduce 25 ml. of 0.5 *N* HCl into the covered dish; and heat over Bunsen burner to a gentle boiling for 5 minutes. Filter on a 9 cm. filter, catching the filtrate in a 250 ml. Erlenmeyer flask; wash the dish and the filter three times with hot water. Back-titrate excess acid with 0.5 *N* NaOH against phenolphthalein. The net ml. of 0.5 *N* acid used, times 5, expresses the neutralization value of the slag in terms of $CaCO_3$.

TABLE 6.—*Neutralization values of slags of two types as determined by the acetate method in relation to values computed from the analyses of Table 1*

SLAG		$CaCO_3$—EQUIVALENCE						
		BY ACETATE METHOD					COMPUTED	DEVIATION BY ACETATE
TYPE	NO.	1	2	3	4	AV.		
Blast furnace	1	92.0	91.8	92.3	91.8	92.0	93.0	−1.0
	2	87.8	87.5	88.3	—	87.9	89.4	−1.5
	3	95.0	95.3	95.0	—	95.1	96.1	—1.0
	4	88.5	88.5	89.0	—	88.7	91.5	−2.8
	5	87.3	87.8	87.8	—	87.6	89.4	−1.8
	6	89.5	89.5	89.8	—	89.6	92.1	−2.5
	7	92.0	91.0	91.5	—	91.5	93.9	−2.4
	8	89.0	88.8	88.5	—	88.8	90.8	−2.0
Phosphate furnace	349	85.5	85.8	85.8	—	85.7	85.6	0.1
	693	83.0	82.8	—	—	82.9	84.2	−1.3
	795	84.5	83.8	84.5	—	84.2	83.0	1.2
	920	94.3	94.3	94.5	94.3	94.3	93.8	0.5
	921	86.5	86.5	86.3	86.3	86.4	85.5	0.9
	1053	84.8	84.5	83.8	84.5	84.4	82.8	1.6
	1058	84.3	84.3	—	—	84.3	82.2	2.1
	1083	82.0	82.1	81.5	—	81.9	82.1	−0.2
	1084	81.5	81.8	81.8	82.0	82.4	81.8	0.6

RESULTS BY THE ACETIC ACID METHOD

The neutralization values registered by the acetate procedure on slags from the two types of furnace are given in Table 6, in comparison with the "computed" values. The values obtained for the blast furnace slags were compared with values indicated by determined content of hydrochloric acid-soluble calcium + magnesium; those for the phosphate furnace slags were derived from total calcium + magnesium content after correction for component F_2 and PO_4. Seven of the nine phosphate furnace slags show a mean value 0.9 percent higher than the computed value, with individual increases in the range of from 0.1 to 2.1 percent. There

was one significant exception, a minus deviation of 1.3 percent. The positive deviations can be accounted for easily by the small content of magnesium and by undetermined incidence of sodium and potassium. The values indicated for the blast furnace slags by the acetic acid procedure are lower than those computed from the hydrochloric acid-soluble calcium+magnesium. The deviation may be due in part to incidence of calcium sulfate and in part to incomplete dissolution.

THE HYDROCHLORIC ACID METHOD

The advantage of hydrochloric acid over acetic acid is virtually restricted to its being a more effective dissolvent for the unquenched blast furnace slags. In the hydrochloric acid solution, the silica precipitates as a gel near the end of the evaporation, whereas in the acetic acid solution it precipitates as gelatinous flakes during the digestion.

Procedure.—Weigh a 0.5-gram charge into a 250 ml. beaker. Moisten and introduce 25 ml. HCl (1+4) while swirling the solution; stir continuously for 2 minutes and then boil gently 5 minutes with frequent stirring. Place in boiling water bath, improvised by use of a 400 ml. beaker containing 50 ml. of boiling water, and evaporate to dryness. Add 35 ml. of HCl (1+4), dilute with 25 ml. of water, and heat to boiling; neutralize with NH_4OH (1+1) to the yellow color of methyl red. Digest 5 minutes on hot plate, filter through a 9 cm. filter into a 100×50 mm. Pyrex, lipped crystallization dish. Rinse the beaker three times and wash the filter five times with hot neutralized 2 percent NH_4Cl solution. To the filtrate add 20 ml. of concentrated HNO_3 and evaporate in watch-glass-covered dish on hot plant, preferably overnight. Take up with 25 ml. of water and add 10 ml. of a 10 percent solution of oxalic acid. *Under hood,* evaporate to dryness in the dish, suspended in a 1-liter beaker containing boiling distilled water. Take up with 10 ml. of water and again evaporate to dryness; remove water bath, cover with Pyrex watch-glass, and heat on hot plate until virtually all of the oxalic acid on cover-glass has volatilized. Heat the dish in a muffle 10 minutes at 550°C.; remove and allow to cool. Moisten the powdery residue with 10 ml. of water; through lip of the dish, introduce 25 ml. of 0.5 *N* HCl and boil gently 5 minutes. Filter, and complete the determination as directed under "Acetic acid procedure."

RESULTS BY THE HYDROCHLORIC ACID PROCEDURE

Every value given by the hydrochloric acid procedure in Table 7 is 1–4 percent lower than the corresponding computation from determined calcium+magnesium content, whereas the direct titrations to $pH_{e.p.}$ give a mean value of only 0.6 percent below the analogous mean obtained by the hydrochloric acid-oxalate procedure. Upon exclusion of the single exceptionally low titration of sample No. 7, the mean deviation is lessened to minus 0.3 percent. This agreement is as good as could be expected from results by such two widely different procedures. When the mean for the more practical procedure of titration to pH 5 is used in a similar comparison, the deviation is minus 0.4 percent. As would be expected, the individual deviations by this simplified titration procedure are greater than those among titrations to $pH_{e.p.}$. The data of Table 7 indicate that, where de-

TABLE 7.—*Neutralization values of blast furnace slags as determined by HCl indirect titration procedure in relation to those obtained by other procedures, in terms of $CaCO_3$*
(Results expressed as per cent)

SLAG NO.	BY HCl-OXALATE METHOD					BY Ca+Mg ANALYSIS		BY TITRATION TO $pH_{e.p.}$		BY TITRATION TO pH 5	
	1	2	3	AV.	AVERAGE DEVIATION	NEUTR. VALUE	DEVIATION*	NEUTR. VALUE	DEVIATION*	NEUTR. VALUE	DEVIATION*
1	92.0	92.0	91.5	91.8	±.2	93.6	1.8	92.0	0.2	92.5	0.7
2	88.0	88.5	88.0	88.2	±.2	89.4	1.2	88.0	−0.2	89.3	1.1
3	95.5	95.3	95.3	95.4	±.1	96.5	1.1	94.5	−0.9	94.0	−1.4
4	90.3	90.0	90.5	90.3	±.3	91.2	0.9	89.0	−1.3	89.3	−1.0
5	88.0	87.8	88.3	88.0	±.2	89.3	1.3	87.5	−0.5	88.0	0.0
6	91.0	91.5	91.0	91.3	±.3	93.1	1.8	90.8	−0.5	91.2	−0.1
7	92.3	92.8	92.5	92.5	±.3	93.3	0.8	90.8	−1.7	90.5	−2.0
8	89.0	89.0	89.5	89.2	±.2	91.1	1.9	89.3	0.1	88.5	−0.7
Average				90.8		92.2	1.4	90.2	−0.6	90.4	−0.4

* From the values determined by the HCl-oxalate procedure.

viations of ±1 percent are admissible, *the potentiometric titration to pH 5 is the simplest method for the determination of the calcium carbonate equivalence of fluorine-free slags.*

INDETERMINATE ERROR IN THE CHEMICAL EVALUATION OF BLAST FURNACE SLAGS

The effect of component sulfides was not considered in the foregoing discussions. The calcium of the sulfide has no capacity to effect neutralization in soils when no volatilization of sulfur occurs, yet it registers analytically as though it were in silicate combination. The phosphate furnace slags (*4*) carry about 0.3 percent of sulfur, or a one percent calcium carbonate-equivalence, whereas the calcium carbonate-equivalence of the sulfur content of the blast furnace slags amounts to nearly 5 percent. When blast furnace slags, Nos. 1 and 8, were digested with aqua regia, in lieu of hydrochloric acid, the resultant decreases of 5.2 and 2.2 percent in calcium carbonate potential were respectively equivalent to the sulfur content of the two slags.

COMPARISON OF RESULTS BY THE ACETIC AND HYDROCHLORIC ACID PROCEDURES ON PHOSPHATE FURNACE SLAGS

The proposed acetate procedure meets the imperative need for a dependable procedure for the determination of neutralization values of phosphate furnace slags. Its chief disadvantage lies in the time required for the dehydration of the acetate residues and the slowness with which unquenched slags undergo dissolution, whereas the dissolution of slags of all types is accomplished expeditiously by the more involved hydrochloric acid method, which moreover is superior to the acetic acid procedure for blast furnace slags. On phosphate slags, however, the results by the hydrochloric acid procedure were invariably higher than those by the acetate method, the plus errors being accounted for by the calcium of fluoride and phosphate. Analysis of experimental slags fortified with fluorides and phosphate served to confirm the relationship between magnitude of the plus error and the equivalences of the added compounds. The findings of Table 8 indicate that F_2 and PO_4 were eliminated in other than calcic combination through the treatments prescribed, and preceding the titration of the ignited residue. The removal of PO_4 can be ascribed to ammoniacal precipitation as aluminum phosphate, but the mechanism of fluoride elimination is not apparent. Some fluorine may be evolved during the evaporation with nitric acid and some through the treatment with oxalic acid. It is obvious, however, that the *hydrochloric acid procedure does not give the true calcium carbonate equivalence of slags that contain appreciable quantities of fluorides.* Apparently, component fluorides and phosphates do not affect the acetic acid method of evaluation of phosphate furnace slags as they do in the hydrochloric acid procedure. The

TABLE 8.—*Effect of fluorine and phosphate components upon the neutralization values of phosphate furnace slags as obtained by the HCl-oxalate procedure*
(Results expressed as per cent)

SYSTEMS TITRATED	$CaCO_3$-EQUIVALENCE				
	OF P_2O_5	OF F_2	COMPUTED[a]	DETERMINED	DEVIATION
Slag No.					
920	0.4	—	93.8	94.5	0.7
921	0.4	6.3	85.5	92.5	7.0
1058	4.9	6.1	82.2	92.5	10.3
1084	2.8	5.1	81.8	88.9	7.1
Control mixtures					
$CaSiO_3+AlCl_3$	—	—	90.0	90.0	—
$CaSiO_3+AlCl_3$	—	5.26	90.0	95.3	5.3
$CaSiO_3+AlCl_3$	4.22	5.26	90.0	99.5	9.5

[a] By correction for determined Ca in PO_4 and F_2 combinations.

fluorine is retained as calcium fluoride in the acetate procedure and the phosphate is not precipitated as aluminum phosphate when the acetate solution is ammoniated. Such a precipitation would cause the ignited residues to show increases of nearly three per cent in titration value, whereas the results in Table 6 show no such general increase. The occasional higher values are no greater than those computed without allowance for magnesia content.

The residue from the acetic acid dehydration of the silica contained from $\frac{2}{3}$ to $\frac{3}{4}$ of the initial PO_4 content of the slag. The analytical conditions are conducive to the formation of calcium fluorphosphate, but verification of this probability would require detailed analytical work and is not essential to the present objective. It is believed that the results warrant the conclusion that the more expeditious acetate procedure is adequately accurate for the determination of the neutralization value of phosphate furnace slags, and also for blast furnace slags, quenched and unquenched, *provided complete dissolution of the slag is effected.*

SUMMARY

The A.O.A.C. titrative procedure for liming materials is not applicable to the determination of neutralization value of the calcium silicate slags. This inadequacy prompted the attempt to evolve a rapid and dependable procedure for the determination of the calcium carbonate-equivalence of such slags from both blast and phosphate furnaces.

Preliminary studies established that dissolution of 0.5-gram charges of blast furnace slags is completed by 5-minute boiling in 35 ml. of 0.5 *N* acid. The proportion of 25 ml. of the acid to 0.5 gram, although carrying a 30 per cent excess beyond the calcium-magnesium equivalence of most

slags, did not effect the complete dissolution of slags of high aluminum content, even when the digestion was prolonged.

By means of the glass electrode, potentiometric titration curves were obtained for guidance in the studies as to titration end point for blast and phosphate furnace slags. The transition points in the titration curves for blast furnace slags did not register the equivalences computed from determined calcium+magnesium content.

Titration curves for solutions of calcium silicate, containing aluminum chloride, demonstrated that the equivalence *p*H is governed by the ratio of $SiO_2:Al_2O_3$ in the titrated system. In general, the equivalence *p*H of the blast furnace slag solutions falls within the range of *p*H 4.8–*p*H 5.4. Potentiometric titrations to *p*H 5.0 gave satisfactory results for experimental blast furnace slags.

Fluorine content is the chief cause of irregularity in the titration curves for phosphate furnace slags, the equivalence *p*H of which develops in the region between *p*H 6 and 8, wherein the alumino-silicate precipitate exerts considerable cation sorption.

A study of the indicators appropriate for the titration of blast furnace slags demonstrated that phenolphthalein gave a minus error of about 6 per cent in calcium carbonate-equivalence, in contrast to a plus error of from 6 to 24 percent by the use of methyl orange. Titrations against bromocresol green to *p*H 5.2 gave only proximations of true values. Accuracy of the colorimetric readings of direct titrations was impaired by undissolved matter and by precipitates formed by the titratant.

Two procedures are proposed to obviate both direct titration, technic and complete analysis of slags. One procedure directs the dissolution of the slag in acetic acid, dehydration of silica and its elimination jointly with R_2O_3, evaporation of the acetate solution, ignition and titration of the resultant calcine. The other prescribes dissolution in hydrochloric acid, joint elimination of silica and R_2O_3, evaporation of filtrate with nitric acid, oxalate precipitation and ignition, and titration of the calcine. The first-mentioned procedure is especially applicable to slags that contain fluorides and phosphates, and to slags in general when complete acetic acid dissolution of the charge is effected. The last-mentioned procedure was found appropriate for slags devoid of fluorides and phosphates.

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DETERMINATION OF FLUORINE IN MINERALS AND BONES*

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The determination of fluorine in biological materials, especially feed, bone and mineral samples, is of considerable importance in establishing tolerances of this element for animals. A number of methods have been published, but they are not satisfactory in all respects. The procedure finally adopted in this laboratory is a combination, with several modifications, of three published methods: Crutchfield,[1] Willard and Winter[2] and Dahle *et al.*[3] The fundamental steps include ashing with magnesium acetate, distillation from perchloric acid, and titration with thorium nitrate and sodium fluoride. Some refinements which might be made in the procedure are described by Clifford.[4]

PROCEDURE

The ashing of the sample is carried out in a manner similar to that described by Crutchfield.[1] The sample (which should contain .05–.25 mg. of fluorine) is weighed into a 200 ml. nickel crucible. One ml. of 25 percent magnesium acetate solution is added to the sample and mixed by swirling. The crucibles are placed in an automatically controlled electric muffle furnace at room temperature, and the temperature is raised gradually to

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[1] *Ind. Eng. Chem., Anal. Ed.*, **14**, 57 (1942).
[2] *Ibid.*, **5**, 7 (1933).
[3] *This Journal*, **21**, 435 (1938).
[4] *Ibid.*, **27**, 246 (1944).

570°C.; the rate is determined by the amount of moisture and organic matter to be removed. The samples remain in the furnace at 570°C. until all the carbon is burned off, which usually requires about 6 hours.

The distillation procedure is similar to that of Willard and Winter.[2] Approximately 5 ml. of water is added to the ash followed by 5 ml. of 60 percent perchloric acid. This completely dissolves the bone ash but does not dissolve all types of ash. The sample is washed into a 50 ml. round-bottomed flask, 24/40 standard taper joint, with a fine stream of water, making the volume up to approximately 20 ml. Five ml. of 60 percent perchloric acid, about 0.1 gram of sand, and three small pieces of sintered glass (which is more effective than glass beads in preventing bumping) are added to the flask, and the distillation is carried out until nearly 200 ml. of distillate has been collected in a 200 ml. volumetric flask. The temperature of the liquid in the distilling flask is kept at 135° ± 3°C. The rheostat of the 250-watt heater is kept on full throughout the distillation. The use of 350-watt heaters shortens the distillation time further.

After nearly 200 ml. of distillate has been obtained it is made to volume, and an aliquot is taken for titration. The titration is carried out according to the back titration described by Dahle, Bonnar, and Wichman.[5] It is usually not necessary to use more than 0.2 ml. of the 0.05 *N* sodium hydroxide for the neutralization of the acid in a 40 ml. aliquot of distillate. The end point is quite distinct if the size of the sample is adjusted so that 1–5 ml. of the standard sodium fluoride is required for the titration. For proper illumination of the Nessler tubes during the titration, a titration illuminator, with a base 18 by 7 inches containing two 15-watt fluorescent tubes under a milk glass plate has been found to be satisfactory.[6] Shadowless-bottomed 50 ml. Nessler tubes are placed in two holes bored side by side in a wood base which covers the top of the illuminator. The tubes slide into cylinders of cardboard which prevent other light from interfering. A slightly darkened room aids in observing the color change.

Since a single distillation requires 2.5–3 hours, a means was developed to carry out several distillations simultaneously. After making several changes in methods of adding the water and investigating the use of steam distillation, the writers found the apparatus shown in Figure 1 to be satisfactory. Four units can be managed at the same time. The temperature of the liquid in the flask is maintained by adjusting the screw clamp so that water runs in at about the rate necessary. The setting of the screw clamp may not need to be disturbed during a distillation. If the temperature drops below 132°C., the flow of water can be stopped by closing the stopcock. If the temperature goes above 138°C., water can be added rapidly by turning the stopcock so that the water runs through the unrestricted arm of the stopcock. The water in the 2 liter Pyrex bottle

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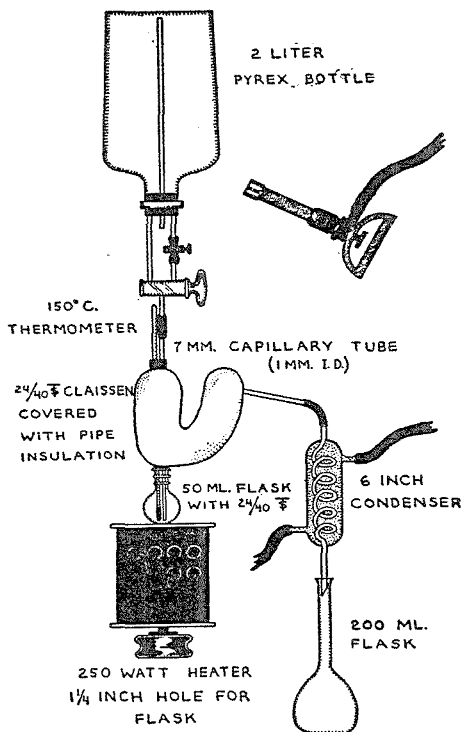
[5] *This Journal*, 21, 459 (1938).
[6] Supplied by Arthur H. Thomas Co., Philadelphia, Pa.

reservoir is kept warm by direct application of heat from the burner at the point indicated in the figure. The use of warm instead of cool water speeds up the distillation.

Dahle[3] suggests that a double distillation is sometimes necessary, especially with samples high in chlorides. This is necessary to overcome the



FLUORINE DISTILLATION APPARATUS

Fig. 1

high acidity in the distillate due to the volatilization of the chlorides (McClure)[7]. In the work in this laboratory with bones and minerals the double distillation has not been found necessary.

RESULTS

Although most of the fluorine determinations reported were made on bone samples, several minerals, such as defluorinated phosphate, rock phosphate, superphosphate, and limestone, have been analyzed. Table 1

[7] *Ind. Eng. Chem., Anal. Ed.*, 11, 171 (1939).

TABLE 1.—*Fluorine determinations on minerals and bones and recovery from solutions of sodium fluoride*

SAMPLE NO.	MATERIAL	FLUORINE FOUND
		per cent
1	Steamed bone meal	0.044–0.044
2	Steamed bone meal	0.044–0.045
3	Steamed bone meal	0.047–0.048
1	Raw rock phosphate	3.3 –3.4
2	Raw rock phosphate	3.3 –3.5
1	Defluorinated phosphate	0.100–0.110
2	Defluorinated phosphate	0.020–0.021
3	Defluorinated phosphate	0.040–0.041
1	Superphosphate	1.6 –1.7
2	Superphosphate	1.5 –1.6
1	Melted rock phosphate	0.29 –0.30
1	Limestone	0.034–0.034
2	Limestone	0.035–0.036
3	Limestone	0.020–0.020
1	Mineral feeds*	0.092–0.094
2	Mineral feeds*	0.360–0.380
1	Sheep bones	0.018–0.019
2	Sheep bones	0.027–0.028
3	Sheep bones	0.100–0.110
4	Sheep bones	0.160–0.160
5	Sheep bones	0.59 –0.60
		microgram
1	10 ml. NaF solution containing 10 micrograms of F.	9.6
2	10 ml. NaF solution containing 10 micrograms of F.	9.9
3	10 ml. NaF solution containing 10 micrograms of F.	9.8
4	10 ml. NaF solution containing 10 micrograms of F.	9.5

* Analysis by E. D. Schall, Purdue University Department of Agricultural Chemistry.

presents some of the results obtained. The average deviation between duplicate samples amounted to 3.6 percent. Recovery of fluorine from solutions of known content was satisfactory, although the results were consistently low by almost 3 percent.

SUMMARY

A procedure is described for the determination of fluorine, which is a combination of three published methods.

Improved apparatus facilitates the distillation and titration of fluorine. Results show an average deviation between samples of 3.6 percent. Recoveries of 95–99 percent of fluorine from solutions of known content were obtained.

APPARATUS FOR THE DETERMINATION OF VOLATILE CITRUS OILS*

By E. T. BARTHOLOMEW and WALTON B. SINCLAIR (University of California Citrus Experiment Station, Riverside, Calif.)

Determinations of volatile oils in various portions of the peel of four varieties of citrus fruits at different stages of maturity are being made in this laboratory. Several methods and types of apparatus have been recommended for such purposes, by Wilson and Young,[1] Klevenger,[2] Scott,[3] Wilson,[4] and others, but none of these appeared to be entirely satisfactory for the present studies. This paper describes an apparatus (Figure 1) which has been so constructed as to overcome most of the shortcomings of the earlier designs, gives directions for its use, and presents enough data to indicate the degree of accuracy that may be obtained by using it.

PROCEDURE

The lemons used in the present studies were allowed to stand overnight in the laboratory to lose turgidity. They were then shellacked to prevent loss of oil while disks of the peel were being removed with a cork borer having an inside diameter of 15.7 mm. A hundred of these disks, with 200–300 ml. of distilled water, were placed in a Waring Blendor and ground very fine. The material was then transferred to the 2 liter flask illustrated in Figure 1 (A), made up to 1 liter, and boiled until 200 ml. of distillate had passed through the 250 ml. receiving flask (B), the outlet tube (C), the rubber tube (D), and the leveling tube (E) into the graduated cylinder (F). The receiving flask (B) was filled with water before distillation began, and the leveling tube (E) adjusted so that the water level came to within about 2 cm. of the top of the flask after the end of the condenser (H) had been inserted. The source of heat was an electric flask heater, so regulated that distillation was completed in approximately 30 minutes after boiling began. The oil (G) was caught in the lower end of the Ful-Jak Liebig-type condenser (H), which had an air-outlet tube (I) sealed in just below the 60 cm. jacket.

At the end of the distillation period, the pinchcock on the rubber tube was closed, and the receiving flask, which rested on a movable support, was lowered and moved from under the condenser. The calibrated measuring tube (J), which had a capacity of 5 ml, and was marked with 0.01 ml. subdivisions 0.98 mm. apart, was then attached to the receiving flask; the end of the rubber tube was changed from the leveling tube to the

* Paper No. 526, University of California Citrus Experiment Station, Riverside, Calif.
[1] *Ind. Eng. Chem., Anal. Ed.*, **9**, 959 (1917).
[2] *J. Am. Pharm. Assoc.*, **17**, 345 (1928).
[3] *This Journal*, **24**, 165 (1941).
[4] *Ibid.*, **27**, 201 (1944).

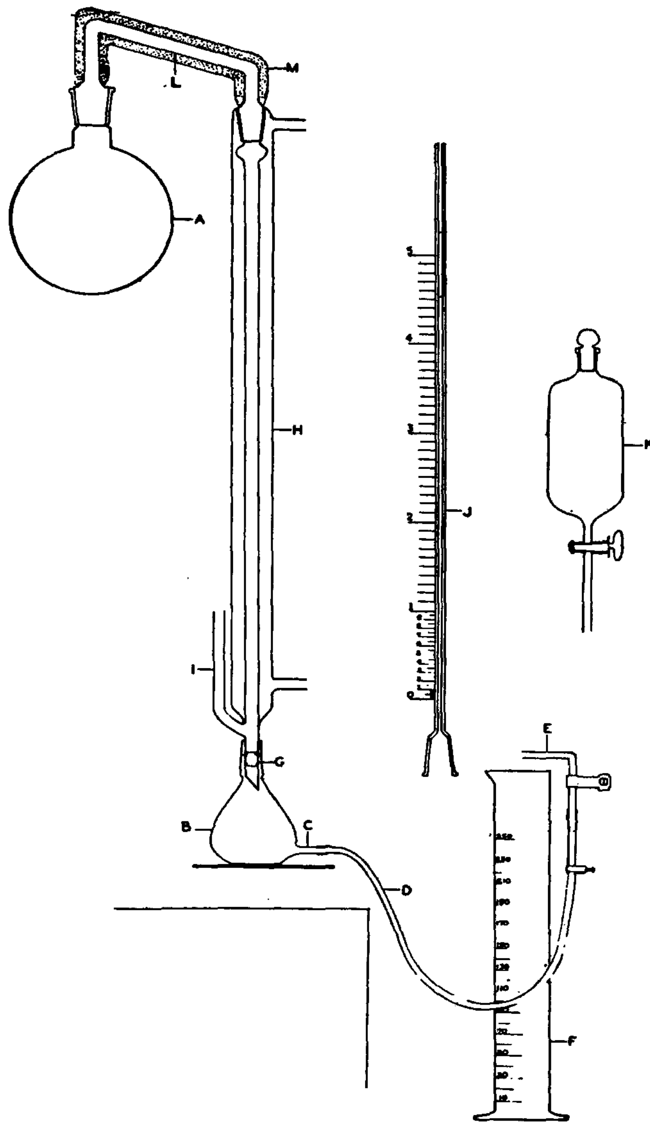


FIG. 1.—Apparatus for the determination of volatile oils.
(See text for description)

water reservoir (K), and the pinchcock was opened. The water from the reservoir forced the oil into the calibrated tube where it could be read. It

should be noted that the connecting tube (L) between the flask (A) and the condenser (H) was insulated with cork (M).

EXPERIMENTAL RESULTS

Length of Distillation.—To determine how long distillation should continue and how much distillate should be caught in order to extract all the oil from the ground peel, oil readings were made after distilling over each of four consecutive 50 ml. aliquots of distillate. The total time consumed in obtaining the four aliquots was approximately 30 minutes. The results (Table 1) show that from 95 to 99 percent of the oil came over with the first 50 ml. aliquot. In the fourth aliquot there was only a trace of oil, a small droplet, on the water meniscus.

TABLE 1.—*Amount of oil obtained from consecutive 50 ml. aliquots of distillate from lemon peel*

ALIQUOT NO.	TEST 1		TEST 2		TEST 3		TEST 4	
	ml.	*per cent**	*ml.*	*per cent*	*ml.*	*per cent*	*ml.*	*per cent*
1	1.19	97.6	1.59	98.8	1.57	94.9	1.47	95.8
2	0.02	1.6	0.02	1.2	0.075	4.5	0.055	3.6
3	0.01	0.8	Trace		0.01	0.6	0.01	0.6
4	Trace		Trace		Trace		Trace	

* All percentages are based on the total amount of oil determined.

Recovery of Oil Added to Distilled Water.—In several tests (Table 2), 2 ml. of freshly distilled lemon oil was taken up in a standardized 2 ml. pipet and put into the 2 liter flask with 1 liter of water for distillation by the procedure described above. This was to determine whether all the oil could be recovered or whether some of it would be lost, perhaps because of the use of direct heat instead of an oil bath or steam distillation, or through some fault of the apparatus itself. The results show that recoveries were both good and consistent.

Recovery of Oil Added to Previously Distilled Ground Lemon Peel.—The oil was distilled from a sample of ground peel in the usual manner (see "Procedure"), the flask and contents were cooled, 2 ml. of oil was added, distillation was repeated, and the recovered oil was measured. Again the flask and contents were cooled, another 2 ml. of oil was added, and the oil was recovered and measured. The next day two more 2 ml. samples of oil were recovered from a similarly treated sample of peel. The results show very good recovery values (Table 3). They also indicate that a small amount of the heavy fraction of the oil remained in the peel after the preliminary extraction. This is indicated by the fact that the recoveries for

TABLE 2.—*Recovery of oil added to distilled water*

TEST NO.	OIL ADDED	OIL RECOVERED	
	ml.	*ml.*	*per cent*
1	2.0	1.985	99.3
2	2.0	1.985	99.3
3	2.0	1.980	99.0
4	2.0	1.985	99.3
5	2.0	1.985	99.3

tests 1 and 3 were slightly greater than those for tests 2 and 4, and a little greater than the recoveries made when oil was added to distilled water (Table 2).

TABLE 3.—*Recovery of oil added to previously distilled ground lemon peel*

TEST NO.	OIL ADDED	OIL RECOVERED	
	ml.	*ml.*	*per cent*
1	2.0	1.995	99.8
2	2.0	1.980	99.0
3	2.0	2.000	100.0
4	2.0	1.990	99.5

Oil Distilled from Fresh Lemon Peel.—In connection with the more extensive studies previously mentioned, many determinations have been made on the oil content of different portions of the peel of citrus fruits at different stages of maturity. A few of the results with lemon peel are presented in Table 4, to show how nearly the determinations on comparable samples were duplicated when the apparatus illustrated in Figure 1 was used.

For a set of four determinations, as shown in Table 4, 50 lemon fruits of the desired age were taken, one from the north side and one from the south side of each of 25 trees. When these fruits had been prepared as described in "Procedure," 100 disks of peel were taken, one at what might be termed the 6 o'clock position and one at the opposite or 12 o'clock position on the stem end of each fruit. After the oil in this sample had been determined, a second lot of disks was taken in the same manner from the 3 and 9 o'clock positions on the stem ends of the fruits. Determinations were made on similar samples of disks from the stylar ends of the fruits.

The results of these tests (Table 4) show that the amounts of oil obtained from comparable samples were the same or nearly the same; that there was less oil in the peel at the stem end than at the stylar end of the fruit; and that the peel of light silver (nearly mature) lemons contained more oil than that of lemons at the other stages of development.

TABLE 4.—*Oil obtained from peel of stem and stylar ends of lemons at different stages of maturity*

FRUIT COLOR AND TEST NO.	OIL OBTAINED PER 100 SQ. CM	
	STEM-END PEEL	STYLAR-END PEEL
	ml.	*ml.*
Dark green		
1a	0.57	0.65
1b	0.57	0.66
2a	0.58	0.67
2b	0.58	0.67
Light green		
3a	0.67	0.83
3b	0.69	0.86
4a	0.66	0.81
4b	0.67	0.82
Light silver*		
5a	0.74	0.87
5b	0.75	0.85
6a	0.74	0.86
6b	0.75	0.86
Yellow (tree ripe)		
7a†	0.51	0.57
7b†	0.51	0.57
8a‡	0.61	0.64
8b‡	0.62	0.65

* Intermediate between light green and yellow.
† Thin peel.
‡ Thick peel.

DISCUSSION

The apparatus illustrated in Figure 1 appears to have several distinct advantages over other types of apparatus used for the determination of volatile oils. There is little chance, in this apparatus, for the vaporized oil to escape, unless it is too volatile to be condensed after having passed down the entire length of the condenser; nor do the condensed oil and the water drip from the end of the condenser onto the oil in the trap below, as in some of the earlier designs, a condition which favors emulsification and tends to drive droplets of oil down into the water in the receiving flask, where it may be lost. This objection has been eliminated recently by means of a modified trap[4] in which the condensate falls on an inclined wall in the trap, and not on the oil. In this modified apparatus, however, a

reflux condenser is used, and if distillation is too rapid there is a chance that some of the lighter fractions of the vaporized oil may escape around the top of the condenser. This would be true especially if bumping occurred, which would more or less violently agitate the contents of the flask and produce strong puffs of air-laden vapor. There is also the possibility that fractions of the oil having a lower boiling point than water may condense on the upper part of the refluxing condenser where they cannot be washed down by the condensed water vapor.

The air-outlet tube sealed into the condenser just below the jacket (Fig. 1, I) reduces to a minimum the possibility of loss of condensed oil by evaporation and by being driven into the water in the receiving container (Fig. 1, B), especially when steam distillation is used. The amount of condensed oil that adheres to the inner wall of the lower end of the condenser, when the receiving flask is removed, is negligible. In one type of apparatus described[3] the droplets of oil may be driven down through the constricted portion of the receiver into the water reservoir below. This does not occur in the apparatus described in this paper.

The calibrated measuring tube (Fig. 1, J) is long enough so that small amounts of oil can be measured accurately, the 0.01 ml. graduations being 0.98 mm. apart. To determine its physical properties, the oil may be recovered by forcing it out of the top of the tube into a suitable container. There was some tendency toward emulsification in the connecting tube (Fig. 1, L) before it was insulated (M), but not afterward.

Another important characteristic of the apparatus shown in Figure 1 is that all internal walls are readily accessible and easily cleaned. After cleaning, all parts of the apparatus should be kept filled with distilled water until just before they are to be used. Excess water should be drained from the calibrated tube for 2 or 3 minutes before it is used.

SUMMARY

An apparatus for the determination of volatile oils is described. It has been found to have the following advantages:

(1) Chances for vaporized oil to escape, or for condensed oil to adhere to the condenser where it cannot be washed down, are reduced to a minimum.

(2) The condensed oil remains in a single small mass; it is not broken into droplets by the dripping effect of condensed liquids or by puffs of air originating in the distilling flask.

(3) The apparatus is so constructed that puffs of air do not tend to drive the condensed oil down into the water in the receiving flask, or to evaporate it.

(4) The calibrated measuring tube is long enough so that comparatively large amounts, as well as small amounts, of oil can be measured accurately.

(5) All inner walls of the apparatus are readily accessible for cleaning.

Results of determinations of volatile oils in samples of lemon peel are presented to demonstrate the usefulness and accuracy of the apparatus.

SOME CHECKS ON METHODS AND SOLUTIONS USED IN THE ANALYSIS OF FERTILIZERS*

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

It is always desirable in a control laboratory to obtain results as close to absolute accuracy as possible. In this laboratory over a period of years it has been found that such accuracy is not easily obtained in routine fertilizer analysis. It has been observed that different chemists using the same A.O.A.C. methods often obtain considerably different results on the same sample. Some of the causes for variation may be use of inaccurate weights, inaccurate volumetric apparatus, inaccurate values for standard solutions, or faulty technic. A study was made in this laboratory of various means of checking the A.O.A.C. methods used in fertilizer analyses, principally by analysis of pure compounds, and the procedures developed have proven advantageous.

The check fertilizer samples sent out by E. W. Magruder for a number of years have served a valuable purpose to fertilizer chemists in providing standards by which each chemist could check his analyses. A study of the results on these samples for 1944 show that while many analysts obtained closely agreeing results, the over-all variations were too great. Averages of the five highest results and of the five lowest results for nitrogen, available phosphoric acid, and potash for Samples 1, 2, 3, 4, and 5 are given in Table 1. The portions of these samples sent to this laboratory were uniform in appearance and about 96 per cent of mixed-fertilizer Samples 1 and 2 passed a 0.5 mm. sieve, and the remainder of these two samples was only slightly larger in particle size. The small particle size and the care with which these samples were prepared preclude the possibility that much of the variation in results is due to differences in the individual portions sent to each analyst. Further, if the variations were caused by differences in the separate portions, results should be closer for the one-component Samples 3, 4, and 5 than for the mixed-fertilizer Samples 1 and 2.

It is believed that a wider use of check determinations with pure compounds as outlined in this paper would result in better agreement by analysts. Many analysts use check determinations regularly, and some of the procedures described in this paper will of course not be new to them.

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* This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director. Presented before the Division of Fertilizer Chemistry at the 108th Meeting of the American Chemical Society, New York, N. Y.

MOISTURE

The moisture results on the Magruder check samples have varied more than the results for any other component. A temperature of 100°C. is too high for satisfactory moisture determination in fertilizers. Whittaker and Ross (*11*) have adequately discussed the reasons for this. There is need for a rapid low temperature method for determination of moisture in fertilizers.

NITROGEN

The nitrogen determination may be checked by use of a sample of reagent grade sodium nitrate and by a similar sample of ammonium sulfate, which are dried at 120°C. The nitrogen in the sodium nitrate is determined by the official A.O.A.C. Kjeldahl method to include nitrate nitrogen (*3*), using a 0.3502 gram sample. Sufficient time must be allowed for complete solution of the nitrate in the salicylic-sulfuric acid mixture before digestion. This laboratory obtained 16.23 per cent nitrogen when the sample was allowed to stand only 10 minutes in this mixture and 16.48 per cent, the theoretical amount, when the time was increased to 30 minutes. For the determination with ammonium sulfate a 1.4008 gram sample (or 14.008 times normality of standard alkali if this normality is slightly different from 0.1 *N*) is transferred to a 200 ml. volumetric flask, dissolved in water, and made to volume. The nitrogen is determined in the usual way, using a 50 ml. aliquot of this solution. It is preferable to use the normality of the standard alkali solution as determined by titration in the same buret that was used for titration with standard alkali in the routine analysis. Slight changes in the standard alkali due to the action of the alkali on the glass, to condensation of moisture on the sides of the bottle, or to changes in temperature may be corrected by direct titration of the alkali with a standard acid and by making the necessary change in the weight of the sample. If 0.1 *N* alkali and a 1.4008 gram sample are used, the per cent nitrogen may be found by subtracting the titration reading from the acid-alkali blank and by moving the decimal point one place to the left. If the alkali is not exactly 0.1 *N*, the weight of sample is 14.008 times normality of alkali and the per cent nitrogen may be obtained as described. Considerable inaccuracy in the buret may be overcome by this procedure. Ammonium sulfate has a theoretical nitrogen content of 21.20 per cent, or the equivalent of 5.30 per cent for the aliquot used. By preparing a larger volume of the ammonium sulfate solution, the nitrogen determination may be checked at any time by adjusting the solution to the original temperature and taking the aliquot.

TOTAL PHOSPHORIC ACID

The standards used to check total phosphoric acid are the Bureau of Standards rock phosphate sample and reagent grade primary potassium

phosphate. The rock phosphate is dried at 105°C. and the potassium phosphate is dried over sulfuric acid. The rock phosphate analysis is made according to the A.O.A.C. method (*4*), using 8 (b) for solution of sample. A 1.25 gram sample of the primary potassium phosphate is transferred to a 250 ml. volumetric flask, 50 ml. of water and 5 ml. of nitric acid are added, and the solution is boiled. A 0.05 gram aliquot is taken for analysis. The ammonium phosphomolybdate precipitate is agitated by shaking at room temperature.

Richardson (*7*) attributed the high results when precipitation is made in the presence of sulfates to the formation of an ammonium sulfomolybdate which is acidic in nature. This was confirmed by Falk and Suguira (*2*). In 1929 the A.O.A.C. volumetric method was changed to require precipitation at room temperature with shaking. Ross (*9*) concluded that samples containing considerable organic matter should be digested in sulfuric acid and that satisfactory results could be obtained if the precipitation was made at 25°–30°C. with continuous stirring. Collaborative work with only one sample was conducted on this phase of his investigation.

Investigation in this laboratory indicates that the volumetric procedure may give high results in the presence of sulfate even when the precipitation is made at room temperature, and it seems probable that the presence of sulfate causes the formation of some ammonium sulfomolybdate at 25°–30°C. High results were obtained by the volumetric procedure when the two standard samples were digested in sulfuric acid (Table 2). Digestion with sulfuric acid gave theoretical results when the gravimetric method was used.

POTASH

Reagent grade potassium chloride dried at 120°C. is used as a check on the potash determination. The A.O.A.C. method for potash salts (*5*) is applied, using a 0.125 gram aliquot. This serves as a check on the purity of the chloroplatinate solution and on the washing procedure, rather than on the method applied to mixed fertilizers.

The results in Table 3 compare the use of 95, 84, and 80 per cent alcohols for preparation of the acid alcohol and for transferring and washing potassium chloroplatinate precipitates. The results in Column 3 were obtained by rewashing these precipitates with five successive 5 ml. portions of the respective alcohols, after which the crucibles were dried and reweighed. The potassium chloroplatinate was then dissolved in hot water, the crucibles were again dried and weighed, and the per cent potash was determined. The acid alcohols were prepared by mixing the respective strengths of alcohol with hydrochloric acid in the proportion specified in the A.O.A.C. method for 80 percent alcohol. The specific gravity of the 84 percent alcohol was 0.845 at 25°/25°C. and that of the 80 percent alcohol was 0.857 at 25°/25°C.

The extreme differences obtained by varying the strength of the alcohol and the amount of washing are nearly as great as the differences in the results on the Magruder check sample 4. The low results obtained with 80 percent alcohol are evidently due to solubility of potassium platinic chloride in this alcohol. The high results obtained with 95 percent alcohol may be due to the failure of this alcohol to wash out all excess platinum chloride or ammonium chloride. Acid alcohol made with 84 per cent alcohol, and 84 percent washing alcohol seem to be satisfactory. Duplicate determinations using another platinum chloride solution prepared by dissolving pure platinum, gave similar results. Differences were negligible on two groups of determinations in which the temperature of the alcohols was kept at 25° and 30°C., respectively.

In determining potash in mixed fertilizers variable amounts of insoluble residues are sometimes found in duplicate analyses. An investigation revealed that when the 1 ml. of normal sodium hydroxide used in the determination was from a freshly prepared solution, much smaller residues resulted. It appears that when the alkali solution stands for some time in glass bottles, even in Pyrex, the dissolved constituents of the glass increase the insoluble residue in the potash determination. The average insoluble residue in nine determinations in which freshly prepared sodium hydroxide solution was used was 0.26 mg. In nine duplicate determinations using a recently filtered sodium hydroxide solution which had stood in a Pyrex bottle for several months the average insoluble residue was 1.6 mg. For accurate results it is necessary to dissolve the potassium chloroplatinate and reweigh the crucible. Platinum dishes were used in all determinations, since Allen (*1*) found that larger insoluble residues resulted when ignition was made in silica dishes.

ACID AND NON-ACID-FORMING QUALITY

A reagent grade calcium carbonate dried at 120°C. is used as a standard. A 0.5 gram sample and 35 ml. of normal hydrochloric acid are preferable. Here, as in all of the determinations with pure compounds, theoretical results do not insure absolute accuracy in the same determination with mixed fertilizers or materials. However, if theoretical results are not found with pure salts, accurate results will not be obtained with mixed fertilizers.

NEUTRAL AMMONIUM CITRATE SOLUTION

Robinson (*8*) originally recommended "that a neutral solution of ammonium citrate be considered as one in which the ratio of ammonia to anhydrous citric acid is 1 to 3.794 and which shall contain 45.33 grams of ammonia and 172.00 grams of anhydrous citric acid per liter at 20°." This was determined by analysis in triplicate of eight solutions of carefully prepared neutral ammonium citrate by the method of Patten and Marti (*10*). Using a glass electrode to determine *p*H the writer duplicated very

TABLE 1.—*Variations in results of Magruder check fertilizer samples*

	NITROGEN	AVAILABLE PHOSPHORIC ACID	POTASH
	per cent	*per cent*	*per cent*
	Sample 1, 4-10-4		
Av. 5 highest analyses	4.47	10.85	4.48
Av. 5 lowest analyses	4.20	10.32	4.01
Difference	0.27	0.53	0.47
Max. difference	0.45	0.62	0.87
	Sample 2, 4-9-3		
Av. 5 highest analyses	4.23	10.04	3.56
Av. 5 lowest analyses	3.98	9.49	3.13
Difference	0.25	0.55	0.43
Max. difference	0.31	0.75	0.54
	Sample 3, 0-19-0		
Av. 5 highest analyses		20.40	
Av. 5 lowest analyses		19.51	
Difference		0.89	
Max. difference		1.01	
	Sample 4, 0-0-62		
Av. 5 highest analyses			63.09
Av. 5 lowest analyses			62.07
Difference			1.02
Max. difference			1.36
	Sample 5, 8-0-0		
Av. 5 highest samples	8.52		
Av. 5 lowest samples	8.16		
Difference	0.36		
Max. difference	0.58		

closely the results of Robinson. The present A.O.A.C. method (*6*) specifies that the solution shall have a specific gravity of 1.09 at 20°C. and a *p*H of 7.0. The usual summer laboratory temperature here is considerably higher than the 20°C. specified; and further, different hydrometers do not give uniform readings. Therefore it has been found advisable to standardize this solution at room temperature, after adjusting it to *p*H 7.0, to contain the amounts of ammonia and citric acid recommended by Robinson. With the hydrometer in use in this laboratory the specific gravity of the solution is about 1.0895 at 25°C. and 1.0885 at 30°C. A hydrometer having an over-all length of 250–350 mm. is preferable to the shorter types. It is not necessary to analyze the solution each time it is made, provided the hydrometer has been checked at the prevailing room temperature. This procedure amounts to standardization of the hydrometer at the temperature used. For practical purposes, standardization at only 25° and 30°C. is necessary.

The methods used for determining ammonia and citric acid are slight modifications of the methods of Patten and Marti (*10*). A 50 ml. portion of the ammonium citrate is diluted to 500 ml. To determine ammonia, 10 ml. of this solution is transferred to a Kjeldahl flask and diluted to 300

TABLE 2.—*P_2O_5 in standard samples as determined by various procedures*

SAMPLE	DIGESTED WITH HNO_3—HCl		DIGESTED WITH H_2SO_4	
	VOLUMETRIC	GRAVIMETRIC	VOLUMETRIC	GRAVIMETRIC
	per cent	*per cent*	*per cent*	*per cent*
KH_2PO_4*	52.30	52.16	53.00	52.16
Rock Phosphate†	31.40	31.34	32.00	31.32

* Theory 52.16%.
† Bureau of Standards Sample No. 56. Theory 31.33%.

TABLE 3.—*Effect of different strengths alcohol and of amount of washing on determination of potash in KCl**

		USUAL PROCEDURE	WEIGHED K_2PtCl_6 REWASHED†	LOSS IN WEIGHT OF K_2PtCl_6‡
Per Cent		*Per Cent K_2O*	*Per Cent K_2O*	*mg.*
95	Acid alcohol			
	and	63.40	63.37	0.2
95	Washing alcohol			
95	Acid alcohol			
	and	63.26	63.15	0.8
84	Washing alcohol			
84	Acid alcohol			
	and	63.15	63.00	1.0
84	Washing alcohol			
80	Acid alcohol			
	and	62.70	62.46	1.5
80	Washing alcohol			

* Theory 63.17% K_2O. Results are average of two or more analyses.
† Figures in this column are results of rewashing K_2PtCl_6 precipitates with five successive 5 ml. portions of the 95, 84, and 80% alcohol, respectively.
‡ In second washing.

ml.; a slight excess of alkali is added; and the ammonia is distilled, collected, and titrated in the usual way (ml. of 0.1 N alkali$\times$1.7032 = grams of ammonia per liter). To determine the citric acid, 10 ml. of the same solution is transferred to an Erlenmeyer flask; 4 ml. of 40 per cent neutral formaldehyde and 20 ml. of water free from carbon dioxide are added;

the flask is stoppered; and the mixture is allowed to stand 30 minutes. The liberated acid is titrated with 0.1 *N* alkali, free from carbonates, using phenolphthalein indicator (ml. of 0.1 *N* alkali$\times$6.403 = grams of citric acid per liter). Blank determinations should be made for both the ammonia and citric acid analyses.

Moderate differences in the ammonia and citric acid contents of the ammonium citrate solution do not appreciably change the citrate-insoluble P_2O_5 result in the average mixed fertilizer; however, there are many possible sources of error in this determination, and two or more small errors in the same direction can affect the result. Some work has been done on determination of specific gravity of this solution by means of the pycnometer, but this does not appear to be practical for routine work.

SUMMARY

Averages of results on the Magruder check samples show that the overall variations between different analysts are too great. Procedures for checking nitrogen, phosphoric acid, and potash determinations on standard samples are given, and reasons for some variations in results are stated. Advantages of using the normality of the standard alkali as determined by titration in the same buret used in the routine nitrogen determination are pointed out. It is shown by analysis of standard samples that high results may be obtained with the A.O.A.C. volumetric method for phosphoric acid when sulfuric acid is used for solution of sample. Differences of about 1 per cent potassium oxide may be obtained in analysis of reagent grade potassium chloride by varying the strength of the alcohol from 80 to 95 per cent and by varying the amount of washing. An acid alcohol prepared with 84 per cent alcohol and 84 per cent washing alcohol gives theoretical results. A procedure is given for preparing the neutral ammonium citrate solution at the prevailing room temperature by determining the ammonia and citric acid contents. This serves also to standardize the hydrometer at this same temperature.

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APPLICATION OF A MODIFIED RED-COLOR TEST FOR ROTENONE AND RELATED COMPOUNDS TO *DERRIS* AND *LONCHOCARPUS*

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Since published by Gross and Smith, the red-color test for rotenone and related compounds (rotenoids)[1] has undergone several modifications by various authors designed to make it more precise or to apply it to other compounds. A bibliography of the method is given in a review by Jones.[2] Great interest was aroused in the method when it was shown by Jones *et al.*[3] that of several criteria tested the red-color test was most closely correlated with toxicity to houseflies. This result bore out the contention of many workers in this field that some non-rotenone constituents of the total extractives contribute to insecticidal vlaue and that the red-color test is a fair criterion of toxicity. However, as pointed out by Haller,[4] since this test is not specific for rotenone, it should be applied with caution to plants outside the genera *Derris*, *Tephrosia*, and *Lonchocarpus*.

In this paper attention is directed toward modifications that lead to increased precision in the results of the red-color test and to the applicability of the method to various plant materials. The method used is a modification of that given by Goodhue.[5]

METHOD

REAGENTS

(a) Dilute 1 volume of H_2SO_4 to 4 volumes, with water at room temperature (27°C.).

(b) Dissolve 1 gram of $NaNO_2$ in 10 ml. of water and dilute to 1 liter with 95% alcohol previously purified by refluxing for 1 hour with 5 grams of zinc and 8 grams of KOH and then distilling off the alcohol.[6]

(c) Dissolve 40 grams of KOH in water and dilute to 100 ml.

(d) Mix 1 volume of reagent (c) with 7 volumes of reagent (b). This solution need not be prepared daily.

DETERMINATION

To 2 ml. of an acetone extract containing the rotenone or related compounds in a 50 ml. conical flask, add 2 ml. of reagent (d), swirl, and place in a water bath maintained at 30°C. by means of a thermostat. After 5 minutes quickly invert an ordinary ½ inch test tube containing 5 ml. of reagent (a) into the flask and swirl for a few seconds, keeping the flask partly submerged in the water bath. After an additional 5 minutes, determine the transmittance of the red solution by comparing it with water in a Coleman double monochromator spectrophotometer with a 30 mμ slit set at the wave length 540 mμ. (Other methods of color comparison may be used if desired.)

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MODIFICATIONS

When ordinary alcohol was used in reagent (**b**) it was noted that reagent (**d**) after a day or so became light yellow and gave a higher transmittance for a given rotenone sample. On the assumption that this was due to browning of impurities in the alcohol by the potassium hydroxide, purified alcohol was tried, with the result that the reagent remained clear and colorless for weeks and gave the same transmittance for a given amount of rotenone. It was noted, however, that contact between this reagent and rubber caused rapid discoloration.

The most important source of error in the original method was variation of the temperature at which the red color was developed. A lower temperature of the water in which the flask was immersed when the acid was added led to more rapid dissipation of the heat of neutralization and dilution with consequent lower transmittance of the red color corresponding to a given amount of rotenone. Thus the transmittance of the red color developed from rotenone at a concentration of 0.08 mg./ml. was 8.3, 15.3, and 32.3 per cent at 25°, 30°, and 35°C., respectively. Although lower temperatures make the test more sensitive, there are two reasons for not using such temperatures. First, it is more convenient to set a thermostat a little above ordinary room temperature since provision need not be made for cooling the water; second, the more sensitive the test, the greater the relative error of a determination due to the fixed instrumental and methodical error. For example, when replicate samples were tested, the transmittances were within a range of 1 per cent due to combined methodical errors including that due to the instrument itself. This error of 1 per cent would cause an error in the determination itself which increases in proportion to the steepness of the transmittance-concentration curve.

The second important source of error was the manner in which the acid, reagent (**a**), was added to the alkaline solution of rotenone. Slow addition caused an erratic increase of several per cent in the transmittance and resulted in a more or less evanescent white precipitate in the flask. The difficulties were overcome by measuring the acid into a test tube and then inverting the tube into the flask at the exact time desired while the flask was being swirled partly submerged in the water bath. By this method the results were more precise and there was no trouble with a precipitate. Other variables tested were the effect of intensity and color of the light falling on the flask while the test was being made, and the effect of the age of the sodium nitrite solution. Variations due to these factors were found to be of the same magnitude as the error of the instrument.

To find the optimum amount of acid to develop the color, trials were made on portions of 2–7 ml. The transmittance increased with the amount of acid, but with small amounts there was more difficulty in dissolving the evanescent, white precipitate formed when the first part of the acid was mixed with the solution. The maximum extinction per unit volume

was found when 4 ml. of acid was used, but since the precipitate was somewhat persistent it was decided to use 5 ml. to avoid this difficulty.

The effect of the elapsed time between mixing the sample with the potassium hydroxide-sodium nitrite reagent and subsequent acidification was also studied. Using a solution of rotenone in acetone, and an acetone extract of powdered *Derris* root, it was found in both cases that as the time between mixing with reagent (**d**) and acidification was increased from 0.5 to 3 minutes the resulting transmittance steadily decreased. With intervals of from 3 to 6 minutes a minimum transmittance was obtained. If more than 6 minutes elapsed the transmittance began to increase. Therefore the time was taken as 5 minutes. Since the differences caused by changing the time from 5 to 4 minutes, or to 6 minutes, were found to be of the same order of magnitude as the error of the method, the time need not be measured exactly but can be taken as 5 ± 0.5 minutes.

MEASUREMENT OF THE RED COLOR

A Coleman double-monochromator spectrophotometer, Model 10S, using the 30 mμ slit in conjunction with a Coleman electrometer, Model 310, was highly satisfactory for the measurement of the red color. The transmittance at 540 mμ was constant with time, but at lower wave lengths it changed with time. Therefore, in order to obtain a spectrogram of the red color at a certain time after mixing the rotenone solution with the first reagent, it was necessary to measure transmittances at all wave lengths as a function of time and then interpolate to obtain the reading corresponding to a fixed time. This was done as follows: From an aliquot of a standard rotenone solution a red color was developed and the solution was transferred to the photometer cell at 10 minutes after mixing; transmittance readings were made at several wave lengths at intervals of 30 mμ, and the time of reading was recorded; this operation was repeated twice so that the transmittance at each wave length was measured as a function of time after mixing. The process was repeated with additional aliquots over the spectrum available on this instrument (380–1000 mμ). From the plot of the transmittance vs. time, the transmittance at 15 minutes after mixing was read off for each wave length and plotted to obtain the spectrogram of the red color at 15 minutes after mixing. The plot showed maximum absorption at 540 mμ and minimum from 700 to 1000, and another at 430 mμ.

Upon testing solutions of graded rotenone concentrations, it was found that Beer's law was valid, i.e. the plot of log transmittance vs. concentration was a straight line. However, since at high concentrations the transmittances sometimes tend to be too high, it is best to use concentrations that give transmittances of over 20 per cent.

RED COLOR GIVEN BY SOME COMPOUNDS RELATED TO ROTENONE

The red color values of some compounds related to rotenone are presented in the form of a spectrogram in Figure 1. The compounds were rotenone (m.p. 163°C.), 1-elliptone (m.p. 157°), deguelin (m.p. 169°),

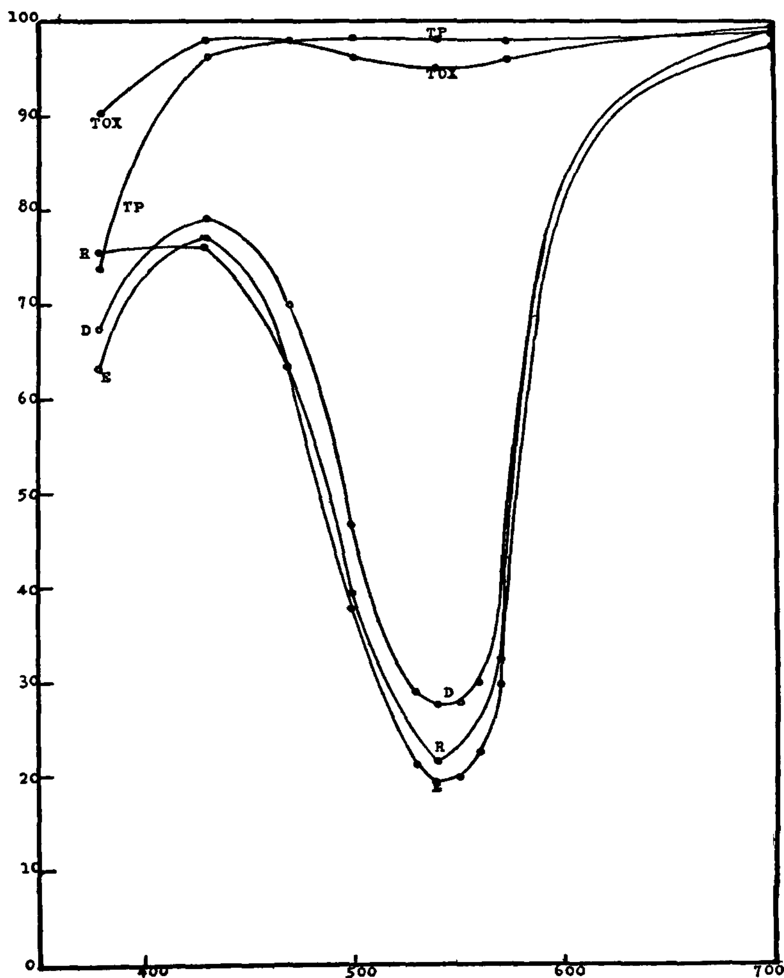

Fig. 1.—Spectrogram of the color produced from rotenone (R), 1-elliptone (E), deguelin (D), Toxicarol (TOX), and tephrosin (TP) by the red-color test. All compounds were 0.06 mg. per ml.; the reference solution was a blank.

toxicarol (m.p. 209°), and tephrosin (m.p. 195°).* All were tested at a concentration of 0.06 mg. per ml. For all of these tests the reference solution was a blank prepared from pure acetone plus the reagents, since at the low wave lengths the slight turbidity of the red solution, invisible to the eye, caused the transmittance to be low. Such a blank compared with water gave about 56 per cent transmittance at 380 mμ. (The instrument is a good turbidimeter at low wave length.)

The figure shows that the compounds have different spectrograms but that the general form of each is the same. It is also apparent that rotenone-bearing plant material containing these compounds as well as others will have a red-color value greater than that due to rotenone content.

On the basis of these data an attempt was made to estimate the concentrations of the components in mixtures of these compounds by measuring transmittances at several wave lengths and solving simultaneous equations for concentrations. However, it became clear that the method was definitely limited because the spectrograms of the constituents were not sufficiently different, and further because the number and nature of the components in the plant extracts were not known.

RELATION BETWEEN ROTENONE CONTENT AND RED-COLOR VALUE IN SAMPLES OF *DERRIS* AND *LONCHOCARPUS* ROOT

Since the red-color test cannot be used to measure rotenone in plant extracts, it became of interest to investigate the relation between red-color value and rotenone content. In this work rotenone was determined by the official A.O.A.C. gravimetric method[7] and the red-color value by the method given in this paper. The red-color value is expressed as the percentage of rotenone that would have to be present in a given sample to give the density of red color found by the red-color test. In other words, it is the rotenone equivalent as measured colorimetrically.

In preliminary trials it was found that 500 mg. samples of root that had been ground through a ½-mm. sieve in a No. 1 Wiley mill could be satisfactorily extracted by soaking for 16 hours in 50 ml. of acetone. Soaking for 15 minutes yielded about 87 per cent, for 3 hours about 94 per cent. Extraction for 12 hours in a Soxhlet apparatus, followed by standing overnight before removal, failed to yield more rotenone. With some plant materials it actually gave poorer results, probably because of heat-induced decomposition. Shorter Soxhlet extractions were not so efficient.

Therefore, small samples of the ground material (usually a little less than 500 mg.) were rapidly weighed on a microtorsion balance, 50 ml. of acetone (U.S.P. or better) was added, and the flasks were swirled intermittently until the following day, when the samples were cooled in a bath

* The 1-elliptone and deguelin were furnished by L. D. Goodhue, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md. Attempts to obtain deguelin from *Lonchocarpus chrysophyllus* roots grown at this station showed that only traces were present. This was confirmed by analyses made by Goodhue.

[7] *Methods of Analysis, A.O.A.C.*, 1940, 64.

of ice water and filtered. After the liquid had returned to room temperature, an aliquot of the filtrate, usually 1 ml. with high quality samples, was diluted to 50 ml. with acetone, and 2 ml. of this mixture was taken for the red-color test.

The samples were handled in groups of 10 or less, and reagent (**d**) was added to each. After 5 minutes the acid tubes were inverted into the samples in the water bath in the same order so that all samples reacted 5 minutes with reagent (**d**) before reagent (**a**) was added. In each group there was included at least one standard rotenone sample as a check. (Since the acid in the tubes was not kept at 30°, the red-color value of the standard changed slightly with the room temperature.)

Seven samples from regional test plots of *Derris* grown in Puerto Rico gave ratios of rotenone (gravimetric) to red-color value ranging from 0.343 to 0.387, with a mean of 0.364 ± an average deviation of 0.012. The ratios for 29 samples of *Derris* grown at this station for an age-at-harvest experiment varied from 0.360 to 0.427, with a mean of 0.390 ± an average deviation of 0.013.

In 23 *Derris* samples collected in Guatemala the ratios were from 0.082 to 0.529, with a mean of 0.369 ± an average deviation of 0.089. However, it is important to note that in this group the results for rotenone (gravimetric) had a comparatively large relative error in some samples of low rotenone content. In the sample having a ratio of 0.082 the rotenone content was 0.22 per cent. It is obvious that the smaller the numerical values that make up the ratio, the more sensitive the ratio will be to small errors in either the numerator or denominator. Considering only the 14 samples having over 1.5 per cent rotenone (dry basis) the average ratio was 0.438 ± an average deviation of 0.055.

A similar wide range of ratios was found in a group of samples of *Derris* grown in the station greenhouse; here the ratios ran from 0 to 0.504 with a mean of 0.364 ± an average deviation of 0.107. When only the 27 samples having over 1.5 per cent rotenone were considered, the ratios ranged from 0.330 to 0.504, the mean being 0.434 ± an average deviation of 0.043.

With a group of 49 *Lonchocarpus* samples collected in South America ratios of rotenone to red-color value varied from 0.348 to 0.570 with a mean of 0.448 ± an average deviation of 0.028.

Thus far the ratios listed have averaged about 0.4. The only exception encountered here was a group of four *L. chrysophyllus* samples grown in Puerto Rico in which the range of ratios was from 0.754 to 0.819 and the mean 0.779 ± an average deviation of 0.020.

The data are summarized in Table 1.

At present very little is known of the effect of age of the root of young plants on the ratio of rotenone to red-color value. The only data in this laboratory are on two composite samples of root from 2-month-old leafy cuttings of *Derris* in which the rotenone concentrations were 1.07 and

TABLE 1.—*Ratios of rotenone to red-color value for some groups of samples of Derris and Lonchocarpus root powder*

SOURCE	TYPE	NUMBER OF SAMPLES	RANGE OF RATIOS	AVERAGE RATIO	AVERAGE DEVIATION
Puerto Rico, regional plantings	*D. elliptica* Sarawak Creeping	7	0.343–0.387	0.364	0.012
Puerto Rico, age-at-harvest expt.	*D. elliptica* Sarawak Creeping	29	.360– .427	.390	.013
Guatemala	*D. elliptica*	14	.317– .529	.438	.055
Puerto Rico, greenhouse-grown plants	*D. elliptica* Sarawak Creeping	27	.330– .504	.434	.043
South America	*Lonchocarpus* sp.	49	.348– .570	.448	.028
Puerto Rico	*L. chrysophyllus*	4	.754– .819	.779	.020

0.85 per cent and the corresponding ratios 0.310 and 0.271. Although the rotenone concentrations were excessively low, the data indicate that the ratio of rotenone to red-color value would be about the same in young and old roots.

APPLICATIONS OF THE RED-COLOR TEST

Small Samples.—The colorimetric method is particularly useful in the analysis of small samples. Among such samples tested were various parts of root systems being studied to determine whether any part was particularly rich in rotenone, or whether any part could be taken as typical of the whole system. Three plants were excavated from field plantings, and the position and course of roots were noted. After drying, each root system was divided into 15–24 portions, and the root diameter and the position in the root system were observed.

The red-color test applied to each of the samples showed no consistent relationship between red-color value and diameter or position. On one plant a rather gnarled root showed a low red-color value, about one-third to one-sixth that of other roots of corresponding diameter. Among roots of a given diameter some were nearly equivalent in red-color value while others showed variations up to about 100 per cent. In general the fine roots (in the neighborhood of 1 mm. in diameter) were inferior. Neither shallow nor deep roots were consistently rich or poor in quality. No particular diameter was found to be optimum for red-color value,* nor did the degree of branching appear to affect the quality of the root.

It was apparent that factors other than the diameter or position deter-

* Previously it had been shown that for large groups of root there was an optimum diameter, around 5 mm., for maximal rotenone percentage. Moore, R. H., Puerto Rico Expt. Sta., U. S. Department of Agriculture, Rpt. 1940, 48–49 (1941).

mined the amount of rotenone to be found therein. However, this furnishes an example of the application of the red-color test to small samples.

Plant Selection.—It can be concluded that if the analyst knows something of the relation of rotenone to red-color value in the material at hand, and if highly accurate results are not required, it is possible to use the red-color test.

Because of the nature of the work, this test would seem to be particularly applicable to plant selection. The analytical problem here resolves itself into finding a rapid method by which large numbers of samples can be tested at a reasonable cost. This last consideration—that of cost of analysis—rules against the use of the official method. Working 48 hours a week a chemist with a helper can maintain a rate of about 36 samples per week by the official method for rotenone; in the same length of time the same man and helper would be able to analyze about 5 times as many samples by the red-color method.

When the difficulty of selecting a representative sample of root material from a growing plant is considered, especially under ordinary field or jungle conditions, it seems futile to require a rigorous analysis. Furthermore, it must be remembered that cuttings may not produce the same quality root as the parent plant from which they were taken, because of the marked effects of climate and soil on the rotenone-forming mechanism of the plant. A more feasible method would be to use the red-color test and multiply the red-color value by 0.4 to obtain an estimate of the rotenone content (unless one is dealing with a class of material known to have a different ratio of rotenone to red-color value); or the selection could be based on red-color value alone. In this manner low rotenone clones could be discarded and promising clones could be propagated for further study. The official method could then be used to check samples of particular interest.

SUMMARY

(1) Modifications are described for the red-color test for rotenone and related compounds present in samples of plant material.

(2) The analysis of mixed plant extracts by differential spectrophotometric analysis of the red-color test was found impracticable.

(3) The ratio of rotenone to red-color value was measured for root samples of *Derris* and *Lonchocarpus*.

(4) The applicability of the red-color test to the analysis of small samples and in plant selection was discussed.

ACKNOWLEDGMENT

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COMPARISON OF SEVERAL METHODS FOR DETERMINING THE SULFUR CONTENT OF FEEDS*

By ROBERT JOHN EVANS and J. L. ST. JOHN (Division of Chemistry, Washington, Agricultural Experiment Station, Pullman, Washington)

No methods are given in the official methods of the A.O.A.C. for the determination of the total sulfur in cereal foods, dairy products, grain and stock feeds, meat and meat products, or other foods. Two methods are described as official for the determination of sulfur in plants, the magnesium nitrate method and the sodium peroxide method (*1*). A number of methods have been suggested for the determination of sulfur in feeds and related materials. Several of these were compared during an investigation of the sulfur amino acids of certain feeds. The results are presented here since they should be of interest to those studying feeds, foods, and plant materials.

The Parr bomb has long been used for the determination of sulfur in feeds (*10*). Only 0.5 gram samples are ordinarily used. This does not allow the degree of accuracy desired for feeds low in sulfur. In this study the Parr bomb method (*10*) was used as a standard in comparisons with the magnesium nitrate method (*1*), the sodium peroxide method (*1*), and several other methods used in the analyses of fish meal, soybean oil meal, and ground wheat.

METHODS

Parr bomb method (*10*), magnesium nitrate method (*1*), and sodium peroxide method (*1*).

Alkaline permanganate method A is the Potter and Jones (*12*) modification of the method of Pollock and Partansky (*11*).

Alkaline permanganate method B is the procedure of Stotz (*16*).

The Benedict-Denis procedure used is Frear's (*4*) modification for the determination of sulfur in plants.

Nitric-perchloric acid method A is a determination of sulfate on a solution prepared by Gerritz's (*5*) procedure for calcium and phosphorus.

Nitric-perchloric acid method B is the method of Evans and St. John (*3*).

RESULTS AND DISCUSSION

Table 1 presents a comparison of the Parr bomb and magnesium nitrate methods for total sulfur. Samples of herring fish meal, casein, soybean oil meal, cottonseed meal, ground peas, and ground wheat were used. The results were low by the magnesium nitrate method in all cases and also variable. For example, values of 0.568, 0.813, and 0.874 were obtained on

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one sample of herring fish meal by the magnesium nitrate method. The low results obtained in some cases may have been caused by mechanical losses due to the very fine ash formed, although St. John (*14*) did not find that what appeared to be losses from "fly ash" affected the results in the ash determination. The use of watch-glasses to prevent loss did not result in values as high as those obtained by the Parr bomb method, though in

TABLE 1.—*Comparison of the Parr bomb, with the magnesium nitrate and sodium-peroxide methods for total sulfur determination*

SAMPLE	PARR BOMB	MAGNESIUM NITRATE	$\frac{\text{MG.}(NO_3)_2}{\text{PARR BOMB}} \times 100$	SODIUM PEROXIDE	$\frac{\text{SODIUM PEROXIDE}}{\text{PARR BOMB}} \times 100$
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Herring fish meal	0.942	0.568	60.3	0.947	100.5
	—	0.813	86.3	—	—
	—	0.874	92.8	—	—
Herring fish meal	0.961	0.713	74.2	—	—
	—	0.629	65.5	—	—
Herring fish meal	0.958	0.808	84.3	0.955	99.7
Casein	0.655	0.490	74.8	—	—
Casein	0.675	0.520	77.0	0.675	100.0
Meat meal	0.483	—	—	0.475	98.3
Soybean oil meal	0.445	0.400	89.9	—	—
Soybean oil meal	0.414	—	—	0.416	100.5
Soybean oil meal	0.409	0.314	76.8	0.434	106.1
	—	0.396	96.8	—	—
Soybean oil meal	0.444	0.376	84.7	—	—
Cottonseed meal	0.496	0.414	83.5	0.476	96.0
Cottonseed meal	0.418	—	—	0.400	95.7
Alaska peas	0.152	0.128	84.2	—	—
Ground wheat	0.127	0.077	60.6	0.134	105.5
	—	0.113	89.0	—	—
Ground corn	0.139	—	—	0.134	96.4
Average	—	—	80.0	—	99.9

the case of some of the plant products there was occasional fairly close agreement.

The magnesium nitrate method was developed for use with seeds (*6, 7, 8*). Latshaw (*6*) obtained results in fair agreement among the sodium peroxide method, the magnesium nitrate method, and the Parr bomb method with soybean oil meal, cottonseed meal, and mustard seed. The results in Table 1 do not agree with Latshaw's though the magnesium nitrate method showed a trifle better agreement on seed products than on animal products.

The Parr bomb and sodium peroxide methods for total sulfur are also compared in Table 1. The agreement between these two methods is good. The sodium peroxide method showed some definite disadvantages. These

include a tendency for the sample to explode or ignite when an incorrect amount of sodium peroxide was added or when the sample was heated too rapidly. Too much sodium peroxide caused the ignition of the sample during the preliminary fusion, but too little caused it to ignite on final fusion because of incomplete preliminary fusion. The quantities of sodium peroxide and the required consistency of the sample varied with the type of material analyzed. A high salt concentration after solution caused much bumping, and frequent loss of the determination during precipitation of the barium sulfate. The sodium peroxide method was also the most time consuming and laborious of any of the methods used.

The results of the comparison of several methods for determining the sulfur in fish meal, soybean oil meal, and ground wheat are presented in Table 2. The only methods that gave as high a sulfur content for each of

TABLE 2.—*Comparison of several methods for determining total sulfur in feeds*

METHOD	HERRING FISH MEAL	SOYBEAN OIL MEAL	GROUND WHEAT
	per cent	*per cent*	*per cent*
Parr bomb	0.942	0.409	0.127
Magnesium nitrate	0.624	0.322	0.086
Sodium peroxide	0.947	0.434	0.135
Alkaline permanganate A	0.724	0.336	0.116
Alkaline permanganate B	0.726	0.384	0.142
Benedict-Denis	0.726	0.342	0.122
Nitrid-perchloric acid method A	0.634	0.286	0.109
Nitric-perchloric acid method B	0.943	0.416	0.127

the three samples as did the Parr bomb method were the sodium peroxide method and the nitric-perchloric acid method B. Next to the magnesium nitrate method the nitric-perchloric acid method A gave the lowest results. Evans and St. John (*3*) showed that nitric-perchloric method A does not oxidize more than approximately 7.0 per cent of the methionine sulfur to sulfate. Other papers from this laboratory such as that of St. John and Midgley (*15*) have in general shown more complete recovery through the use of perchloric acid. The Benedict-Denis and the two alkaline permanganate methods apparently do not recover all of the sulfur. Callan and Toennies (*2*) showed that the low values obtained by the alkaline permanganate methods are due to incomplete oxidation of the methionine sulfur to sulfate, since they could recover none of the sulfur as barium sulfate after oxidizing methionine by these methods. Potter and Jones (*12*) reported complete recovery of the sulfur from both cystine and methionine by both the alkaline permanganate method and the magnesium nitrate method. Results presented here suggest that methionine may be incompletely oxidized to sulfate by the alkaline permanganate method. Painter and Franke (*9*) and Rutenber and Andrews (*13*) obtained low re-

sults for the sulfur of methionine with the Benedict-Denis procedure, but Painter and Franke obtained good recovery of the sulfur in cystine.

SUMMARY

A comparison was made of the official A.O.A.C. magnesium nitrate and sodium peroxide methods, the alkaline permanganate method, the Benedict-Denis method, and two nitric-perchloric acid digestion methods, with the Parr bomb method for determining the total sulfur content of feeds. Only one of the nitric-perchloric acid digestion methods and the sodium peroxide method gave as high values as did the Parr bomb method. The magnesium nitrate method gave low and variable values for the sulfur content of the feed samples studied.

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DETERMINATION OF THIOCYANATE NITROGEN IN ORGANIC THIOCYANATES AND MIXTURES

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This paper is an account of a phase of work pursued during recent years in the interest of administration of the Economic Poisons Article, of the Agricultural Code of California. When thiocyanate insecticides were first introduced into the economic poisons field, routine examinations were con-

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fined to a determination of total nitrogen or sulfur from which the percentage of organic thiocyanate present was calculated. The methods were satisfactory provided no other compound containing an additional amount of the element was present, but they were of little value for analysis of mixtures, and a method was sought for direct determination of thiocyanate radical. Attention has been given this radical rather than the accompanying inorganic groups since it is to this constituent that the insecticidal value of the compounds is largely attributed.

Kemp[1] reported a method based on the reaction:

$$2RSCN+2KOH=2\ RS+KOCN+KCN+H_2O$$

The amount of thiocyanate present was calculated from the amount of cyanide produced as determined colorimetrically with alkaline picrate solution. Trial of this procedure gave good results when applied to normal butyl thiocyanate of technical grade dissolved in kerosene, but attempts to use it in the analysis of certain fly sprays proved unsuccessful, possibly because of masking of color by extractives in the samples.

Panchenko and Smirnov[2] suggested a method based on the theoretical reaction:

$$2RSCN+Na_2S=R_2S+2NaSCN.$$

Their procedure involves refluxing with alcoholic sodium sulfide and subsequent dilution with water, removal of hydrogen sulfide and determination of thiocyanate by Volhardt's method. They state the method is applicable to aliphatic, aliphatic-aromatic, and aromatic compounds, but in this laboratory it failed to give quantitative results when applied to fly sprays.

EXPERIMENTAL

The materials used in the following work included four commercial products:

"A," said to be a solution of beta-butoxy-beta-thiocyano diethyl ether in petroleum oil and standardized to contain total combined nitrogen 3.76 per cent.

"B," said to be a solution of mixed aliphatic thiocyanates in petroleum oil and standardized to contain total combined nitrogen 2.85 per cent.

"C," said to be a solution of beta-thiocyano ethyl esters of natural mixtures of higher fatty acids in petroleum oil and standardized to contain total combined nitrogen 2.52 per cent.

"D," said to contain secondary terpene alcohol thiocyanyl acetate with a minimum of total combined nitrogen 4.14 per cent.

A supply of normal butyl thiocyanate of technical grade was also avail-

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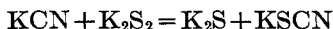
[1] *Analyst*, **64**, 648 (1939); *C. A.*, **33**, 8903 (1939).
[2] *J. Gen. Chem.* (*U.S.S.R.*), **2**, 193–196 (1932); *C. A.*, **27**, 245 (1932).

able. The nitrogen content (11.78 per cent) indicates a maximum purity of 96.9 per cent.

Various changes in the Panchenko and Smirnov procedure were introduced in an effort to ascertain wherein the difficulty lay. Better results suggested the discarding of the alcoholic medium for reaction in favor of an aqueous one; and sodium hydroxide and a polysulfide were used in addition to sodium sulfide. The polysulfide serves to convert any cyanide to thiocyanate[3] according to the reaction:

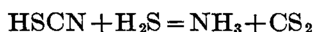


$$KCN + K_2S_2 = K_2S + KSCN$$

Upon dilution of the mixture after reaction it was found feasible to extract neutral oils with petroleum benzin, and finally there was obtained a water solution suitable for determination of thiocyanate radical. These modifications gave results sufficiently accurate to warrant further study.

It was observed in some of the experimental work that lower results were obtained if the volume of the aqueous phase in the initial reaction was less than that of the oil phase, the effect probably being due to the lesser contact between reagents and thiocyanate. Since a suitable quantity of fly spray for analysis does not usually exceed 25 grams, a standard volume of 35 ml. of aqueous solution of reagents was adopted as satisfactory.

Stability of thiocyanic acid.—Various statements in the literature[3,4,5] were noted to the effect that thiocyanic acid is unstable and volatile. To determine how rapidly decomposition or loss takes place under the acid conditions used in the method, 0.138 gram of potassium thiocyanate in 300 ml. of water was boiled for 15 minutes with 2 ml. of dilute sulfuric acid, and no loss was detected as determined by Volhardt's method of analysis. Statements were also found in the literature[6] that thiocyanates are decomposed by hydrogen sulfide according to the reaction:



$$HSCN + H_2S = NH_3 + CS_2$$

To investigate this matter further, 0.277 gram of potassium thiocyanate in solution was diluted to 300 ml., 10 ml. of "strong potassium polysulfide solution," described under reagents, was added, the mixture was immediately neutralized with sulfuric acid (1+4), and 2 ml. excess acid was added. The solution was then boiled 8 minutes, and thiocyanate was determined as directed in the method given below. Recoveries of 99.3 per cent and 99.5 per cent on two such determinations indicate thiocyanic acid is neither appreciably decomposed nor lost under these conditions.

Comparison of treatments for freeing thiocyanate radical from the organic group.—To determine conditions required for completion of the initial

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[3] Treadwell-Hall, "Analytical Chemistry," 6th ed., vol. I, pp. 333, 343.
[4] Prescott and Johnson, "Qualitative Chemical Analysis," 7th ed., p. 280.
[5] Viehoever and Johns, *J. Am. Chem. Soc.*, 37, 601 (1915).
[6] Molinari, "General and Industrial Organic Chemistry," Part I, p. 429 (1921).

operation of freeing the thiocyanate radical from the organic group and whether incidental changes in them would affect results, analyses of product "B" were carried on under varying conditions, which yielded the data shown in Table 1.

TABLE 1.—*Results of analysis of organic thiocyanate under varying conditions*

TIME AND CONDITIONS OF TREATMENT	THIOCYANATE NITROGEN FOUND
	per cent
Shaking 2 min. at 70°C.	2.65
Shaking 5 min. at 70°C.	2.73
Shaking 10 min. at 70°C.	2.75
Shaking 15 min. at 70°C.	2.73
Shaking 10 min. at room temp.	2.67
Shaking 10 min. at room temp. and 15 min. at 70°C.	2.75
Refluxing 15 min.	2.77
Refluxing 30 min.	2.72
Refluxing 60 min.	2.74

The figures in Table 1 indicate that the thiocyanate radical is readily liberated from an organic compound in a relatively short time at 70°C. It is best not to heat the solution without preliminary shaking at room temperature as release of increased pressure in the flask at that stage might result in loss of thiocyanate. A proper procedure is subsequently outlined under the heading "Shaking."

A further comparison of results obtained by four variations in treatment is shown in Table 2.

Variation I consists in shaking the organic thiocyanate dissolved in 20 ml. of kerosene with 20 ml. of strong potassium polysulfide, 10 grams of sodium sulfide, and 6 grams of potassium hydroxide with water to make approximately 35 ml. of aqueous phase. The shaking is carried out for 10 minutes at room temperature followed by an equal time at 70° C.

Variation II is identical with Variation I except that the concentration of reagents in the aqueous phase is reduced by half.

Variation III is identical with Variation II, except that the shaking at the higher temperature is conducted for 15 minutes instead of 10 minutes.

Variation IV consists in refluxing for 30 minutes with the same reagents used in Variation II.

The lack of agreement between total nitrogen and thiocyanate nitrogen in the case of "B" and "C" indicates that these materials contain thiocyanates that do not react under the conditions, or that they contain other combined nitrogen. It was thought possible that the difference might be due to isothiocyanate, which upon hydrolysis would yield amines[1,7] as follows:

$$RNCS + 3KOH = RNH_2 + KSH + K_2CO_3.$$

[7] Perkin and Kipping, "Organic Chemistry," p. 327 (1911).

Table 2.—*Analyses of organic thiocyanates*
(Comparison of the four variations)

Material	Total nitrogen guaranteed	Total nitrogen found (Kjeldahl)	Variation I		Variation II		Variation III		Variation IV	
			Nitrogen found	Recovery	Nitrogen found	Recovery	Nitrogen found	Recovery	Nitrogen found	Recovery
	per cent		per cent		per cent		per cent		per cent	
"A"	3.76	3.76	3.71	98.8	3.74	99.4	3.76	100.0	3.71	98.6
							3.77	100.2		
"B"	2.85	2.88	2.76	96.8	2.73	95.7	2.75	96.5	2.75	96.5
							2.76	96.8	2.77	97.2
"C"	2.52	2.65	2.40	90.5	2.32	87.5	2.38	89.7	2.29	86.5
							2.33	87.9	2.27	85.5
							2.39	90.2		
"D"	4.14	4.32	4.25	98.5	4.26	98.8	4.28	99.3	4.26	98.8
Butyl-thiocyanate	—	11.78	11.67	99.0	11.60	98.6	11.53	98.0	11.53	98.1
									11.49	97.6

However, no test for these compounds was obtained by Hoffmann's carbylamine reaction[8] after treatment with alcoholic potassium hydroxide. It is believed that material "C" is a comparatively crude product, supporting the conclusion that non-thiocyanate nitrogen is present.

Differences in results obtained by these variations on all materials except "C" are not large. In the analysis of "C," Variations I and III gave the highest results. In general, Variation III seems to be least subject to interference. The attack on other constituents of the sample is more severe in the refluxing than with shaking at 60°–70°C. Refluxing is difficult to carry out in the presence of vegetable oils or other materials readily affected by the hot alkali as the mass becomes quite thick and severe bumping may occur. If difficulties are encountered in attempting to apply it to a given material it is suggested that Variation III be used.

The shaking procedure was somewhat time-consuming, but its use was continued in preference to refluxing since preliminary attempts with the latter method seemed unsatisfactory. When more dilute solution was used, it was later found that refluxing gave results only slightly lower than did shaking. Refluxing for longer periods of time resulted in no increased yield as shown by refluxing Sample "A," 0.5, 1.0, and 2.5 hours with results of 3.71 per cent, 3.71 per cent, and 3.72 per cent nitrogen, respectively. These figures also show that no decomposition of the thiocyanate radical occurs during refluxing.

Estimation of thiocyanate.—Following removal of hydrogen sulfide from the solution, preliminary attempts were made to titrate thiocyanate directly by Volhardt's procedure. These proved unsuccessful owing to the presence of sulfur compounds. Treatment with freshly precipitated lead carbonate did not solve the difficulty since thiosulfate was not removed, and this subsequently reacted in the ultimate titration with silver nitrate. Separation of thiocyanate as the cuprous salt was found possible, but the precipitate was impure and reconversion to potassium thiocyanate by boiling with potassium hydroxide resulted in a solution which still gave unsatisfactory results by the Volhardt method. Weighing the compound was impracticable owing to the difficulty of washing the precipitate and also on account of its impurity. However, it was found that nitrogen in the cuprous thiocyanate could be quantitatively converted to ammonia by Kjeldahl digestion with sulfuric acid and determined by the official method.[9]

Presence of other nitrogen compounds.—With the exception of cyanides, which become thiocyanates when heated with polysulfides, no interference was encountered from other nitrogenous compounds. A common ingredient accompanying organic thiocyanates in fly sprays is isobutyl-

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[8] *Ibid.*, p. 212.
[9] *Methods of Analysis, A.O.A.C.*, 1940, 21, 26.

undecylenamide added as an intensifier. In the absence of other forms of nitrogen, the percentage of this material may be estimated in the presence of organic thiocyanates without resorting to an acid hydrolysis for the determination of amide nitrogen. Total nitrogen is determined by the Kjeldahl method and thiocyanate nitrogen subtracted from it, the difference being calculated to isobutylundecylenamide. To demonstrate that the presence of this compound does not interfere with determination of thiocyanate, some of the material was added to a solution of "B" in highly refined kerosene. The mixture was analyzed and thiocyanate nitrogen 2.76 per cent was found, which agrees with results obtained when no isobutylundecylenamide was present. Similar results were obtained in the presence of 3–5 dinitrocresol.

THIOCYANATE NITROGEN IN ORGANIC THIOCYANATES AND MIXTURES

Following are details of the method finally adopted for determination of thiocyanate nitrogen in fly sprays.

REAGENTS

(a) *Strong potassium polysulfide solution.*—Dissolve 180 grams of KOH in 120 ml. of water, which will make approximately 200 ml. of solution. Saturate 100 ml. with H_2S, while cooling, which will require about 42 grams. Add the other 100 ml. of KOH solution and 80 grams of sulfur. Shake until dissolved.

(b) *Sodium sulfide* ($Na_2S \cdot 9H_2O$).

(c) *Mixed sulfide solution.*—To 100 ml. of (a) add 50 grams of (b), 50 grams of KOH, and 200 ml. of water.

(d) *Sodium bisulfite* ($Na_2S_2O_5$ or $NaHSO_3$).

(e) *Sulfur dioxide.*

(f) *Copper sulfate solution* (20% $CuSO_4 \cdot 5H_2O$).

(g) *Potassium hydroxide solution* (10%).

(h) *Sulfuric acid.*—(1+4).

(i) *Wash solution.*—To 300 ml. of water add 1 ml. of (h), 1 gram of (d), 10 ml. of (f), 12 grams of Na_2SO_4, and pass SO_2 into the solution for 10 minutes.

PROCEDURE

Weigh an amount of sample preferably containing ca. 0.03 gram of thiocyanate nitrogen. (If the percentage is very low the weighed amount should not be increased unduly without correspondingly increasing the quantity of mixed sulfide solution (c) used; 20–25 grams of fly spray is usually sufficient.)

First treat the sample by refluxing or by shaking to free the thiocyanate radical from the organic compound, as follows:

Refluxing.—Weigh a suitable sample into a 200 ml. Erlenmeyer flask fitted with a cork stopper carrying a reflux condenser, and add 35 ml. of the mixed sulfide solution (c) to the flask. Add about a gram of 10–20-mesh carborundum crystals and place in the flask a glass tube enlarged at the top to fit loosely over the lower end of the condenser to act as a funnel leading the condensate through the mixed sulfide solution during refluxing. Connect to the reflux condenser and boil over a small flame for 30 minutes. Cool, and continue as directed under the heading "Removal of petroleum oil."

Shaking.—As an alternative procedure, weigh the sample into a 250 ml. glass-stoppered Erlenmeyer flask and add 35 ml. of the mixed sulfide solution (c). Shake vigorously at room temperature for 10 minutes, during which time reaction is fairly completed; next heat to 70°C. on a steam bath, carefully releasing the pressure resulting from heating; and shake at the temperature of 70°C. for 15 minutes more. Cool.

Removal of petroleum oil.—Dilute and transfer to a separatory funnel with about 200 ml. of water. Add 50 ml. of petroleum benzin, shake, and draw off the aqueous layer into a 600 ml. beaker. Wash the petroleum benzin layer with two 10 ml. portions of water, which add to the main solution. (If emulsions occur during the washing process, they may be broken by acidifying with the H_2SO_4 (h). The aqueous layer may then be drawn off and the petroleum benzin layer washed with water as directed.) Discard the petroleum benzin layer.

Determination of thiocyanate nitrogen.—Dilute the water solution to about 300 ml. and neutralize with the H_2SO_4 (h), using a piece of litmus paper as outside indicator. Add 2 ml. of the H_2SO_4. Bring mixture to a boil quickly and boil for 8 minutes to remove H_2S. Cool. If fatty acids or other oils are present at this stage, transfer to a separatory funnel, extract with petroleum benzin, and return the aqueous phase to the original beaker. Filter through a small Büchner funnel and transfer the filtrate to a beaker. Neutralize to litmus paper with the KOH (g) and add 1 ml. of the H_2SO_4. Add about 1 gram of the $Na_2S_2O_5$ and stir until dissolved. Add excess (about 15 ml.) of the $CuSO_4$ solution (f) and pass SO_2 into the solution for 10 minutes. Allow the precipitated cuprous thiocyanate to settle for 15 minutes and filter through a smaller Büchner funnel fitted to a suction flask, transferring all precipitate to the funnel with the aid of a wash bottle containing the wash solution (i). (Cuprous thiocyanate has a tendency to run through the filter or clog it. The writer uses a 2-inch Büchner funnel coated with a fine suspension of "rock wool," upon which is placed a filter paper and then a suitably adjusted layer of fine asbestos and a little diatomaceous earth. Because of variation of equipment a little experimentation may be required to attain satisfactory filtration.) Wash the filter and precipitate once or twice with the wash solution, continue suction until filter pad is dry, and transfer to an 800 ml. Kjeldahl flask. (This may conveniently be done by folding it in a filter paper together with bits of moist filter paper used to wipe out the Büchner funnel. Then place the whole in the Kjeldahl flask.) Add a few glass beads, 35 ml. of concentrated H_2SO_4, 10 grams of K_2SO_4, and ca. 0.7 gram of HgO, or its equivalent in metallic Hg. Digest till white and for 15 minutes thereafter. Determine the nitrogen by the official method,[9] beginning with the words "After cooling, dilute. . . . " Run a blank analysis on the paper and filtering pad.

ANALYSIS OF DUSTS

Analysis of insecticidal dusts containing organic thiocyanates is carried out by the shaking procedure described in the above method except that a larger volume of the "mixed sulfide solution" (c) may be needed to keep the contents of the flask in a fluid condition—50 ml. is usually sufficient. After the reaction is complete, the solution is cooled, diluted with 200 ml. of water, and filtered on a Büchner funnel. The filtrate is then treated in the same manner as directed for fly sprays, previously described under the heading "Determination of thiocyanate nitrogen."

CALCULATIONS

If the organic thiocyanate concerned is of definite composition the percentage in the sample is found by dividing the percentage of thiocyanate

nitrogen as obtained above by the percentage present in the pure compound and multiplying by 100. For example, 0.24 per cent thiocyanate nitrogen is found by analysis in a fly spray containing beta-butoxy-beta-thiocyano diethyl ether ($C_9H_{17}O_2SN$). Since this compound contains 6.89 per cent nitrogen, the percentage of the compound in the fly spray is $0.24 \times 100/6.89 = 3.48$ per cent.

In the case of insecticides manufactured from mixed thiocyanates, the percentage present in the sample is calculated from the composition of the mixture. For example, a preparation used in the manufacture of a dust contains 54.5 per cent by weight of mixed thiocyanates and 45.5 per cent petroleum oil, and the thiocyanate nitrogen content of the preparation is 2.52 per cent. It follows that the nitrogen content of the mixed thiocyanates is $2.52 \times 100/54.5$, or 4.62 per cent. The dust is analyzed and found to contain 0.22 per cent thiocyanate nitrogen, so the percentage of organic thiocyanate in the dust is therefore $0.22 \times 100/4.62$, or 4.76 per cent.

SUMMARY

A method has been devised for the analysis of insecticidal sprays and dusts containing organic thiocyanates. The thiocyanate radical is separated as cuprous thiocyanate; nitrogen is determined by the Kjeldahl method; and the percentage of organic thiocyanate is calculated in the insecticide.

DETERMINATION OF DEXTROSE, LEVULOSE, INVERT SUGAR, AND SUCROSE-INVERT SUGAR MIXTURES BY COPPER REDUCTION, USING CITRATE-CARBONATE REAGENT

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I. INTRODUCTION

Complex copper solutions in which the alkalinity is furnished by alkali carbonates and bicarbonates were proposed during an early period of reducing-sugar analysis, but such solutions have in general proved less reliable with respect to the reproducibility of analytical results than those containing caustic alkali. It has been shown by Jackson and McDonald[1] that independent operators using Munson and Walker's method for the analysis of pure sugars can attain a precision of about 0.2 per cent within the range of 70–210 mg. of reducing sugar. Lane and Eynon's method[2] has been found to compare well with Munson and Walker's method. Here the skill of the operator is a very important factor in determining the

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* Deceased.
[1] *This Journal*, 24, 767 (1941).
[2] *J. Soc. Chem. Ind.*, 42, 32T, 143T, 463T (1923).

precision of the method. The authors say that titrations on a given sugar solution will agree well within 0.1 ml. Since their tables include values for a titration of 15–50 ml. the careful operator using 25–50 ml. for a titration should obtain a precision well within 0.4 per cent. In spite of this fact, carbonate solutions have been widely advocated for reducing-sugar analysis because the mildly alkaline solutions attack accompanying "non-reducing" substances less than do those containing caustic alkali. The carbonate-bicarbonate solutions have proved particularly serviceable for the determination of small quantities of reducing sugar in the presence of large percentages of sucrose. When caustic alkaline copper solutions are used for analyzing such mixtures, the reducing power of sucrose is relatively large and uncertain. Such analyses are of special importance in European countries where great significance is attached to the reducing-sugar content of raw beet sugar, and consequently a number of methods have recently been elaborated in which, invariably, mildly alkaline copper solutions have been employed.[3] In this country, however, where a somewhat wider variety of sugar products is encountered, the requirements for a reducing-sugar method are more general, and a satisfactory method should be applicable to the entire range of ratios of sucrose to reducing sugar.

In modern reducing-sugar analysis the tendency has been to determine the reduced or unreduced copper in the original reaction vessel by iodometric titration, a procedure that is particularly well adapted to the carbonate methods. Thus convenience and rapidity are added to the other advantages. There remains, however, the fact that the carbonate methods have yielded less reproducible results than have the caustic alkali methods. In the present investigation an effort was made to determine the cause of some of these uncertainties and to ascertain the precision that is attainable under carefully controlled conditions. Since the method selected was rapid and convenient, it was found feasible to conduct a considerable number of analyses and to include a wide range of concentrations of sugars and sugar mixtures.

II. PRELIMINARY EXPERIMENTS

Preliminary to the extended series of measurements described in later paragraphs, a cursory study was made of various complex copper carbonate solutions, and measurements were made of the ratio of reduced copper to the dextrose taken for analysis. Quisumbing and Thomas[4] in comparing the influence of different alkalies in alkaline copper solutions found that the copper reduced by dextrose varied rapidly with change in carbonate concentration. It was also observed in this work that large variations in

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[3] O. Spengler, F. Tödt, and M. Scheuer, *Z. Wirtschaftsgruppe Zucker-ind.*, Bd. **86**, 323 (1936); R. Ofner, *Z. Zuckerind. Czechoslovak.*, **58**, 36 (1933); N. Schoorl, *Chem. Weekblad*, No. 9 (1929); R. F. Jackson and E. J. McDonald, *This Journal*, **26**, 462 (1943).

[4] *J. Am. Chem. Soc.*, **43**, 1510 (1921).

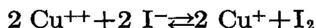
the ratio occurred with change of composition of the copper solution, amounting in extreme cases to about 50 per cent. A partial compilation of such data is given in Table 1. Evidently any change in composition of

TABLE 1.—*Effect of varying composition of copper reagent on ratio of reduced copper to dextrose*

COMPOSITION OF COPPER REAGENT PER LITER		RATIO: MG. OF COPPER / 100 MG. OF DEXTROSE	$\frac{\Delta R}{\Delta S}$[1]
$CuSO_4 \cdot 5H_2O$	ALKALI SALTS		
grams			
17.5	250 K_2CO_3, 100 $KHCO_3$	3.15	−0.0033
25	150 $KNaC_4H_4O_6 \cdot 4H_2O$, 53 Na_2CO_3, 25 $Na_2HPO_4 \cdot 12\ H_2O$	2.653	− .0056
25	75 $KNaC_4H_4O_6 \cdot 4\ H_2O$, 27 Na_2CO_3, 13 $NaHCO_3$	2.572	− .0046
25	81 $K_3C_6H_5O_7 \cdot H_2O$, 70 K_2CO_3, 92 $K_2C_2O_4 \cdot H_2O$, 3.57 KIO_3, 50 KI	2.197	− .0009
25	89 $Na_3C_6H_5O_7 \cdot 2\ H_2O$, 53 Na_2CO_3	2.084	− .0010

[1] The mean change in the ratio caused by 1-mg. increase in concentration of sugar between 40 and 120 mg. of dextrose.

the copper reagent produces a relatively large change in the ratio of reduced copper to sugar taken. Many authors have used as a basis of selection those methods which yield the larger ratios of copper to sugar. It has appeared to the authors that the higher ratios of copper are not necessarily advantageous. The actual determination of copper is the most precise step in the whole analysis, and the error of determination is not measurably diminished by having a slightly increased amount of it. Far more important considerations are the stability of the copper solution, the reproducibility of the analyses, and the smaller variations of the ratios with changing concentration of sugar. These advantages appeared to be combined in greatest degree in the copper citrate-carbonate reagents proposed by Benedict[5] and modified by Shaffer and Hartmann.[6] The latter authors, in a thorough investigation, determined by studying the respective equilibria, the exact conditions required for the quantitative iodometric titration of either cupric or cuprous copper. They found that the reaction



$$2\ Cu^{++} + 2\ I^- \rightleftarrows 2\ Cu^+ + I_2$$

goes quantitatively to the left if the final concentration of copper and of iodide does not exceed 5 millimolar each. Since the cupric ions may be

[5] *J. Biol. Chem.*, **5**, 485 (1908).
[6] *Ibid.*, **45**, 375 (1921).

removed by the addition of potassium oxalate, these authors were able to determine cuprous copper in the presence of cupric copper at higher concentrations. As a result of this study they proposed a procedure whereby an iodide-iodate solution is added to the cold reaction mixture and the reduced copper determined by thiosulfate titration.

In a limited number of experiments Shaffer and Hartmann also showed that this method of copper analysis could be applied to copper reductions carried out in caustic alkaline solution or in citrate-carbonate solution. However, they confined themselves to an exhaustive study of a micro method for determining dextrose by means of a copper citrate-carbonate solution.

As a macromethod, which is the subject of the present investigation, Shaffer and Hartmann suggested a modification of Benedict's solution containing potassium citrate, potassium carbonate, and copper sulfate. For convenience they also added potassium iodate, potassium iodide, and potassium oxalate, thereby combining in a single reagent all the chemicals required for the analysis up to the point of acidification and titration. As an alternative, and mainly for the purpose of diminishing the cost of the chemicals, they described a similar solution containing sodium citrate and sodium carbonate. In this case it was necessary to prepare separate solutions of iodide-iodate and of potassium oxalate, since the latter would have caused the precipitation of sodium oxalate if added to the copper reagent.

1. *Effect of Varying Citrate Concentration.*—The precision with which the alkaline copper reagent must be prepared was first determined. Both sodium and potassium citrates contain water of crystallization, which might conceivably be variable and hence render variable the concentration of dry substance. For reasons which are explained in a later paragraph the sodium salts were selected for preparing the reagents.

Trisodium citrate crystallizes with $5\frac{1}{2}$ moles of water, which is theoretically 27.74 per cent of the substance. On exposure of a pulverized sample to the air it was found to lose more than 10 per cent of this moisture overnight and more than 30 per cent during a period of three days. On long standing it approached a state corresponding to two moles of water. This same crystalline phase is reached by drying at 100°C. for about 36 hours. With this tendency to effluoresce it was anticipated that the substance purchased from the supply houses might vary in water content. A sample of crystals from a freshly opened bottle contained 22.81 per cent of moisture instead of the 27.74 per cent required by theory. Other samples approached the theoretical water content more closely, although in variable degree.

Three alkaline copper reagents were prepared, each containing 25 grams of copper sulfate crystals and 53 grams of anhydrous sodium carbonate in 1 liter. One solution contained 71.435 grams (0.20 mole) of

sodium citrate crystals, the second 89.294 grams (0.25 mole), and the third 107.153 grams (0.30 mole). Each of these solutions was used for the analysis of three 50-ml. solutions of dextrose containing, respectively, 140.1, 113.5, and 56.3 mg. of sugar, the copper being determined after a 5-minute period of boiling by the procedure described in detail in later pages. The results plotted in Figure 1 are expressed as the ratio of reduced copper to the weight of sugar. It is at once evident that variations in the concentration of citrate exert a considerable effect and that as the concentration increases the amount of reduced copper diminishes. The differences in each series due to a change of 0.05 mole (=18 grams) in citrate

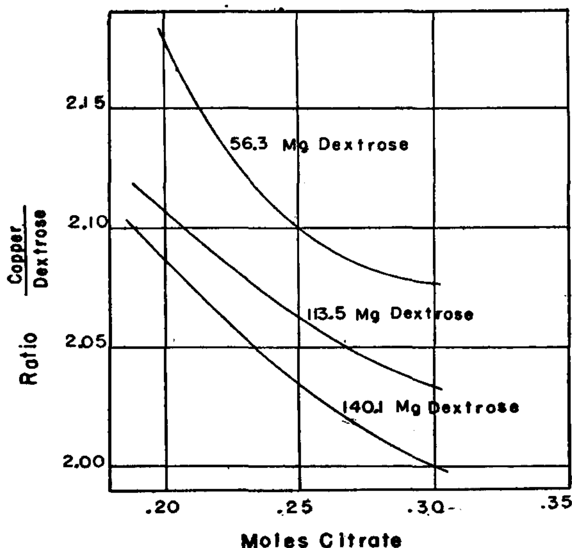


FIG. 1.—Variation in the copper-dextrose ratio with the amount of citrate present.

concentration leads to the conclusion that, in average, such a change causes a difference of 0.04 or about 2 per cent of the ratio. Since a precision of 0.1 per cent is desired the citrate concentration must be adjusted within about 0.9 grams (that is, 0.002/0.04×18) or for a weight of 89.3 grams (0.25 mole) within 1 per cent. It is therefore necessary not only to weigh with this precision but also to determine the dry substance content of the sodium citrate crystals.

To determine moisture in sodium citrate, weigh rapidly a pulverized sample and dry at 100°C. for 24 or 36 hours. Then dry to constant weight at 160°–170°C., preferably allowing the temperature to rise gradually. The substance tends to decrepitate, and it is therefore advisable to cover the drying vessel loosely until the danger of loss is past.

2. *Effect of Varying Carbonate Concentration.*—Variations in the concentration of sodium carbonate also affect the ratio of reduced copper to sugar. Four alkaline copper reagents were prepared in which the copper and citrate were held constant, but in which the concentration of sodium carbonate was varied within wide limits. With these solutions analyses were made of 50-ml. samples, each containing 100 mg. of dextrose. As shown in Figure 2, an increase in sodium carbonate concentration causes

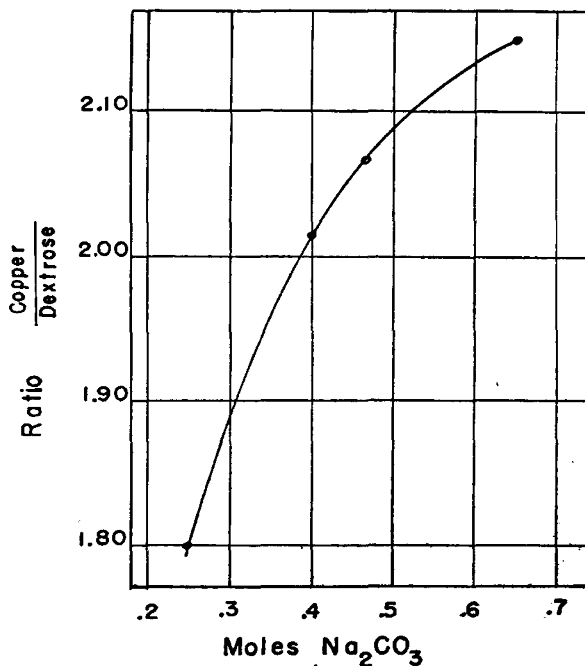


FIG. 2.—Variation in the copper-dextrose ratio with the amount of Na_2CO_3 present.

an increase in the ratio of reduced copper to sugar. Between 0.4643 and 0.65 moles the ratio increases at the rate of 0.0443 for each 0.1 mole, or about 2 per cent. For a precision of 0.1 per cent the variation in the ratio from this cause must not exceed 0.002, and therefore the concentration of carbonate must not vary more than $0.002/0.443 = 0.00475$ mole. If 0.5 mole concentration of sodium carbonate is arbitrarily selected for the preparation of the copper reagent, its concentration must not vary by more than ± 0.00475 mole or about 1 per cent. Many samples of anhydrous sodium carbonate from the supply houses proved to be dry well within this tolerance, while others exceeded it slightly. Moisture can be very simply determined by heating a sample in a covered platinum cruci-

ble to dull redness, but care must be taken to avoid fusion of the salt. For the analyses described in the later paragraphs the copper reagents were prepared with a precision well within the tolerances here indicated.

3. *Relative Merits of Sodium and Potassium Salts for Preparing Reagents.*—Both modifications of Shaffer and Hartmann's reagent were investigated. In one the copper reagent was prepared with tripotassium citrate, potassium carbonate, and oxalate, and contained in 1 liter 3.57 grams of potassium iodate and 50 grams of potassium iodide. Fifty ml. of the copper solution was pipetted accurately, and the copper was reduced by 50 ml. of sugar solution. After the reduction was completed, the reaction mixture was cooled and acidified with 20 ml. of 5 *N* sulfuric acid. The acidification caused the release of the equivalent of 50 ml. of 0.1 *N* iodine, a part of which reoxidized the reduced copper; the remainder was titrated back with standard thiosulfate. When the ratio of copper to sugar was plotted against sugar, the graph showed the same curvature as that with the sodium salts but with slightly different numerical values.

In the other modification the copper reagent was prepared with trisodium citrate and sodium carbonate. It was therefore necessary to prepare in separate solutions saturated potassium oxalate and the standard potassium iodide-potassium iodate solution. After extended experimentation with both of these modifications, the latter was chosen and all of the later analyses were conducted with it alone. It appears at first glance that the convenience of combining all the chemicals in a single reagent has been sacrificed. However, the advantages of the second modification mentioned outweigh those of the combined reagent. The combined reagent always contains the equivalent of 50 ml. of 0.1 *N* iodine, regardless of the amount of copper reduced and, for the smaller concentrations of reducing sugar, requires a long tedious back titration. A blank determination must be run with great precision, since its value enters directly into all calculations. The thiosulfate must be standardized and its constancy assured by separate operations. On the other hand, when using the second modification in which the solutions of iodide-iodate and potassium oxalate are added just before titrating, one can gauge roughly the quantity of the former to add in order to produce the slight excess necessary. Since potassium iodate can be obtained as a very pure chemical, the iodate-iodide can be prepared as a standard solution merely by weighing out the chemicals. Thus the working solution becomes the fundamental standard and no additional standardization is required. If made slightly alkaline and if tightly stoppered, the solution keeps indefinitely.

4. *Iodide-Iodate Solution.*—Shaffer and Hartmann proposed an iodide-iodate solution containing 5.4 grams of potassium iodate and 60 grams of potassium iodide in 1 liter. This solution upon acidification yields an equal volume of 0.1514 *N* iodine. This solution was modified by taking a weight of 5.611 grams of potassium iodate and 60 grams of potassium

iodide. This solution upon acidification yields an equal volume of 0.1573 *N* iodine. This factor is the reciprocal×10 of the atomic weight of copper and 1 ml. is exactly equivalent to 10 mg. of copper. With the iodide-iodate solution adjusted to 0.1573 *N*, the calculation of reduced copper becomes very simple. The ratio of concentration of the standard iodate solution to that of 0.1 *N* thiosulfate is determined by titration of not less than 10 ml. of the former. The back titration of thiosulfate in the analytical determination is then multiplied by this ratio factor and the thio-

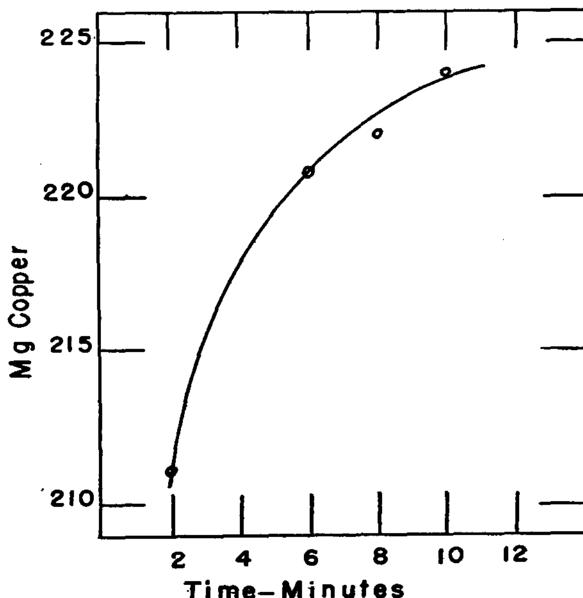


FIG. 3.—Effect of time of boiling on weight of copper reduced by 100 mg. of dextrose.

sulfate thus converted to iodate. This is deducted from the measured volume of iodate added, yielding directly after multiplication by 10 the number of milligrams of copper reduced.

It is of course important that the potassium iodide be pure and free from iodate. Occasionally potassium iodide becomes discolored after long standing, possibly indicating free iodine. The writers have made a practice of washing it with chloroform, which restores its white color, and allowing it to dry in air. The iodide-iodate solutions were prepared at a known temperature, and when necessary the measured volumes were corrected for variations of temperature by assuming an expansion coefficient of 0.0002.

5. *Effect of Varying Boiling Time.*—As is true of all reducing-sugar

methods, the 5-minute duration of boiling arbitrarily specified does not effect a maximum reduction of copper. It does, however, serve adequately for an arbitrarily selected terminal point. Figure 3 shows the rate of increase of precipitation of copper by 100 mg. of dextrose for the several periods of boiling time. At the 5-minute point the ratio (2.195) is increasing at the rate of 0.01 per minute. Therefore an error of 0.1 minute in judging the boiling time results in a difference of less than 1 part per thousand.

6. *Determination of Blank.*—Blank determinations were made frequently, by boiling the copper reagent with 50 ml. of water. The mean value of copper found was 0.6 mg. with a mean deviation of 0.09 mg. Practically this same value was found when the copper reagent itself was treated as in the analysis but without boiling. In other words, the value of the blank is not caused by autoreduction of the copper reagent, but is conceivably the result of a slight reduction of the copper by the iodine. This view is at variance with the findings of Shaffer and Hartmann that the reaction given on page 373 runs quantitatively to the left in the presence of potassium oxalate of sufficient concentration, but nevertheless the writers were unable to get the same titration values in the presence of copper and oxalate as in their absence.

These blank values are remarkably reproducible, and therefore all the data for reduced copper in the following tables have been left uncorrected for the blank. These uncorrected values are the more serviceable, since the analyst who makes only occasional analyses seldom makes a blank determination. If he does make a blank determination and finds a value different from 0.6 mg., he has merely to apply the difference as a correction.

III. DETAILS OF METHOD

REAGENTS

(a) *Modified Benedict's solution.*—Dissolve the equivalent of 64.52 grams of anhydrous trisodium citrate (or 89.29 grams of crystals containing 5½ moles of water, the moisture content of which has been corrected for), and 53 grams of anhydrous sodium carbonate in about 600 ml. of water. Add with vigorous agitation a solution of 25 grams of $CuSO_4 \cdot 5H_2O$ in about 150 ml. of water. (Both solutions must be cool before mixing.) Make to 1 liter and filter.

(b) *Standard potassium iodate solution.*—Dissolve 5.611 grams of pure KIO_3 and 60 grams of KI and make to 1 liter.

(c) *Potassium oxalate solution.*—Saturated.

(d) *Sulfuric acid.*—Approximately 5 *N* (250 grams of concentrated acid in 1 liter of solution).

(e) *Sodium thiosulfate.*—Approximately 0.1 *N* (25 grams of crystals in 1 liter).

PROCEDURE

Transfer 50 ml. of solution (a) to a 300-ml. Erlenmeyer flask and add 50 ml. of a solution containing not more than 150 mg. of reducing sugars. Mix with a rotary motion, cover the flask with a small inverted beaker, and place on a plate of asbestos gauze about 4.5 cm. above the top of a gas burner (electric heating may be used).

Heat to boiling in 4 minutes and continue the boiling for exactly 5 minutes. In judging the beginning of the 5-minute period disregard the early sporadic appearances of boiling and start the final period at the moment when the ebullition suddenly becomes violent (this occurs usually about 20 seconds after the first appearance of bubbles).

At the expiration of the prescribed period of boiling immerse the flask without agitation in a cold water bath, and allow it to remain until cool. Add accurately a volume of standard iodate (b) in excess of that required for oxidation of the Cu_2O and add ca. 20 ml. of saturated $K_2C_2O_4$. Add, by means of a rapidly delivering pipet, 20 ml. of 5 *N* H_2SO_4, and agitate with a rotary motion until the cuprous salts are completely dissolved. Examine the mixture by holding the flask above the level of the eye. Any undissolved Cu_2O or $CuSO_4$ can be clearly discerned and must be dissolved by continued agitation. Dilute to about 200 ml. and add from a buret standard thiosulfate until the solution starts to turn from green to blue-green. Add starch indicator and complete the titration dropwise to the disappearance of deep blue starch iodide, the final additions of thiosulfate being in split drops.

Determine the ratio of concentrations of thiosulfate to iodate and by means of the factor thus obtained convert the thiosulfate volume to its equivalent volume of iodate. Deduct this from the volume of iodate added, and multiply the result by 10 to obtain the number of milligrams of Cu reduced. From Table 4 obtain the amount of reducing sugar corresponding to the weight of Cu found.

IV. STANDARDIZATION

For the purpose of standardization, eight series of analyses were carried out. These included the reducing powers of pure dextrose, levulose, and invert sugar and of sucrose-invert sugar mixtures taken in such proportions that all possible ratios of the two were included. The reducing sugar was varied from various low concentrations to about 150 mg. in 50 ml. In each of the five series of analyses of sucrose-invert sugar mixtures the sucrose was kept at a constant value and the invert sugar was varied.

The sucrose and dextrose were National Bureau of Standards Samples Nos. 17 and 41, respectively. The sucrose was free from a measurable amount of moisture, and the dextrose contained 0.01 percent moisture, which was corrected for when the sample was weighed. The levulose was prepared by crystallization, once from water and three times from aqueous alcohol. When the crystals were dried for several hours at 60°C. to constant weight, a small sample was found to suffer no further loss at 70°–75°C. The levulose contained less than 0.002 percent of ash.

In preparation for analysis a 500-ml. solution was prepared to contain about 1.5 grams of the sugar, accurately weighed. Aliquot portions of this solution were taken by means of a series of odd-volume pipets at roughly 2–5-ml. intervals. These pipets, by frequent calibration, were found capable of delivering the respective volumes of solution with a precision of 0.002–.003 ml. The sucrose solutions were prepared in such concentration that 10 ml. contained the weight of sugar desired. For the 10-gram sucrose series the dry sugar was weighed directly into the reduction flask, and 6 ml. of water was added to make a total calculated volume of 12.19

ml. of solution. Invert sugar solutions and water were added in such quantity that the total volume became 50 ml.

Invert sugar was prepared in the earlier measurements by inverting sucrose for 35 minutes at 70°C. in the presence of 0.1 *N* hydrochloric acid, cooling, and neutralizing the acid with sodium hydroxide. Comparative measurements were made with invert sugar solutions prepared by weighing equal quantities of dextrose and levulose. Since no measurable difference was detected, the later measurements were made solely with the synthetic invert sugar.

When the Erlenmeyer flasks used for the reduction had dried in the air for several days the reaction mixtures boiled without bumping, but if the flasks were used more frequently, bumping occurred. The addition of talc in amounts of 2 mg. or less prevented bumping and did not obscure the start of boiling.

V. EXPERIMENTAL RESULTS

The results of the reduction measurements made as described in previous pages are assembled in Table 2. For each sugar a formula was devised to express the relation of sugar present to copper reduced. These formulas were prepared by the method of averages.

It is difficult to make an exact estimate of the precision of the method. The curves, Figure 4, showing the relation of the copper-sugar ratio to copper, especially in the case of invert sugar, may shift an appreciable amount if the analysis is not carried out under exact conditions. Since the variations in gas pressure seemed to be sufficient to cause such a shift in the curves, a series of analyses was made over a period of time in which the heating was done electrically. The voltage was adjusted by a "Variac" and stabilized by a voltage regulator. The flask was placed in a conical heater in such a way that the surface of the liquid was just below the top of the heater. Table 3 gives the results of this series of analyses.

The pure sugars—dextrose, levulose, and invert sugar—yield roughly parallel curves. As the concentration of sugar is diminished from 150 mg. the reducing powers pass through maxima in the vicinity of 40 mg. of sugar or approximately 90 mg. of copper, and then abruptly and very rapidly diminish as shown in Figure 4. The form of these curves is similar to that produced by levulose[7] in its reaction with Ost's solution at 55°C., the maximum reducing power in the latter case being at about 60 mg. of levulose. The very rapid change of the ratios with concentration of sugar at the lower concentration levels makes it questionable whether a satisfactory precision of analysis can be attained with these dilute solutions. For this reason and because the number of experiments is insufficient to define these rapidly varying curves accurately, there is recorded

[7] Jackson and Mathews, *This Journal*, 15, 204 (1932).

TABLE 2.—*Milligrams of copper reduced by dextrose, levulose, invert sugar, and sucrose-invert sugar mixtures*

Dextrose

SUGAR TAKEN	COPPER	SUGAR BY FORMULA[1]	ERROR
145.5	295.9	145.1	−0.4
132.3	272.4	132.7	+ .4
117.9	243.8	117.9	0
98.6	205.9	98.7	+ .1
87.3	183.0	87.2	− .1
80.3	169.3	80.4	+ .1
72.9	154.5	73.2	+ .3
65.1	137.4	64.8	− .3
58.3	123.8	58.3	0
54.4	115.4	54.3	− .1
48.1	102.9	48.3	+ .2
40.2	85.7	40.1	− .1
32.2	69.3	32.4	+ .2
29.1	61.8	28.9	− .2
24.1	51.3	24.1	0
16.1	33.5	15.9	− .2

Levulose

SUGAR TAKEN	COPPER	SUGAR BY FORMULA[1]	ERROR
146.7	305.8	146.9	+ .2
133.3	279.1	133.3	0
118.9	250.4	118.9	0
108.7	229.4	108.5	− .2
99.4	211.0	99.4	0
88.0	187.3	87.9	− .1
73.4	157.5	73.5	+ .1
58.8	126.2	58.7	− .1
44.1	95.0	44.1	0
29.3	62.9	29.3	0
20.7	44.3	20.9	+ .2
14.6	30.2	14.5	− .1

Invert sugar +10 g. of sucrose

SUGAR TAKEN	COPPER	SUGAR BY FORMULA[1]	ERROR
134.1	290.2	133.5	− .6
120.2	264.9	120.3	+ .1
106.4	238.3	106.8	+ .4
88.7	202.5	89.0	+ .2
71.3	165.4	71.0	− .3
53.3	127.1	53.1	− .2
44.3	107.7	44.2	− .1
35.5	89.3	35.9	+ .4
25.1	64.3	24.9	− .2
17.7	47.9	17.7	0

Invert sugar

SUGAR TAKEN	COPPER	SUGAR BY FORMULA[1]	ERROR
149.5	307.1	149.5	0
148.2	304.0	147.8	−0.3
141.0	290.6	140.9	− .1
140.2	289.4	140.2	0
135.8	280.8	135.8	0
134.7	279.2	135.0	+ .3
127.4	264.8	127.5	+ .1
120.1	250.7	120.4	+ .3
113.6	238.1	114.0	− .4
100.4	210.8	100.3	− .1
95.0	199.9	94.8	− .2
84.6	179.7	84.9	+ .3
74.8	159.3	74.9	+ .1
74.2	157.8	74.4	+ .2
59.4	126.4	59.3	− .1
56.5	120.8	56.6	+ .1
56.2	119.7	56.1	− .1
59.9	127.4	59.8	− .1
44.9	95.8	44.8	− .1
42.4	90.5	42.4	0
37.4	79.4	37.2	− .2
44.5	95.1	44.5	0
42.1	89.8	42.0	− .1
35.0	74.6	35.0	0
29.6	63.1	29.6	0
19.8	42.0	20.0	+ .2
14.0	28.9	14.1	+ .1

Invert sugar +0.1 g. of sucrose

SUGAR TAKEN	COPPER	SUGAR BY FORMULA[1]	ERROR
148.4	306.6	149.0	+ .6
140.8	290.1	140.4	− .4
133.0	275.7	133.0	0
117.7	245.3	117.5	− .2
98.3	207.4	98.4	+ .1
78.8	167.6	78.8	0
59.0	126.5	59.0	0
49.1	105.4	49.0	− .1
39.3	84.6	39.3	0
27.7	59.6	27.7	0
11.8	24.6	11.8	0

Invert sugar +0.3 g. of sucrose

SUGAR TAKEN	COPPER	SUGAR BY FORMULA[1]	ERROR
148.4	306.4	148.1	−0.3
141.1	292.7	141.1	0
133.0	277.4	133.2	+ .2
117.7	246.9	117.8	+ .1
98.3	208.3	98.4	+ .1
78.8	168.1	78.8	0
59.0	126.7	58.9	− .1
49.1	105.9	49.0	− .1
39.3	84.9	39.2	− .1
27.7	60.3	27.8	+ .1
19.6	42.3	19.6	0
11.8	25.2	11.8	0

Invert sugar +1 g. of sucrose

SUGAR TAKEN	COPPER	SUGAR BY FORMULA[1]	ERROR
148.0	307.9	147.9	− .1
140.7	292.3	139.9	− .8
132.6	278.7	132.9	+ .3
117.4	249.2	118.0	+ .6
98.0	208.8	97.8	− .2
78.6	169.2	78.5	− .1
58.8	128.6	59.0	+ .2
48.9	107.4	49.0	+ .1
39.2	86.0	39.0	− .2
27.7	61.2	27.6	− .1
19.5	43.8	19.6	+ .1
11.7	26.6	11.9	+ .2

Invert sugar +3 g. of sucrose

SUGAR TAKEN	COPPER	SUGAR BY FORMULA[1]	ERROR
148.4	308.4	147.5	− .9
140.8	296.8	141.5	+ .7
133.0	281.4	133.4	+ .4
117.7	250.6	117.4	− .3
98.3	213.6	98.6	+ .3
78.8	173.6	78.8	0
59.0	132.0	58.8	− .2
49.1	110.7	48.8	− .3
39.3	89.6	39.0	− .3
27.7	64.7	27.6	− .1
19.6	47.3	19.8	+ .2
11.8	29.5	11.9	+ .1

[1] Dextrose $=0.786+0.4476\ Cu+0.000135\ Cu^2$.
Levulose $=1.009+0.4432\ Cu+0.000110\ Cu^2$.
Invert sugar $=0.936+0.4435\ Cu+0.000131\ Cu^2$.
When 0.1 g. of sucrose is present, Invert sugar $=0.792+0.4442\ Cu+0.000128\ Cu^2$.
When 0.3 g. of sucrose is present, Invert sugar $=0.498+0.4457\ Cu+0.000118\ Cu^2$.
When 1 g. of sucrose is present, Invert sugar $=-0.023+0.4438\ Cu+0.000119\ Cu^2$.
When 3 g. of sucrose is present, Invert sugar $=-0.922+0.4308\ Cu+0.000165\ Cu^2$.
When 10 g. of sucrose is present, Invert sugar $=-2.429+0.4117\ Cu+0.000195\ Cu^2$.

in Table 4 the milligrams of sugar corresponding to a minimum value of 100 mg. of copper.

The precision of this method can be estimated by considering the deviation of analytical results from the calculated values. Of the 76 analy-

TABLE 3.—*A series of invert sugar analyses*

INVERT SUGAR TAKEN	INVERT SUGAR SOUND						MAXIMUM ERROR		AVERAGE ERROR	
	AUG. 2	AUG. 3	AUG. 4	AUG. 5	AUG. 7	AUG. 11				
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*mg.*	*per cent*
140			140.4	139.7		139.6	0.4	0.29	0.32	0.23
—	—	—	140.2	—	—	—	—	—	—	—
120	119.8	120.1	120.1	120.4	120.0	119.9	.4	.33	.15	.12
100	99.9	100.1	100.5	100.0	100.4	99.8 99.9	.5	.5	.20	.20
80	79.9	80.0	80.3	80.2	80.2	79.9 80.0	.3	.38	.12	.15
60	59.7	60.0	60.2	59.9	—	—	.3	.50	.20	.33

ses falling within the range included in Table 4, the number of analyses in which results deviate from the calculated values by specific amounts are as follows:

Number of analyses	*Deviation per cent*	*Number of analysis*	*Deviation per cent*
3	0.6–0.5	15	0.3–0.2
7	0.5–0.4	22	0.2–0.1
7	0.4–0.3	22	0.1–0

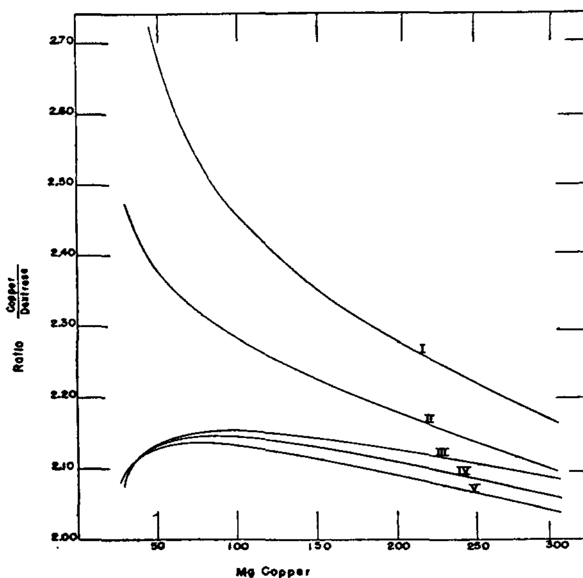


FIG. 4.—Change of the copper-sugar ratio with variation in the amounts of reduced copper.

Curve I. Invert sugar +10 grams of sucrose; II. invert sugar +3 grams of sucrose; III. levulose; IV. invert sugar; V. dextrose.

Ninety-six percent vary less than 0.5 percent, with 77 percent less than 0.3 percent.

The results of analyses of invert sugar solutions given in Table 3 are in agreement with these findings. When working with pure sugar solutions under carefully controlled conditions the accuracy attained within the range given in Table 4 agrees well with the precision of the method. If one is working with very dilute solutions or those in which more than about 300 mg. of copper is reduced (the total amount of copper present is 318.2 mg.), slight variations in the conditions may shift the curves given in Figure 4 so that the results become less accurate, although duplicate analyses may agree well within the limits of the precision of the method. The precision of a reducing-sugar method in the analysis of products other than pure sugar solutions is so dependent upon the composition of the product that a general statement cannot be made. However, it can be said that in the analysis of five molasses samples investigated by this method, the end point could readily be determined and duplicate determinations agreed within 0.5 percent.

TABLE 4.—*Table for calculating dextrose, levulose, invert sugar, and invert sugar in the presence of sucrose (0.1, 0.3, 1.0, 3.0 and 10 g.)*

COPPER	DEXTROSE	LEVULOSE	INVERT SUGAR	INVERT SUGAR				
				PLUS 0.1 G. OF SUCROSE	PLUS 0.3 G. OF SUCROSE	PLUS 1.0 G. OF SUCROSE	PLUS 3.0 G. OF SUCROSE	PLUS 10.0 G. OF SUCROSE
100	46.9	46.4	46.6	46.5	46.2	45.5	43.8	40.7
110	51.7	51.1	51.3	51.2	51.0	50.2	48.5	45.2
120	56.4	55.8	56.0	55.9	55.7	54.9	53.2	49.8
130	61.3	60.5	60.8	60.7	60.4	59.7	57.9	54.4
140	66.1	65.2	65.6	65.5	65.2	64.4	62.6	59.0
150	71.0	70.0	70.4	70.3	70.0	69.2	67.4	63.7
160	75.9	74.7	75.2	75.1	74.8	74.0	72.2	68.4
170	80.8	79.5	80.1	80.0	79.7	78.9	77.1	73.2
180	85.7	84.4	85.0	84.9	84.6	83.7	82.0	78.0
190	90.7	89.2	89.9	89.8	89.4	88.6	86.9	82.8
200	95.7	94.1	94.9	94.8	94.4	93.5	91.8	87.7
210	100.4	98.9	99.8	99.7	99.3	98.4	96.8	92.6
220	105.8	103.8	104.8	104.7	104.3	103.4	101.8	97.6
230	110.9	108.8	109.9	109.7	109.2	108.4	106.9	102.6
240	116.0	113.7	114.9	114.8	114.3	113.4	112.0	107.6
250	121.1	118.7	120.0	119.8	119.3	118.4	117.1	112.7
260	126.3	123.7	125.1	124.9	124.4	123.4	122.2	117.8
270	131.5	128.7	130.2	130.1	129.4	128.5	127.4	123.0
280	136.7	133.7	135.4	135.2	134.5	133.6	132.6	128.2
290	141.9	138.8	140.6	140.4	139.7	138.7	137.9	133.4
300	147.2	143.9	145.8	145.6	144.8	143.9	143.2	138.7

It is interesting to note that, quite in contrast to their relative reducing powers in the caustic alkali-copper reagents, levulose has in the copper carbonate solutions a greater reducing power than dextrose.

VI. CONCLUSIONS

In general, and in harmony with previous experience with the carbonate method, it is concluded that this method is inferior in respect of precision to the methods of Munson and Walker and of Lane and Eynon in which caustic alkali is a constituent of the copper reagent. It is, however, valuable, because of its convenience for rapid work that does not require a precision greater than one-half percent. It is particularly useful to those who make only occasional analyses, since if care is taken to preserve the standard iodate solution no further standardization is required.

EXPERIMENTAL STUDIES ON DECOMPOSITION OF OYSTERS USED FOR CANNING

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This work was undertaken to study biological and chemical aspects of decomposition in oysters used for canning. It was initiated as a result of controversy regarding the objective organoleptic judgment of edibility of certain lots of canned oysters. The point at issue was not a question of improper sterilization or sealing of the canned product but concerned the alleged use of decomposed raw material.

Experimental procedures herein reported were devised to study factors which may affect the odor of the finished canned product and to study the chemistry of spoilage of the unprocessed oyster with special emphasis on the development of a useful chemical criterion of decomposition in canned oysters.

BIOCHEMICAL BACKGROUND OF DECOMPOSITION OF ANIMAL MATTER

It has long been known that decomposition of animal and vegetable matter is accompanied by formation of quantities (usually, at first, traces) of characteristic chemical substances. These reactions have been summarized by various physiological chemists[1,2] and may be briefly stated as follows:

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* Chief, Chemical-Toxicological Section.
† Chief Biologist.
[1] Albert P. Mathews, "Physiological Chemistry," 5th ed., copyright 1930, William Wood & Co., New York.
[2] Simon Mendelsohn, "Embalming Fluids," (1940). Chemical Publishing Co., New York.

The character of putrefactive and fermentative products formed from food is dependent on the species of bacteria present and on the chemical composition of the food. Some facultative anaerobes, for example, ferment glucose and lactose, forming lactic acid, alcohol, and carbon dioxide. Under anaerobic conditions, in the absence of sufficient carbohydrate, skatole and indole are formed from the proteins and hydrogen sulfide from cysteine and cystine. Butyric acid, hydrogen, methane, and other substances are also produced by bacterial decomposition of carbohydrates. In animal tissues, upon passing from a viscous to solid state in cadaveric rigidity, acidity is developed concurrently with the initiation of putrefactive changes. Lactic acid formed under these circumstances apparently originates through resolution of muscle carbohydrate, inasmuch as increase in acidity is definitely associated with a corresponding decline in the glycogen content of the tissue.

It appears that hydrolysis of albuminous matter is catalyzed by enzymes commonly known as tryptases, and represents an initial phase of the reactions which subsequently result in the formation of albumoses, polypeptides, amino acids, ammonia, etc. The amino acids are subject to various changes, eventually yielding phenol, p-cresol, indole, skatole, and numerous amines which are also responsible for some of the characteristic odors of putrefaction. Tryptophane gives rise on putrefaction to indole and skatole. It is believed that intermediate products such as indole-propionic acid and indole-acetic acid are also formed. Osborne and Jones and Okuda *et al.*[3] have demonstrated the presence of tryptophane among other hydrolysis products of crustacean and mollusk proteins.

Indole reacts under certain conditions with paradimethyl aminobenzaldehyde to give a red color. This aldehyde is known as "Ehrlich's aldehyde" and is the principal ingredient in the Ehrlich[4] and Kovacs[5] reagents. It is used by bacteriologists to detect the presence of indole-forming bacteria incubated in tryptone medium.

The measurement of this red color was made quantitative by Chernoff,[6] and was adapted to quantitative determination of indole in butter and cream by Clarke *et al.*,[7] who introduced the use of the photometer in measurement of the color.

Indole is a white crystalline solid, sparingly soluble in cold water, and soluble in hot water, fat, alcohol, and chloroform. It melts at 52°–53°C., boils at 254°C., and can be quantitatively steam distilled. Pure indole in water solution can be determined photometrically with a precision of a fraction of a microgram by the method described herein. Contrary to the impressions of many chemists it is not highly odoriferous in the pure state or in dilute aqueous solutions. Its homologue, skatole (methyl indole), is more odorous, but neither has enough stench in the pure state to account for the bad odor of putrid oysters. It is believed that one or more of the other chemical products of decomposition of animal matter account

[3] Winton, "Structure and Composition of Foods," John Wiley & Sons.
[4] Stitt, Clough and Clough, "Practical Bacteriology, etc." 9th ed. Blakiston.
[5] *Z. Immunitäts.*, 55, 311–315 (1928).
[6] *Ind. Eng. Chem., Anal. Ed.*, 12, 273 (1940).
[7] *This Journal*, 20, 475, (1937).

for most of the organoleptic evidence of decomposition. These facts lend added significance to indole content as an index of decomposition in oysters, independent of the organoleptic condition.

Since oysters contain both protein and carbohydrate (glycogen), it is reasonable to conclude from the foregoing discussion of the general reactions involved in the fermentation and putrefaction of animal matter that similar or related reactions proceed in an oyster during spoilage. It is highly probable that many if not all the products of decomposition are formed, which combine to give the bad odor of decomposed seafood. It is not definitely known which of the foregoing reactions involved are strictly autolytic or bacteriological or both.

Hunter and Linden[8] identified the organisms isolated from decomposing oysters and found that the bacterial flora of the oyster was composed of common water and soil organisms including cocci, nonspore-forming gram-negative aerobic bacilli, spore-forming gram-positive aerobic (and anaerobic) bacilli, lactobacilli, streptococci, and yeasts.

In considering any chemical approach to determination of decomposition in canned oysters, it is necessary to work with chemical products of decomposition which can be determined in minute amounts. The organoleptic test can detect traces of some of the most odoriferous products. This test, however, is confined to the volatile decomposition products. It is obvious that many of these may be lost in the drastic steaming, washing, and soaking processes through which oysters pass prior to canning. The organoleptic test has a further disadvantage in that the sensitivity of the sense of smell is different for different individuals, giving rise, in borderline cases, to differences in opinion as to whether or not a given sample of food is decomposed. Although there is much evidence that an individual's sense of smell can be trained to detect certain faint odors, acceptable chemical tests, on the other hand, are characterized by their reproducibility by different analysts.

EXPERIMENTAL PROCEDURES

Preliminary experiments in this laboratory showed that no indole is formed during steaming and processing of good oysters and that oysters allowed to spoil without being washed free of their own shell liquor contain indole. With the foregoing in mind the experimental procedures described below were followed in an effort to throw light on the biological and chemical aspects of canned oysters prepared from decomposed raw material.

EFFECT OF ENVIRONMENT ON DECOMPOSITION OF RAW OYSTERS

To ascertain the effect of environment on the decomposition of raw oysters two one-gallon lots of commercially shucked Louisiana oysters

[8] *J. Agr. Research*, 30, 971–976 (1925).

were allowed to decompose. One lot was thoroughly washed with running, cold, city tap water. The other lot was not washed. The shell liquor was discarded with the exception of that which adhered to the meat or was mechanically transferred to the shucking pail. The two lots were shucked at different times—about one week apart. Both lots were allowed to spoil at a temperature of 20°–25°C. under conditions noted below. Organoleptic and indole* determinations were made at intervals as indicated (Tables 1 and 2).

TABLE 1.—*Decomposition of washed, shucked, raw oysters*
(The oysters were allowed to stand in a loosely covered gallon oyster can)

INTERVAL	INDOLE†	ORGANOLEPTIC
hours	*micrograms/100 gram*	
0	1.0	Fresh
24	1.5	Slightly stale odor but not definitely decomposed.
48	1.8	Oysters were gassing (gaseous fermentation)—faint sweetish odor of decomposition.
72	1.0	Offensive sour and yeasty odor, resembling yeasty cream.
120	2.1	Fermented yeasty and sour odor more pronounced than at 72 hours.
144	4.0	Same but more pronounced.
288	5.3	Same as above—oysters by this time had almost completely liquefied.

† This includes "apparent indole" resulting from a slightly variable photometer reading due to a yellowish-brown to orange and occasionally light pink color obtained from good oysters upon application of the indole method. The term "apparent indole" as used throughout this paper refers to "indole reacting material" which, while causing absorption in the spectrophotometer, nevertheless does not register the curve characteristic of indole.

It appears from the foregoing experiments that the nature of decomposition of raw oysters depends on the conditions under which they are allowed to spoil, especially with respect to the external environment. Washing the shucked oysters with clean water seems to remove putrefying agents whether they be autolytic agents or micro organisms, leaving those which favor fermentation and souring rather than an ammoniacal or putrefactive type of decomposition.

In order to determine the character of decomposition of oysters *in the shell* a sack of cultivated oysters from California Point in Breton Sound were set aside inside a New Orleans shucking plant. After 6 days they were inspected and found to contain approximately 60 per cent "gapers." † All oysters were moist, apparently with exuding shell liquor from the gapers. The gapers were shucked separately. All were in an advanced state of decomposition with a sickeningly putrid odor. None was dehy-

* Indole determined according to method described later in this paper.
† The term "gapers" is applied in the industry to dead oysters whose adductor muscle and locking mechanism have permanently relaxed and whose valves are partially open.

TABLE 2.—*Decomposition of unwashed, shucked, raw oysters*
(These oysters were placed in a covered collander. After 48 hours oysters were placed in a large covered pan in a thin layer)

INTERVAL	INDOLE RAW	INDOLE AFTER CANNING*	ORGANOLEPTIC
hours	*micrograms/100 grams*		
0	1.6(2.4)	1.8	Fresh
24			No odor of decomposition.
48 top oysters	3.1		Oysters on surface had a distinct though faint odor of putridity—oysters below surface and on bottom were not putrid. These had an odor of sour milk.
bottom oysters	1.1		
72	5.8	7.0	Between the 48- and 72-hour intervals the remaining oysters were spread in a thin layer in a large pan and covered with paper. Odor at 72-hour period was that of sour cream. Oysters were gassing, and their stomachs were ruptured. Any putrid odor present was masked by the sour odor. The oysters after canning had a faint putrid odor. Putrid odor was not at all pronounced. Many of the oysters were perforated.
168	21.3		Oysters had a cheesy odor with an underlying putrid odor. Oysters had begun to dry out and were beginning to turn black. A white growth was noted on their surface.

* Canned according to commercial procedure described later. Indole on basis of 100 grams of drained meats.

drated, but instead all bore more or less the shape of a normal oyster. Discoloration was noted in many. Numerous maggots were noted in the oyster meats as well as outside the shell. These oysters, after being shucked, were steamed and canned under conditions similar to commercial canning operations.*

The closed oysters were shucked. Out of a representative number examined all were dead and decomposed. Most were putrid, although the putrid odor was not as pronounced as in the case of the gapers. These were also canned as above. Two days later one can each of these oysters was opened and examined with the following results:

* One quart of the shucked meats was steamed in a small pressure cooker on an 8-mesh brass sieve for 12 minutes at 10 lbs. pressure (240° F.). They were then washed 1–2 minutes in cold, running, tap water, followed by draining and a 5 minute soaking in 15% brine. After draining they were packed 7.25 oz. to the No. 1 can and covered with boiling 2% brine; the can was closed, and processed 13 minutes at 15 lbs. pressure (250°F.) and cooled immediately under water.

Condition of Shell Stock	*Indole (microgram per cent)**	*Organoleptic*
Closed	16.1	Putrid odor† Some discolored oysters present.
Gapers	89.8	Putrid odor† More pronounced than in the closed oysters.

* Micrograms/100 grams of drained meats.
† The putrid odor was greatly decreased by the canning operation.

CHEMICAL AND ORGANOLEPTIC CHARACTERISTICS OF OYSTER REEF MUD AND WATER

In order to determine the indole content and organoleptic characteristics of oyster reef mud and water, samples were taken at each of 6 stations designated by the Conservation Department as typical oyster beds representing Louisiana waters with respect to tides, variation in salinity, *p*H, and oyster food. These stations are located at the following points. The water samples were taken from the bottom.

Station	*Location*
A	Grand Pass
B	Creole Gap
D	Johnson's Bayou
E	West Karako Bay
F	Half Moon Island (Grand Island)
G	Three Mile Bayou

The samples were kept refrigerated and examined 4 days later. Results are expressed in Table 3.

TABLE 3.—*Indole and H_2S formation*

STATION	ORGANOLEPTIC TEST	"APPARENT INDOLE" CONTENT	H_2S (BY ODOR AND Pb ACETATE PAPER TEST ON STEAM DISTILLATION)
		(micrograms/100 grams)	
	On steam distillation of oyster reef mud		
A	No odor of decomposition	1.3	positive
B	No odor of decomposition	0.9	positive
D	No odor of decomposition	1.4	positive
E	No odor of decomposition	0.7	positive
F	No odor of decomposition	0.7	positive
G	No odor of decomposition	1.4	negative
	On steam distillation of oyster reef bottom water		
A	No odor of decomposition	1.0	none
B	No odor of decomposition	1.0	none
D	No odor of decomposition	0.6	none
E	No odor of decomposition	0.6	none
F	No odor of decomposition	1.0	none
G	No odor of decomposition	0.7	none

"INDOLE REACTING MATERIAL" IN FRESH OYSTERS

In order to determine the base line for the "apparent indole" content (that is, the substance in fresh canning stock oysters which gives a yellowish-brown to orange and light pink color exhibiting a positive photometer reading at a wave length of 560mμ) the following series of packs were made at Grand Pass, La. The oysters were "steam stock" (canning stock) oysters obtained from reefs which serve as their normal source. They were obtained by tonging or dredging. The dredged oysters were obtained from Mississippi boats which had caught them in Louisiana waters. Grand Pass is a port of entry through which all oysters caught in Louisiana must pass before being taken out of the State for canning. The dredged samples were taken from the decks of the boats and information regarding each source was obtained from the captain of the boat.

The oysters were carried through the commercial process for canning oysters. This work was done at the Louisiana State Conservation Department Marine Biological Laboratory at Grand Pass. The detailed procedure is outlined as follows:

The shell stock was carefully sorted and any gapers or oysters with broken bills were discarded. Any cluster of oysters containing mud which bore putrid oyster liquor was discarded. They were then steamed 10–12 minutes at 240°F. (10 lbs. pressure) and shucked, and each individual oyster was tested organoleptically at this point. Any oyster which exhibited an odor of decomposition after shucking was discarded. The shucked oysters were then washed for 1 or 2 minutes in running cistern water, drained, soaked 5 minutes in 10 percent brine, drained again, packed 7.25 ounces to a No. 1 can, covered with boiling 2 percent brine, closed, and processed 13 minutes at 250°F. Results of analysis are shown in Table 4.

TABLE 4.—*"Apparent indole" content* of fresh canning oysters from various sources*

SOURCE OF OYSTERS	"APPARENT INDOLE" CONTENT	SOURCE OF OYSTERS	"APPARENT INDOLE" CONTENT
	(micrograms/ 100 grams)†		*(micrograms/ 100 grams)*†
Black Bay (Fortuna Point)	2.0	Callega Bay	1.9
Le Petit Pass	1.9	Ditto (different boat)	2.2
Eloi Bay (Deadman Point)	1.7	Grand Pass (tonged)	1.7
West Karako Bay (Boudreau Bay—Dead Man's Is.)	2.0	Snake Island (Nigger Bay)	1.7
		Mud Grass Island	1.7
Caleko Bay	1.9	Fox Bay	1.6
Catfish Pass	1.6	Creole Gap (tonged)	2.0
Raccoon Point	1.6	Bayou Pierre (tonged	1.4

* Based on photometer reading. Spectrophotometric curves shown in Fig. 1 demonstrate that other substances than indole cause the absorption in solutions prepared from good oysters.
† The organoleptic test on the canned oysters showed no decomposition.

EFFECT OF SOURCE OF OYSTERS ON TYPE OF DECOMPOSITION

In order to determine if the source of the oysters has any effect on the formation of indole during spoilage the following experiment was started on March 20 and concluded on March 28, 1944.

Six lots of canning shell stock from different sources were put on a screened porch covered with a roof at Grand Pass. Five lots were obtained from passing oyster boats. One lot was tonged in Bayou Pierre. They were allowed to stand for eight days at a mean temperature of approximately 75°F. All lots were fresh at the beginning of the experiment with exception of the lot from Dead Man's Island, which contained some gapers and spoiled closed oysters when set aside.

After 8 days all lots contained some gapers and some closed oysters. Each lot was carried through the usual canning procedure as described previously. On shucking the steamed oysters all lots were found to contain decomposed oysters. Results of examination of the canned oysters are shown in Table 5.

TABLE 5.—*Indole content and organoleptic characteristics*

SOURCE	TIME OUT OF WATER	INDOLE CONTENT OF CANNED OYSTERS	ORGANOLEPTIC TEST ON CANNED OYSTERS
		(micrograms/ 100 grams)	
	Canned decomposed oysters from various sources		
Dead Man's Island	Unknown (at least 8 days)	17.2	Faint putrid odor
Bayou Pierre	8 days	10.5	Mild putrid odor
Fox Bay	Unknown (at least 8 days)	82.3	Strong putrid odor
Calego Bay	Unknown (at least 8 days)	105.0	Strong putrid odor
Snake Island	Unknown (at least 8 days)	141.0	Strong putrid odor
Mud Grass Island	Unknown (at least 8 days)	40.5	Strong putrid odor

INDOLE CONTENT AND ORGANOLEPTIC CHARACTERISTICS OF MIXTURES OF GOOD AND DECOMPOSED OYSTERS

An experimental lot of wild reef oysters was obtained from California Bay. A portion was steamed and canned while still fresh. The remainder was set aside in sacks until some had begun to gape. Beginning 5 days later and extending through the ninth day a series of 7 cans was put up from this lot. Mean daily temperatures during this period ranged from 52° to 66°F. The oysters were small (about 180 oysters to 7.25 ounces). Upon shucking after steaming, each oyster was examined by odor and placed in one of three classes—passable (edible), decomposed, and decomposed (advanced stage).

Those classed as passable were either fresh or had no definite odor of decomposition. Those classed as decomposed bore a definite, though some-

times faint odor of decomposition. Some of these had a butyric acid or cheesy odor while others had an ammoniacal odor. Those in the advanced stage of decomposition had either a "dead rat" or putrid odor.

Results of examination of these experimental cans of oysters are shown in Table 6.

It will be noted that no strong odor of decomposition could be detected in any of the canned oysters except those in can 8. There could be no question about the odor of these and the odor of the fresh oysters in can 1. Cans 2, 3, 4, 5, 6, and 7, however, had only a slight odor of decomposition, although each contained definitely decomposed oysters. Upon organoleptic examination of the drained meats from these cans, some individuals could not get the decomposition odor. To the experienced nose, however, it was faintly detectable. Indole values in each case show 6.5 or more micrograms per 100 grams.

TABLE 6.—*Results of examination of experimental packs (single cans) of good and decomposed oysters from California Bay*

CAN NO.	WT. OF PASSABLE OYSTERS	WT. OF DECOMPOSED OYSTERS	WT. OF OYSTERS IN ADVANCED STAGE OF DECOMP. (PUTRID)	TOTAL WT. OF DECOMPOSED OYSTERS	ODOR OF DRAINED OYSTER MEATS AFTER CANNING	INDOLE CONTENT OF DRAINED MEATS
	per cent	*per cent*	*per cent*	*per cent*		*(mmg/100 gm*)*
1	100	0	0	0	Good	1.9
7	97.2	2.8	0.0	2.8	Slight odor of decomp.	12.7
3	94.1	5.3	0.6	5.9	Slight odor of decomp.	11.5
2	93.6	5.3	1.1	6.4	Very slight odor of decomp.	8.4
6	92.1	6.6	1.3	7.9	Slight odor of decomp.	13.2
5	90.3	9.7	0.0	9.7	Slight odor of decomp.	11.5
4	89.5	10.2	0.3	10.5	Slight odor of decomp.	6.6
8	76.6	13.8	9.6	23.4	Distinct odor of decomposed oysters. Some portions slightly putrid.	34.0

* Average of 2 determinations.

Another lot of fresh dredged canning stock oysters (size, 90–150 per can) was obtained from Half Moon (Grand) Island. A portion was canned while fresh, and the remainder was allowed to stand in sacks, indoors, until the oysters had begun to gape (8 days). Single cans were put up at intervals over a period of 6 days. Mean daily temperatures ranged from 46° to 72°F. The procedure followed was the same as for the California Bay oysters (above). Results are shown in Table 7.

TABLE 7.—*Results of examination of experimental packs (single cans) of good and decomposed oysters from Half Moon Island*

CAN NO.	WT. OF PASSABLE OYSTERS	WT. OF DECOMP. OYSTERS	WT. OF OYSTERS IN ADVANCED STAGE OF DECOMP.	TOTAL WT. OF DECOMP. OYSTERS	ODOR OF DRAINED OYSTER MEATS AFTER CANNING	INDOLE CONTENT OF DRAINED MEATS
	per cent	*per cent*	*per cent*	*per cent*		*(mmg/100 g)**
1	100	0	0	0	Good	1.8
2	Quantity† unknown	Quantity unknown	Quantity unknown	Quantity unknown	No odor of decomp.	3.0
3	88.7	11.3	0	11.3	Slight odor of decomp.	9.2
4	83.1	15.6	1.3	16.9	Slight odor of decomp.	6.5

* Average of 2 determinations.
† Shell stock of lot canned bore numerous "gapers" and, in the aggregate, an odor of decomposition. Some of the individual gapers tested had a distinct odor of decomposition before steaming. After steaming none of the oysters exhibited a detectable odor of decomposition; at least two bore maggots in or around the oyster meat. It is concluded that this can of oysters contained an unknown number of oysters in incipient stage of decomposition.

INCIDENTAL INFORMATION

In the course of the foregoing experimental work the following facts were noted.

Numerous instances of dead oysters with broken bills were noted among commercially dredged oysters. Some clusters bore mud which had a putrid odor. This condition was obviously caused by leaking shell liquor from injured or dead oysters in the cluster which subsequently putrefied on the boat. A number of instances were noted where good oysters bore a large amount of mud inside their closed shells, probably forced in by the dredging operation before the oysters could close their valves.

Oyster reef mud not only has no odor of decomposition when freshly taken, but after standing 15 days in a warm place the samples still evidenced no odor of decomposition. However, when this mud is subjected to steam distillation a strong odor of hydrogen sulfide is produced. Any such odor which may be absorbed by good oysters in the steaming process is apparently removed on washing and soaking since it does not show up perceptibly in the canned product.

Decomposed oysters while still warm, immediately after shucking, are much easier to detect by odor than they are after cooling, washing, soaking, and canning.

METHOD FOR QUANTITATIVE DETERMINATION ON INDOLE IN RAW AND CANNED OYSTERS

The following method, used throughout this work, is a special adaptation of the method reported by Clarke *et al.*[7] in their work on decomposition in butter and cream. Working curves were developed directly from a chloroform solution of Eastman Kodak Co. indole (M.P. 52°–53°C.), after correcting for distillation and reagent blanks. All results on oysters were so corrected. The distillation apparatus was simplified in that no Reichert-Meissel bulb was used. Certain other minor modifications were made in the interest of simplifying and shortening the procedure.

METHOD

I. REAGENTS

(a) *Water.*—Distilled tap water.

(b) *Chloroform.*—U.S.P.

(c) *Acetic acid—ether solution.*—50–50 by volume of acetic acid and U.S.P. ether. Apply the U.S.P. peroxide test to the ether, and if positive, obtain purer ether. Acetic acid should be C.P. glacial.

(d) Hydrochloric acid.—(1+19). Dilute 5 ml. of conc. HCl to 100 ml. with water.

(e) *Phosphoric acid-aldehyde solution.*—Dissolve 0.4 gram of paradimethylamino-benzaldehyde (Eastman White Label, M.P. 72°–73°C.) in 5 ml. of glacial acetic acid, mix with 92 ml. of 85% C.P. phosphoric acid and 3 ml. of conc. HCl.

(f) *Standard indole.*—Eastman white label, M.P. 52°–53°C. Dissolve in $CHCl_3$.

Soln. A—1 ml. = 100 micrograms. Is stable for months if kept refrigerated and protected from light.

Soln. B—1 ml. = 1 microgram. Prepare by dilution just before use.

II. APPARATUS

(1) *Separatory funnels.*—1–500, 1–250, and 1–125 ml. Squibb-type all-glass separatory funnels. No stopcock lubricant should be used. Cut off stem of the 125 ml. funnel to a length of ¼ to ¾ inch.

(2) *Graduated cylinders, glass-stoppered, 50 ml.*—Check graduation at 10 ml. mark.

(3) *Distillation apparatus.*—Use an 800 ml. Kjeldahl flask equipped with safety tube and boiling tube for steam generator. Connect by rubber tubing to a 500 ml. Kjeldahl flask equipped with a bent glass tube and two-holed rubber stopper set up for steam distillation. Connect by glass-tubing, vertical, straight-bore condenser. Use shortest connections possible and arrange entire unit on a single ring stand. Protect receiving flask from heat. Collect distillate in a 500 ml. Erlenmeyer flask.

All apparatus must be rinsed carefully with 95% alcohol followed by distilled water before each use.

III. SAMPLING

Canned oysters.—Drain entire content of can for 2 minutes on an 8-mesh sieve. Weigh the meats and thoroughly disintegrate and mix them in a "Waring Blendor." Weigh 100 grams of the mixture and transfer to the distillation flask with a minimum quantity of water. If the "Blendor" is of a type that will not operate successfully on drained meats, add a weighed amount of the drained liquid (in some cases all of the liquid may be required). Weigh out 100 grams of the mixture as before and calculate the indole result to 100 grams of drained meats.

Raw oysters.—Comminute the sample in the "Blendor," weigh 100 grams of the mixture, and transfer to the distillation flask with a minimum quantity of water.

IV. DISTILLATION

Steam distil until 325 ml. of distillate is collected at such a rate that 40–50 minutes is required for the operation. Shake flask containing oysters occasionally. At the end, turn off condenser water and continue distillation until steam is evolved through the end of the condenser.

V. EXTRACTION

Transfer distillate to the 500 ml. separatory funnel, rinsing flask into the funnel with the portions of $CHCl_3$ used below. Add 5 ml. of the dilute HCl.

Add 25 ml. of $CHCl_3$ and shake for 1 minute at the rate of about 150 times per minute. Allow to settle several minutes, swirling the funnel occasionally during this interval. Draw off the $CHCl_3$ layer into the 250 ml. separatory funnel containing 25 ml. of water. Shake vigorously 10 times and allow to settle. Finally pass the $CHCl_3$ layer through a small pledget of surgical cotton (previously moistened with $CHCl_3$) placed in a small glass funnel, into a clean, *dry* 125 ml. separatory funnel. Take care not to allow any of the wash water to go through the cotton. Repeat the above extraction operation twice more, using 20 ml. and 15 ml. portions of $CHCl_3$, successively.

VI. DEVELOPMENT OF COLOR

Add 10 ml. of the phosphoric acid-aldehyde reagent (accurately measured) and shake vigorously exactly 2 minutes at a frequency of approximately 200 per minute. Allow to stand 10 minutes and draw off exactly 9.0 ml. into the graduated glass-stoppered 50 ml. cylinder, *taking care not to get any of the $CHCl_3$ layer into the cylinder.* Dilute the mixture in the cylinder to about 40 ml. with the acetic acid-ether reagent, mix, and cool. Fill with the acetic-ether mixture to the 50 ml. mark and mix. If not brilliantly clear, filter the solution through a small paper, keeping the funnel covered with a watch-glass to prevent evaporation. Transfer the solution to a suitable photometer cell and determine the scale reading. Reading should be made within 15 minutes. If the color is too deep to read in the smallest cell used for the standard curve, make appropriate dilutions with the acetic-ether mixture. Subtract from the scale reading the blank, which includes reagent blank and distillation blank, if any. Convert the corrected scale reading to micrograms of indole by referring to the standard curve described below. Calculate indole in terms of micrograms per 100 grams of oyster meats.

VII. DETERMINATION OF BLANKS

Determine distillation blanks on each still.

VIII. PREPARATION OF STANDARD CURVE

Add sufficient $CHCl_3$* to the 125 ml. separatory funnel to total 50 ml. when the standard indole solution is added. Pipet into the funnel sufficient standard indole solution (solution B) to make a series of standards of 1, 2, 5, 7, 10, 15, and 25 micrograms. Proceed as directed under VI, *Development of Color,* and read on the photometer at a wave length of 560 mμ. Plot a separate curve for each cell used after subtracting the reagent blank determined as above, using pure $CHCl_3$ and the phosphoric acid-aldehyde reagent without addition of indole.

In order to study qualitatively the colors obtained from good oysters and bad oysters and those obtained from pure indole and skatole and reagent blank a series of spectrophotometric curves (see Fig. 1) was prepared with a Beckman Quartz Spectrophotometer. These curves show that the principal substance measured in bad oysters is indole. They show further that the yellow to brown, to light pink color, obtained from good oysters is not due to indole. Any skatole formed along with the indole does not appreciably affect the photometer reading. When the indole color fades in diffused light the characteristic shape of the indole curve is maintained.

In limited trials the greater part of the indole of decomposed oysters appears to reside in the oyster meats. A can of fresh oysters, after standing

* The presence of approximately 0.75% alcohol used as preservative in U.S.P. $CHCl_3$ apparently has no effect on the photometer reading.

for eleven months, still exhibited no indole—an "apparent indole value" of 2.1 microgram percent being obtained, as compared with 2.2 obtained on another can from the same lot analyzed a few days after canning.

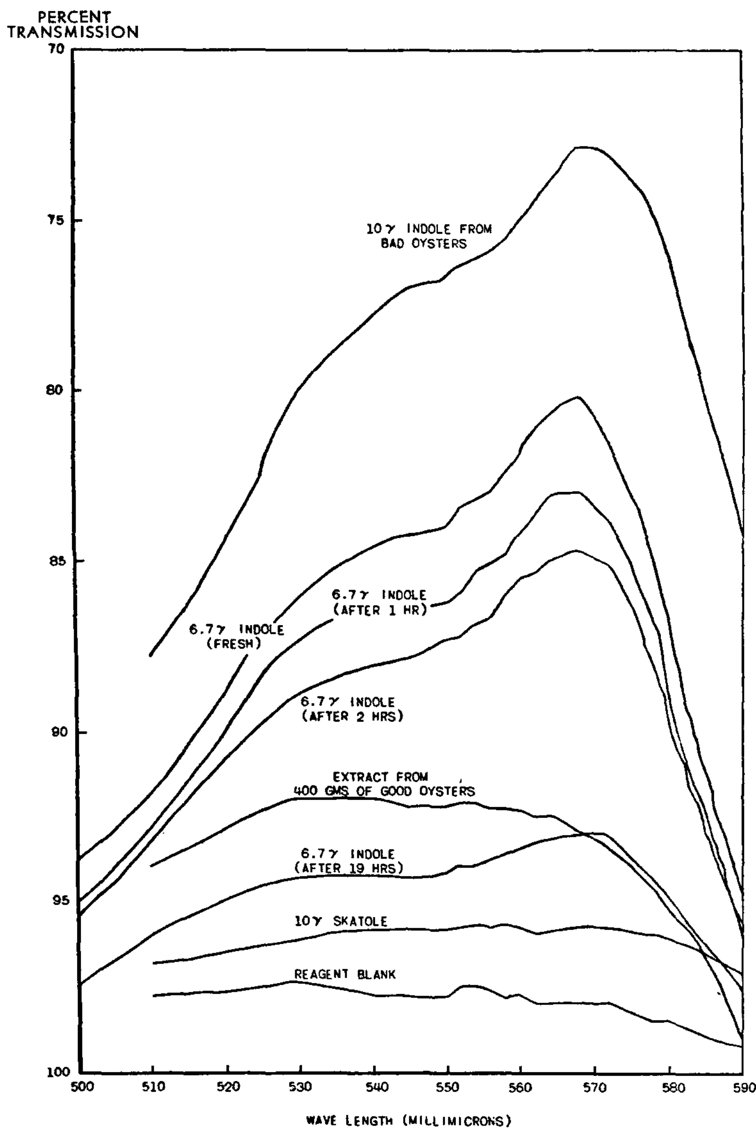

FIG. 1.—Spectrophotometric Curves.

SUMMARY

A brief review of the literature concerning the chemical nature of decomposition of animal matter is given. A method for determination of indole in canned oysters was adapted from recently published methods. The indole content, or "indole reacting material," of 15 edible lots and 8 decomposed lots of canning stock oysters obtained from various points in the Louisiana bays and inlets, along the shores of the Gulf of Mexico, was determined by the method, after canning by the usual commercial procedure. Mixtures of good and decomposed oysters were canned and analyzed for indole. Two lots of shucked raw oysters were studied with respect to formation of indole on decomposition. A study was made of oyster reef water and mud, obtained from 6 stations typical of Louisiana oyster bottoms, with respect to odor, hydrogen sulfide production on steaming, and indole content, together with the relation to the organoleptic properties of such mud and water in canned oysters obtained from these areas.

ACKNOWLEDGMENT

The authors wish to express appreciation to A. H. Seibert of the Shilstone Testing Laboratory, New Orleans, for development of the spectrophotometric curves on their Beckman Quartz Spectrophotometer. Sincere thanks are also extended to James N. McConnell, Director, Division of Oysters and Water Bottoms, Department of Wildlife and Fisheries, for his generous cooperation in connection with all the work reported herein.

GENERAL COLORIMETRIC METHOD FOR DETERMINATION OF SMALL QUANTITIES OF SULFONATED OR SULFATED SURFACE ACTIVE COMPOUNDS

By J. H. JONES* (Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.)

Surface active agents have valuable uses in many processes and products. Frequently, the concentration of the active compound required for effective action is quite small. Except for soaps, the surface active compounds most widely used at present are the anionic, "sulfonated" compounds. The number of commercially available products of this class is large.[1,2,3] A simple general method for the determination of small quantities of such compounds is, therefore, needed.

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* Present address: Armour Foundation, Chicago, Ill.
[1] Van Antwerpen, F. J., *Ind. Eng. Chem.*, **35**, 126 (1943).
[2] Bulletin of the National Formulary Committee, Vol. X, No. 8–9 (1942).
[3] "Wetting Agents" Bulletin No. 9, The American Perfumer and Essential Oil Review, New York (1939).

More or less satisfactory methods are available for the determination of the "active" ingredients in the concentrated commercial materials, but these methods have not been applied to the determination of small quantities. Several methods[4,5,6] have been proposed for the determination of small quantities of the "sulfonated" compounds; however, these methods are not generally applicable, and in most cases they cannot be used if soap is present.

It has been found that "sulfonated" surface active compounds form colored salts with methylene blue. The reaction appears to be

$$(1) \qquad RSO_3^- + MB^+ = RSO_3MB,$$

where RSO_3^- is the anion of a "sulfonated" surface active compound and MB^+ is the cation of methylene blue. These colored salts are soluble in chloroform and, under suitable conditions, can be quantitatively extracted from an aqueous solution without extraction of any uncombined methylene blue. The amount of surface active compound present may, therefore, be calculated from the concentration of the colored salt in the chloroform extract. A colorimetric method for the determination of "sulfonated" surface active compounds based on these facts is described in this report. This method appears to be generally applicable and few substances seem to interfere.

The proposed method depends upon the combination of a colored cation (methylene blue) with a colorless anion (the "sulfonate"). A method, essentially the inverse of this procedure (*i.e.*, the combination of a colored anion with a colorless cation), has been used for the determination of high molecular weight quaternary ammonium salts.[7]

METHOD

APPARATUS

The spectrophotometric data on which the proposed method is based were obtained with a General Electric Recording Spectrophotometer having an 8 mμ slit. Any good photometer with a filter transmitting light at 652 mμ should be satisfactory for routine analyses.

REAGENTS

(a) *Methylene blue chloride.*—U.S.P. or certified grade.

(b) *Methylene blue solution.*—a 0.1% aqueous solution of the chloride.

(c) *Chloroform.*—U.S.P.

STANDARDIZATION

(a) *Against methylene blue chloride.*—Determine the dye content of methylene blue by titration with Ti_2Cl_6 as directed in the color chapter of *Methods of Analysis, A.O.A.C.*, 1940. Dissolve 0.250 gram of the standardized dye in exactly 100 ml. of alcohol. By appropriate dilution of the alcoholic solution with $CHCl_3$ prepare standard solutions containing 0.1, 0.2, 0.3, and 0.4 mg. of methylene blue chloride per 100 ml. Determine the instrument reading for these solutions at 652 mμ, in a 1 cm.

[4] Harris, J. C., *Ind. Eng. Chem., Anal. Ed.*, 15, 254 (1943).
[5] Pederson, C. J., *Am. Dyestuff Reptr.*, 24, 137 (1935).
[6] Kling, W., and Puschel, F., *Melliand Textilber.*, 15, (1934).
[7] Auerbach, M. E., *Ind. Eng. Chem., Anal. Ed.*, 15, 492(1943).

or ½ inch cell, and plot (or tabulate) the data obtained. (The photometric measurements should be made within one hour after the solutions are prepared.) These data serve as a general standard for the analysis of "sulfonated" compounds by the proposed method.

(b) *Against surface active agent.*—If a standard sample of the compound to be determined is available, prepare a master solution and analyze aliquots of this solution by the proposed method. Compare the results of the analysis of unknowns with the data obtained on the standard sample.

DETERMINATION

Place an aliquot of the sample containing 0.1–0.4 mg. of "sulfonate" in a separatory funnel and dilute to about 20 ml. with water. Neutralize the solution with HCl and add 3–5 drops in excess. Add 1 ml. of the methylene blue solution and mix thoroughly. Add 20 ml. of $CHCl_3$, shake gently for 1 minute, and let stand for 5 minutes. Drain the $CHCl_3$ layer into a second separatory funnel, add 20 ml. of distilled water, shake gently for 1 minute, and let stand for 5 minutes. Filter the $CHCl_3$-layer through a pledget of cotton, placed in a long-stem funnel, into a 100 ml. volumetric flask. Make three additional extractions with 20 ml. aliquots of $CHCl_3$. Each time, transfer the $CHCl_3$-layer to the second funnel, wash, let stand, and drain into the volumetric flask through the cotton pledget. (For quantitative recovery of some compounds it may be necessary to make one extraction with 20 ml. and four extractions with 15 ml. portions of $CHCl_3$.) Wash the funnel and cotton with $CHCl_3$, make to the mark with the solvent, and mix. Place the colored solution in the photometer cell and determine the instrument reading. Compare this reading with the standardization data to determine the amount of methylene blue extracted.

1 mg. of anhydrous methylene blue chloride
$= 3.13 \times 10^{-6}$ moles of methylene blue,
$= 3.13 \times 10^{-6}$ equivalents of "sulfonate."

(If method (b) for standardization is employed the instrument reading can be compared directly with the standardization values.)

EXPERIMENTAL AND DISCUSSION

Figure 1 shows spectrophotometric curves for solutions of methylene blue chloride in chloroform prepared as directed under "standardization." The values for E_{652} are shown in Table 2. It is apparent that Beer's law holds over the concentration range examined. Quantitative photometric determination of the dye dissolved in chloroform is, therefore, possible.

The proposed method was tested on the compounds listed in Table 1. These compounds were selected because statements of their chemical structure were available in several published lists.[1,2,3]

TABLE 1.—*Surface active compounds*

COMPOUND NO.	TRADE NAME	CHEMICAL COMPOSITION[a]	MOLECULAR WEIGHT[a]
1	Aerosol AY	Sodium diamylsulfosuccinate	360
2	Aerosol OS	Sodium diisopropyl napthalene sulfonate	314
3	Aerosol OT	Sodium dioctylsulfosuccinate	444
4	Duponol C	Sodium lauryl sulfate	288
5	Santomerse No. 3	Sodium alkylbenzene sulfonate	348[b]
6	Tergitol 08	Sodium ethylhexyl sulfate	232

[a] Of the chief component.
[b] Alkyl radical assumed to be dodecyl.

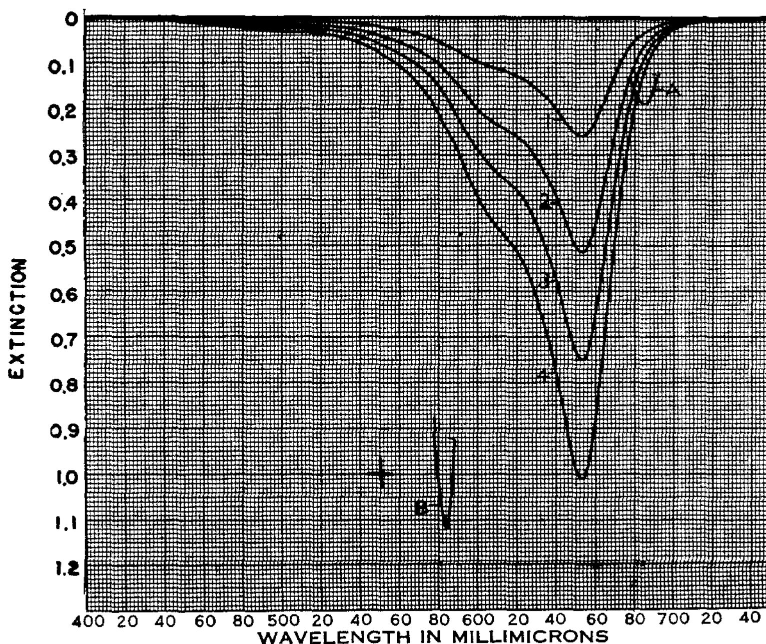

FIG. 1.—Extinction curves of methylene blue chloride in chloroform.
Concentration—
1. 0.1 mg. per 100 ml.
2. 0.2 mg. per 100 ml.
3. 0.3 mg. per 100 ml.
4. 0.4 mg. per 100 ml.
Cell—1 cm.
A = Corning Didymium Glass 512, 6.0 mm. (Absorption peak at 684.8 mμ)
B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 mμ)

Solutions of the compounds listed in Table 1 were prepared, and aliquots were analyzed by the proposed method. Typical results are shown in Table 2, and typical spectrophotometric curves for Duponol C are shown in Figure 2. The spectrophotometric curves for the other compounds have been omitted to save space, but the results in Table 2 show that for all the compounds the amount of color extracted is directly proportional to the sample weight. (The tabulated values are for single determinations. If several determinations are made at each concentration level and averaged, the results for most of the compounds conform to Beer's law within ± 1 per cent.)

The data in Table 2 indicate that any of the surface active compounds tested can be determined by the proposed method if a suitable reference standard is available. The characteristics of the spectrophotometric curves were found to be essentially the same for all the methylene blue

TABLE 2.—*Photometric data obtained by proposed method*

METHYLENE BLUE CHLORIDE*		COMPOUND NO. 1		COMPOUND NO. 2		COMPOUND NO. 3	
mg.	E_{652}	*mg.*	E_{652}	*mg.*	E_{652}	*mg.*	E_{652}
0.1	0.260	0.1	0.265	0.1	0.215	0.1	0.200
0.2	0.510	0.2	0.500	0.2	0.410	0.2	0.395
0.3	0.750	0.3	0.750	0.3	0.610	0.3	0.590
0.4	1.010	0.4	1.000	0.4	0.805	0.4	0.780
COMPOUND NO. 4		COMPOUND NO. 5		COMPOUND NO. 6		BLANK	
mg.	E_{652}	*mg.*	E_{652}	*mg.*	E_{652}	*mg.*	E_{652}
0.1	0.290	0.1	0.220	0.05	0.200	0.0	0.012
0.2	0.555	0.2	0.420	0.10	0.380		0.010
0.3	0.820	0.3	0.660	0.15	0.550		0.016
0.4	0.100	0.4	0.840	0.20	0.720	(Calculated from E in 5 cm. cell)	

Cell—1 cm.
Extinction values determined with General Electric Recording Spectrophotometer.
* Purity, as determined by titration with titanium trichloride, 88 per cent.

—"acid" combinations examined. Hence, any chloroform-soluble methylene blue salt of known purity may be used as a standard if equation (1) is correct, and the extraction is quantitative. Methylene blue chloride is proposed as a standard, however, because it is readily available and its purity is easily determined. It is also sufficiently soluble in chloroform to permit the preparation of standard solutions of the desired concentrations.

To confirm the validity of equation (1) the surface active compounds were analyzed by an independent method, whenever possible, and the results are compared with those obtained by the proposed method. The ratio,

$$\frac{\text{moles of methylene blue extracted}}{\text{equivalents of sulfonate present}}$$

should be 1 in every case if the proposed explanation is correct.

The benzidine method[6,8] is a simple, direct procedure for the determination of the equivalents of "sulfonate" in surface active compounds. Its use as a quantitative procedure is, of course, limited to those compounds which are quantitatively precipitated by benzidine hydrochloride. When, as is frequently the case, the "sulfonate" is only partially precipitated, evidence which supports the validity of equation (1) may still be obtained by this method as follows:

(1) The precipitated benzidine sulfonate is dissolved in hot alcohol and titrated in the usual manner.

(2) The titrated solution is made slightly alkaline and diluted to a

[8] Biffen, F. M., and Snell, F. D., *Ind. Eng. Chem., Anal. Ed.*, **7**, 234 (1935).

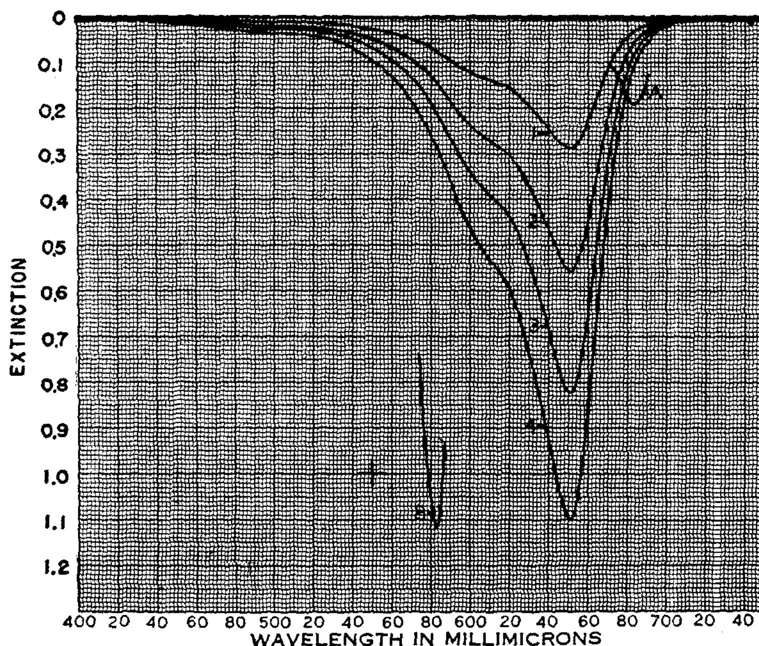

FIG. 2.—Extinction curves of methylene blue "sulfonate" solutions obtained by proposed method.

Sample—

1. Compound 4, 0.1 mg.
2. Compound 4, 0.2 mg.
3. Compound 4, 0.3 mg.
4. Compound 4, 0.4 mg.

Cell—1 cm.

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peak at 684.8 mμ)

B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 mμ)

definite volume with water, and an aliquot is analyzed colorimetrically. The results by both methods may then be compared.

Compounds 3, 4, and 5 are substantially quantitatively precipitated by benzidine hydrochloride. Compounds 2 and 6 are only partially precipitated by this reagent, but sufficient benzidine "sulfonate" can be obtained for analysis as indicated above. A comparison of the benzidine and colorimetric results for compounds 2–6 are given in Table 3. Compound 1 gives no precipitate with benzidine hydrochloride and is therefore not listed in Table 3.

The analyses summarized in Table 3 indicate that the basis of the proposed method is the reaction represented by equation (1), or a similar reaction. It should be pointed out, however, that even when direct analysis

TABLE 3.—*Comparison of benzidine and colorimetric results*

COMPOUND NO.	METHOD OF ANALYSIS BENZIDINE	COLORIMETRIC	RATIO
	equivalents	*moles*	
2	0.185b	0.176b	0.95
2	0.187b	0.174b	0.93
3	0.220a	0.206a	0.94
4	0.302a	0.298a	0.99
4	0.295b	0.288b	0.98
5	0.237a	0.224a	0.95
6	0.202b	0.195b	0.96

[a] Direct determination, results calculated on basis of 0.1 gram sample.
[b] Analysis of benzidine "sulfonate" precipitate.

by both methods gives identical results the recovery of the "sulfonate" is not necessarily complete. Many of the commercial products are mixtures of several more or less closely related compounds and, in some cases, part of the "sulfonate" may not be determined by either method. When the average molecular weight of the compounds present is in the usual range for surface active materials,[9] the fraction which is not determined by the colorimetric method will undoubtedly be small.

Some of the compounds tested are supplied in various grades that contain different amounts of the "active" ingredient, and some of them are quite hygroscopic. Since only one sample of each compound was analyzed the absolute results calculated from the data in Table 2 may not represent the average "purity" of the compounds supplied under these names.

The proposed method has been applied to other "sulfonated" surface active products, including "sulfonated" castor oil and a "sulfonated" amide, not listed in Table 1. In each case the extinction vs. concentration curve was a straight line, but data comparing the colorimetric method with other methods have not been compiled.

The precision with which 0.2 mg. of compound 4 may be determined by the proposed method is shown in Table 4. (The two sets of four determinations each were made on successive days.) The average deviation from the mean is less than 1 percent, and the maximum deviation is 1.8 percent. Similar results were obtained with the other compounds tested.

Moderate variation in the amounts of methylene blue solution, acid, and wash water has a negligible effect on the recovery. Enough methylene blue to combine with all the "sulfonate" must, of course, be used, and the solution in the first funnel must be acid to prevent the extraction of methylene blue base (violet solution in chloroform). Methylene blue chloride is slightly soluble in chloroform, but a single washing with distilled water always reduces the blank to a low value.

[9] Snell, F. D., *Ind. Eng. Chem.*, **35**, 107 (1943).

TABLE 4.—*Study of precision of proposed method*

EXPERIMENT	INSTRUMENT READING	DEVIATION FROM AVERAGE	
	E_{652}	E_{652}	*per cent*
1	0.538	0.004	0.7
2	0.536	0.006	1.1
3	0.534	0.008	1.5
4	0.540	0.002	0.4
5	0.548	0.006	1.1
6	0.542	0.000	0.0
7	0.544	0.002	0.4
8	0.552	0.010	1.8
Average	0.542	0.005	0.9

Sample —0.2 mg. of compound 4.
Cell—1 cm.
Extinction values determined with General Electric Recording Spectrophotometer.

The completeness of the transfer of the colored salt to the chloroform extract depends upon the water-chloroform distribution constant of the salt and the number of extractions made. Compounds 1–5 are quantitatively recovered in four extractions with 20 ml. portions of chloroform. Five extractions are required for compound 6 but this compound has a much lower molecular weight than most of the commercially available "sulfonated" surface active compounds.

The chloroform solution of the colored salt must be perfectly clear for accurate photometric measurements. Filtration of the chloroform extract through cotton appears to remove completely any suspended water, and no difficulty due to cloudy solutions was encountered in the analyses made in the development of the method.

Chloroform solutions of the extracted methylene blue salts are only moderately stable. The rate of decomposition appears to depend largely upon the amount of light to which the solution is exposed. If the solutions are protected from strong direct light the decomposition is usually less than 2 percent in two hours. The standard methylene blue chloride solutions containing alcohol are more stable, but decomposition is apparent after 24 hours. All photometric measurements should, therefore, be made within an hour after the chloroform solutions are prepared.

The chloroform in the colored solutions may be recovered by distillation and used in subsequent determinations.

Dilute aqueous solutions of the surface active compounds are stable for several weeks if preserved by the addition of chloroform.

The proposed method is useful as a qualitative test for "sulfonated" surface active agents, since 0.02 mg. of these compounds gives a definite color to the first 20 ml. of chloroform extract. The reaction represented by equation (1) probably occurs to a certain extent with all sulfonates,

but experiments have indicated that few non-surface active sulfonates respond to the test as such low concentrations. No color is produced by 5 mg. of benzene- or toluenesulfonic acid. One milligram of beta-naphalene-sulfonic acid gives a positive test, but the color obtained from 0.2 mg. of this acid is only slightly greater than the blank. FD&C Orange No. 1 is the only sulfonated azo or triphenylmethane dye tested which interferes appreciably. Sulfonphthaleins, however, give a positive test at approximately the same concentration as do surface active compounds.

Soap, if present in the test solution in relatively high concentration, will give a weekly positive test. The methylene blue extracted from a solution containing 5 mg. of soap corresponds to about 0.02 mg. of "sulfonate." Water-soluble carboxylic acids do not seem to interfere.

A number of methylene blue salts, other than sulfonates, are soluble in chloroform. The perchlorate, chromate, iodide, and picrate fall in this group. All of these salts except the picrate are, however, readily washed from chloroform with distilled water.

Cationic surface active agents interfere in the proposed method; the addition of more than an equivalent amount of a high molecular weight quaternary ammonium salt will completely prevent the extraction of the colored salt. Undoubtedly, in this case, the "sulfonate" combines preferentially with the surface active cation. Since mixtures of anionic and cationic surface active agents have few, if any, practical applications, this interference does not appear to be analytically important.

APPLICATION TO MIXTURES

Mixtures of "sulfonated" surface active compounds with soap are frequently encountered in the analysis of commercial products. The influence of soap on the proposed method is, therefore, of special interest since the determination of small amounts of "sulfonate" in such mixtures by other methods is not feasible. Results of the analysis of "sulfonate"—soap mixtures—by the proposed method are shown in Table 5. The error introduced by the presence of 1 mg., or less, of soap is slight. If a larger amount is present, a correction for the "blank" on the soap alone will allow the "sulfonate" content to be determined with reasonable precision.

The proposed method has been applied to mixtures containing "sulfonated" surface active compounds and various inorganic and organic substances. Typical results are shown in Table 6. Except in one case, where the added material is a sulfonated color, the results appear to be satisfactory. The fact that non-ionic surface active compounds and alkaline detergents do not seem to affect the recovery makes the proposed method particularly advantageous for the analysis of emulsions and detergent solutions.

TABLE 5.—*Analysis of soap—"sulfonate" mixtures*

SURFACE ACTIVE COMPOUND		SOAP* ADDED	RECOVERY
NO.	AMOUNT		
	mg.	*mg.*	*per cent*
3	0.4	0.4	100
4	0.2	0.25	100
4	0.2	0.25	99
4	0.2	1.0	100
4	0.2	5.0	106 (98)†
4	0.2	5.0	108 (100)†
5	0.4	0.4	98
5	0.4	0.4	101
5	0.4	5.0	103 (97)†
6	0.2	0.2	98

* U. S. P. powdered soap.
† Corrected for "blank" on soap alone.

TABLE 6.—*Analysis of mixtures*

SURFACE ACTIVE COMPOUND		OTHER COMPOUND		RECOVERY
NO.	AMOUNT	NAME	AMOUNT	
	mg.		*mg.*	*per cent*
1	0.2	Sodium carbonate	100	99
1	0.2	Sodium sulfate	100	101
4	0.2	Sodium carbonate	100	99
4	0.2	Sodium phosphate	100	101
5	0.2	Ammonia	100	100
5	0.2	Borax	100	100
3	0.2	Glyceryl monostearate	20	101
3	0.2	Propylene glycol	100	99
4	0.2	Triethanolamine	100	100
4	0.2	Alcohol	200	99
5	0.4	FD&C Blue No. 1	1	101
5	0.4	FD&C Orange No. 1	1	111
5	0.4	FD&C Orange No. 1	0.4	105
3	0.2	FD&C Yellow No. 6	1	100
4	0.2	D&C Yellow No. 7	1	101

MODIFIED METHOD

For routine determinations the proposed method may be simplified as follows: A single extraction with a measured volume of chloroform is made. The chloroform layer is washed as before, filtered through dry cotton, and compared with standards. The standards are prepared by analyzing known amounts of the surface active compound in the same manner. The procedure used must be closely standardized to obtain reproducible results. (A sample of 0.02–0.1 mg., extracted with 20 ml. of

chloroform, is convenient if the photometric measurements are made in a 1 cm. or ½ inch cell.)

Results of the application of a modified procedure to Compounds 3 and 4 are shown in Table 7. The extinction vs. concentration curve is a straight line in each case. The modified method is not, however, as precise as the regular method; the average deviation between duplicates is about 3 per-cent.

TABLE 7.—*Photometric data, modified method*

COMPOUND NO. 3		COMPOUND NO. 6	
AMOUNT		AMOUNT	
mg.	E_{652}	*mg.*	E_{652}
0.025	0.20	0.025	0.15
	0.21		0.13
0.050	0.44	0.050	0.27
	0.41		0.26
0.075	0.61	0.075	0.41
0.075	0.61		0.43
0.100	0.82	0.100	0.54
	0.83		0.54
0.000	0.01		
(blank)	0.01		

Volume of chloroform—20 ml.
Cell—1 cm.
Extinction values determined with General Electric Recording Spectrophotometer.

Typical recoveries of "sulfonate" from mixtures by the modified method are shown in Table 8.

TABLE 8.—*Analysis of mixtures, modified method*

SURFACE ACTIVE COMPOUND		OTHER COMPOUND		RECOVERY*
NO.	AMOUNT	NAME	AMOUNT	
	mg.		*mg.*	*per cent*
2	0.05	Sodium phosphate	100	99
3	0.10	Borax	100	102
4	0.05	Sodium carbonate	100	98
5	0.05	Calcium chloride	100	99
2	0.05	Alcohol	100	102
3	0.10	Lanolin	100	101
3	0.10	Dimethylaniline	10	102
4	0.05	Glyceryl monostearate	10	102
5	0.05	Glycerin	100	98
5	0.05	Mineral oil	100	102
4	0.05	Soap	0.05	101
4	0.10	Soap	0.25	94
4	0.10	Soap	1.00	91
5	0.05	Soap	0.05	100
5	0.05	Soap	1.00	91

* Average of duplicate determinations.

Acknowledgment.—The author wishes to thank S. H. Newburger and R. N. Sclar for making the spectrophotometric measurements.

SUMMARY

A colorimetric method for the determination of small amounts of sulfonated or sulfated surface active compounds has been presented and discussed. The proposed method appears to be generally applicable, and the precision is acceptable. The proposed method may be applied to mixtures containing soap, alkaline detergents, and non-ionic detergents.

A modified method, which is more rapid but less precise, is also described.

Typical results obtained by the proposed methods are given.

COMPARATIVE CHEMICAL STUDIES ON PEA SEED AND CANNED SOAKED DRY PEAS*

By V. B. BONNEY and HENRY FISCHBACH (U. S. Food and Drug Administration, Washington, D. C.)

Numerous varieties of sweet, wrinkled peas for commercial canning have been developed. On the other hand, the Alaska pea is the only smooth-skinned variety of real commercial importance.

Certain broad trends in the maturation of peas are now well established. With advance of maturity soluble constituents become less soluble and there is an increase in both insoluble and total solids.

The present investigation was undertaken for the purpose of ascertaining the possibility of chemically differentiating between the smooth-skinned Alaska and the sweet, wrinkled varieties of peas. Data were also obtained on several varieties of pea seed, and corresponding data on these varieties after soaking, blanching, and canning.

MATERIAL

Fourteen varieties of peas, representing over 95 per cent of the seed used by commercial growers, were secured from a well known seed company in the Mid-west. These included one variety of smooth peas, the Alaska, and thirteen of the sweet wrinkled varieties. Subsequently, 29 different samples of Alaska pea seed were obtained for analysis.

CANNING

The aforementioned 14 varieties of pea seed were canned according to the following directions:

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* Contributed from the Food Division, W. B. White, Chief.

Soak 100 grams of seed overnight in water, drain 2 minutes on an 8-mesh screen, and weigh. After treating for 15 minutes in hot water on a steam bath, remove the peas from the steam bath, hold in the water for 1 hour, drain, and weigh. Place the blanched peas in No. 2 cans, cover with hot water, seal, and process for 35 minutes at 240°F.

These peas were canned on January 4, 1944, and stored in the laboratory until opened for analysis between February 3 and 19.

Both the dry seed and the prepared canned soaked dry peas of each variety were analyzed by the following methods:

METHODS OF ANALYSIS

Preparation of Sample.—

1. Grind the dry seed in a Wiley mill until the powder passes through the 20-mesh sieve, and store in stoppered flasks until analyzed.

2. Drain the canned peas as prescribed in the official method.[1] Conserve the drained liquor for later analyses. Transfer the peas to a white pan, remove foreign material, wash, and grind as prescribed in the official method.

Total Solids.—Weigh approximately 2 grams of dry peas (or 5–10 grams of drained peas, or 20 grams of the drained liquor) into tared aluminum dishes provided with tightly fitting covers, and evaporate the wet samples on the steam bath to apparent dryness. Follow this pretreatment with an additional 6 hours at 70°C. in vacuo.

Ash.—Evaporate approximately 2 grams of dry peas, 5–10 grams of the drained peas, or 20 grams of the drained liquor, on a steam bath to apparent dryness and ignite in an electric muffle at 550°C.

Sugars.—Add 10 grams of dry peas, or 50 grams of the drained peas, to approximately 150 ml. of water, boil 1 hour, cool, and make to volume in a 250 ml. volumetric flask. Transfer to centrifuge tube and add 2–3 grams of "filtercel." Centrifuge for 15 minutes at 1500–2000 r.p.m. and filter. For the drained liquor make 50 grams to 250 ml. and filter. Add 50 ml. of any of the above sample solutions to a 100 ml. volumetric flask; add an excess of saturated lead acetate solution, make to 100 ml., and filter. Remove excess lead salt with potassium oxalate. Determine reducing substances before inversion and sucrose by the Munson and Walker method.[2]

Water-soluble Solids.—Add 50 ml. of any of the filtrates prepared for the sugar determinations to a tared aluminum dish provided with a tightly fitting cover. Evaporate to apparent dryness on a steam bath and dry for 6 hours in vacuo at 70°C.

Alcohol-insoluble Solids.—Determine alcohol-insoluble solids in the canned peas by the official method.[3] With seed, moisten approximately 3 grams of the dry material with 10 ml. of water, add 300 ml. of 80% alcohol, and proceed with the official method.

Protein.—Digest approximately 2 grams of the pea seed, 10 grams of the drained peas, or 25 grams of the drained liquor, with H_2SO_4, employing Na_2SO_4 and HgO as catalyst as outlined in the Kjeldahl-Gunning-Arnold method.[4] Calculate the distilled ammonia as protein ($N \times 6.25$).

Acid-hydrolyzable Polysaccharides (Calculated as Crude Starch).—To approximately 5 grams of the pea seed, moistened with 10 ml. of water, or to 20 grams of the drained peas, add 300 ml. of 80% alcohol and heat for 1 hour on a steam bath. Filter

[1] *Methods of Analysis, A.O.A.C.*, 1940, 519, 14.
[2] *Ibid.*, 500, 38.
[3] *Ibid.*, 487, 14.
[4] *Ibid.*, 26, 23.

the mixture on a Büchner funnel with suction, wash with hot 80% alcohol, and transfer the precipitate to a 500 ml. Erlenmeyer flask with 200 ml. of hot water. Add 20 ml. of HCl (sp.gr. 1.125), attach to an air condenser, and reflux for 2½ hours by placing the Erlenmeyer flask in a steam bath. Cool, transfer to a 500 ml. volumetric flask, neutralize, make to volume, filter, and determine dextrose by the Munson-Walker method.[3] Calculate the results as crude starch (dextrose×0.9).

Water-soluble Acid-hydrolyzable Polysaccharides (Calculated as Crude Dextrin).—Treat 5 grams of the pea seed, or 20 grams of the drained peas, with 80% alcohol as outlined in the above crude starch determination. Wash the precipitate into a beaker with approximately 150 ml. hot water, boil 1 hour, transfer to a 250 ml. volumetric flask, cool, make to volume, and filter. Evaporate 200 ml. of the filtrate to approximately 25 ml., add with stirring 200 ml. of 95% alcohol previously acidified with 1 ml. of concentrated HCl, permit precipitate to settle for 1 hour, and filter on Büchner funnel with suction. Wash precipitate with 80% alcohol, transfer to a 500 ml. Erlenmeyer flask by means of 200 ml. of hot water, hydrolyze with HCl (sp.gr. 1.125), and determine dextrose as directed in the crude starch determination. Calculate as crude dextrin (dextrose×0.9).

Pectic Acid.—Subsequent to weighing the dry alcohol-insoluble solids, place the residue and filter paper in a 250 ml. beaker and digest for 2 hours on a steam bath with 150 ml. of 0.5% ammonium oxalate solution. Transfer the mixture to a 250 ml. volumetric flask, cool, and make to volume. Add about a teaspoonful of "filter-cel," centrifuge for 15 minutes at 1500–2000 r.p.m., and filter. Determine pectic acid in a 200 ml. aliquot of the filtrate as prescribed in the A.O.A.C. method.[5]

DATA

Tables 1 and 2 give, respectively, the chemical analyses of the seed peas and the canned soaked dry peas. Table 3 combines the results of the foregoing two tables, calculated to the dry basis. Table 4 gives the results on the 29 different samples of Alaska peas.

DISCUSSION

It will be noted that the loss in crude dextrin in the canned peas is not reflected in a corresponding loss in crude starch (Table 3). In a number of instances the loss in crude starch exceeds the loss in crude dextrin. In other cases the reverse is true. These phenomena may be the result of a number of factors such as variety, mechanical and solubility losses in blanching, changes in the carbohydrates during processing, etc. The authors offer no explanation at this time for these phenomena, but merely present these data for the information of those interested in the behavior of pea carbohydrates during the canning procedure.

Although none of the pea seed exhibited determinable amounts of invert sugar, all contained from 4.7 to 10.2 per cent of sucrose. Losses in soaking and blanching considerably reduced the sucrose content of the corresponding canned peas. In any case, the variations in the sucrose content are of little importance in regulatory work since sugar is generally added to canned peas.

Only one sample of Alaska peas was canned as compared with the 13 samples of sweet peas. However, 29 different samples of Alaska pea seed

[5] *Ibid.*, 340, 22.

TABLE 1.—*Composition of dry pea seed* (per cent)*
(Reducing sugars—none)

VARIETY†	MOISTURE		ASH		SUCROSE		WATER-SOLUBLE SOLIDS		ALCOHOL-INSOLUBLE SOLIDS		PROTEIN (N×6.25)		CRUDE STARCH		CRUDE DEXTRIN	
Alaska (Rogers)	10.01	9.95‡	2.71	2.83	4.72	4.73	25.70	24.89	77.09	76.86	23.47	23.36	48.09	46.98	7.63	7.67
Ace (Rogers)	8.26	8.37	3.18	3.20	6.73		28.46	29.14	72.36	72.75	23.78	23.95	39.48	38.62	6.89	7.11
Alderman (Tall Telephone)	8.60	8.51	3.16	3.12	9.55	9.31	33.20		70.10	69.74	26.49	26.51	35.92	34.23	7.57	7.25
Chief (Rogers)	8.52	8.41	3.14	3.12	7.35		31.37	30.95	70.31	70.34	27.01	27.05	34.84	35.47	6.98	7.65
Famous (Rogers)	8.11	8.08	3.28	3.19	8.44	8.57	30.15	29.95	69.55	69.64	24.60	24.82	37.79	37.16	6.24	6.18
Gradus (Improved —Rogers)	8.45	8.27	3.23		9.14	9.08	33.41	33.36	70.71	70.64	29.48	29.66	34.81	35.74	6.79	6.74
Perfection (Rogers)	8.25	8.17	2.93	2.94	8.12	8.33	29.80	28.35	71.58	71.87	23.15	23.12	38.60	39.38	7.17	7.53
Perfection (Wisconsin)	8.09	8.09	2.92	2.98	8.57	8.53	30.50	31.05	70.68	70.88	25.10	25.05	38.34	36.64	5.89	6.38
Pride	8.21	8.09	2.97	2.99	8.22	8.36	31.00	31.35	71.41	71.36	25.92	25.91	38.50	38.11	5.98	6.07
Profusion	8.25	8.15	3.09		8.07	7.90	33.40	33.18	70.66	70.70	28.30	28.30	34.93	35.99	6.41	6.41
Surprise	7.76	7.76	3.09	3.18	8.92	8.89	29.16	29.05	71.13	70.54	26.82	26.60	37.60	35.98	5.73	5.44
Thomas Laxton	8.49	8.54	3.05	3.06	10.10	10.17	31.09	31.35	69.16	69.08	28.70	28.54	30.91	32.67	6.36	6.19
Wisconsin Early Sweet	8.65	8.75	2.97	3.03	8.03	7.97	32.08	32.20	70.82	70.55	28.29	28.45	37.25	38.13	8.11	7.92
Wisconsin Merit	8.33	8.17	3.53	3.61	8.24	8.20	30.00	30.87	70.74	70.72	26.71	26.95	36.25	35.71	5.78	5.81

* Seed furnished by the Rogers Brothers, Nov. 1943, Chicago, Ill.
† Varietal names copied from Rogers Brothers 1943 Catalogue.
‡ Second column under each heading shows duplicate determinations.

TABLE 2.—*Composition of canned soaked dry peas**
(100 grams of seed each variety soaked overnight, blanched 15 minutes in hot water, and canned in No. 2 cans January 4, 1944; analyzed February 1944)

			CANNED PEAS		ANALYSIS OF LIQUOR				ANALYSIS OF DRAINED PEAS									RATIO
VARIETY	WEIGHT SOAKED SEED	WEIGHT BLANCHED SEED	DRAINED WEIGHT	WEIGHT LIQUOR	TOTAL SOLIDS	ASH	PROTEIN (N×6.25)	SUCROSE†	TOTAL SOLIDS	WATER SOLUBLE SOLIDS	ASH	SUCROSE†	ALCOHOL INSOLUBLE SOLIDS	PROTEIN (N×6.25)	CRUDE STARCH	CRUDE DEXTRIN	PECTIC ACID	CRUDE STARCH / PROTEIN
	grams	*grams*	*grams*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Alaska (Rogers)	214	215	235	363	2.46	0.21	1.09	0.57	32.42	2.86	0.45	0.34	31.23	7.66	17.22	0.43	1.04	2.30
					2.44‡	0.21		0.59					31.03	7.37	17.34	0.42	1.04	
Ace (Rogers)	260	262	274	314	3.01	0.27	1.04	0.82	27.63	3.34	0.51	0.57	25.58	7.17	12.08	0.76	1.31	1.65
					3.03	0.27	1.04	0.83	27.46		0.51		25.70	7.21	11.58	0.72	1.30	
Alderman (Tall	266	269	298	304	3.77	0.29	1.19	1.23	25.88	2.93	0.45	0.82	23.18	7.74	11.20	0.82	1.15	1.44
Telephone)					3.77	0.30	1.19	1.22					23.16	7.65	10.95	0.92		
Chief (Rogers)	268	270	291	305	3.46	0.27	1.19	0.99	25.81	4.29	0.51	0.66	23.44	7.51	10.75	0.83		1.41
					3.44	0.27	1.20	0.98	25.92		0.52		23.46	7.56	10.49	0.84		
Famous (Rogers)	262	262	279	319	3.26	0.26	1.03	1.15	26.31	3.61	0.45	0.76	24.22	7.01	10.00	0.60	1.16	1.39
					3.26	0.26	1.03	1.15	26.29		0.49		24.07	7.11	9.61	0.61	1.16	
Gradus (Improved	266	269	305	296	4.77	0.36	1.59	1.19	23.80	4.50	0.43	0.83	21.12	7.86	9.28	0.78	1.00	1.19
Rogers)					4.80	0.36	1.59	1.19					21.04	7.83	9.42	0.88	0.96	
Perfection	261	262	287	311	3.69	0.26	0.95	1.16	26.85	3.44	0.50	0.75	24.61	6.72	10.90	0.72	0.94	1.62
(Rogers)					3.70	0.26	0.95	1.13	27.03		0.50		24.22	6.84	11.05	0.73	1.04	
Perfection	256	250	270	329	3.50	0.26	1.11	1.04	27.50	3.68	0.49	0.73	25.38	7.64	11.52	0.78	1.15	1.50
(Wisconsin)					3.48	0.26	1.12	1.03					25.24	7.69	11.48	0.74	1.17	
Pride	254	260	280	319	3.67	0.28	1.17	1.13	27.20	4.31	0.48	0.73	24.85	7.63	10.76	0.76	1.41	1.42
					3.68	0.29	1.17	1.13					24.55	7.72	11.09	0.82	1.18	
Profusion	266	263	284	319	3.66	0.29	1.29	1.13	26.50	4.01	0.48	0.79	23.95	8.25	8.92	0.53	1.20	1.09
					3.68	0.30	1.33	1.15	26.47		0.48		24.10		9.12	0.63	1.20	
Surprise	265	269	283	314	3.25	0.29	1.11	1.08	26.82	3.57	0.43	0.70	24.33	7.95	10.19	0.55	1.05	1.28
					3.26		1.10	1.06	26.85		0.47		24.19	7.88	10.07	0.55	1.05	
Thomas Laxton	269	266	296	295	4.54	0.35	1.52	1.41	24.43	4.21	0.49	1.01	21.40	7.66	8.82	0.83	1.15	1.15
					4.50	0.34	1.51	1.39					21.54	7.71	8.89	0.76	1.16	
Wisconsin Early	255	251	274	317	3.52	0.27	1.38		26.77	4.18	0.51	0.56	24.50	8.21	11.57	0.80	1.05	1.45
Sweet					3.52	0.27	1.38						24.45	8.23	12.19	0.80	1.08	
Wisconsin Merit	267	269	295	308	3.79	0.31	1.24	1.01	25.21	3.83	0.48	0.68	22.93	7.50	10.13	0.71	1.38	1.31
					3.77	0.32	1.26	1.04					23.02	7.50	9.56	0.79	1.33	

* Seed furnished by the Rogers Brothers, Nov. 1943, Chicago, Ill.
† Includes minute traces of invert sugar.
‡ A second line under each sample shows duplicate determinations.

TABLE 3.—*Composition of pea seed and corresponding canned soaked dry peas on dry basis (per cent)*

VARIETY	WATER-SOLUBLE SOLIDS	ALCOHOL-INSOLUBLE SOLIDS	ASH	SUCROSE	PROTEIN (N×6.251)	CRUDE STARCH	CRUDE DEXTRIN	DEXTRIN IN CANNED PEAS / DEXTRIN IN SEED
Alaska Seed	28.10	85.51	3.08	5.25	26.01	52.79	8.50	0.136
Alaska Canned Peas	18.35	86.00	2.14	3.37	25.42	47.73	1.16	
Ace Seed	31.41	79.13	3.48	7.34	26.03	42.59	7.63	.312
Ace Canned Peas	21.93	82.70	2.64	4.86	27.04	38.16	2.38	
Alderman Seed	36.30	76.46	3.43	10.31	28.98	38.35	8.10	.360
Alderman Canned Peas	22.79	77.95	2.52	6.95	29.96	37.24	2.92	
Chief Seed	34.04	76.82	3.42	8.03	29.53	38.40	7.99	.353
Chief Canned Peas	26.82	79.56	2.68	5.72	29.78	36.03	2.82	
Famous Seed	32.70	75.72	3.52	9.25	26.89	40.78	6.76	.294
Famous Canned Peas	24.43	80.39	2.55	6.91	27.44	32.63	1.99	
Gradus Seed	36.43	77.12	3.52	9.94	32.27	38.49	7.38	.396
Gradus Canned Peas	32.14	74.12	2.74	6.97	32.99	32.88	2.92	
Perfection Seed	31.68	78.14	3.20	8.95	25.20	42.48	8.01	.291
Perfection Canned Peas	24.04	78.89	2.52	6.42	25.23	35.45	2.33	
Wisconsin Perfection Seed	33.48	77.01	3.21	9.30	27.28	40.79	6.68	.358
Wisconsin Perfection Canned Peas	24.97	79.72	2.54	6.25	28.38	36.22	2.39	
Pride Seed	33.94	77.72	3.24	9.03	28.21	41.70	6.56	.383
Pride Canned Peas	27.06	78.71	2.54	6.42	28.69	34.80	2.51	
Profusion Seed	36.26	76.98	3.37	8.70	30.83	38.63	6.98	.272
Profusion Canned Peas	26.58	78.49	2.64	6.77	31.77	29.48	1.90	
Surprise Seed	31.54	76.75	3.40	9.65	28.94	39.86	6.05	.299
Surprise Canned Peas	23.58	79.71	2.53	6.20	30.00	33.28	1.81	
Thomas Laxton Seed	34.12	75.55	3.34	11.08	31.28	34.75	6.86	.398
Thomas Laxton Canned Peas	30.11	74.21	2.86	8.31	31.74	30.59	2.73	
Wisconsin Early Sweet Seed	35.20	77.42	3.29	7.67	31.07	41.28	8.77	.295
Wisconsin Early Sweet Canned Peas	26.75	79.34	2.67	—	31.82	38.52	2.59	
Wisconsin Merit Seed	33.17	77.09	3.89	8.96	29.24	39.76	6.32	.407
Wisconsin Merit Canned Peas	26.63	78.66	2.75	5.98	30.16	33.74	2.57	

TABLE 4.—*Composition of dry Alaska pea seed*

SAMPLE NO.	MOISTURE	SUCROSE	PROTEIN	STARCH	STARCH / PROTEIN
0-952	10.72	5.48	22.23	47.48	2.14
0-94617	9.42	5.36	23.19	45.30	1.95
0-94630	9.06	5.49	22.53	47.03	2.09
0-94629	9.02	5.55	22.18	46.89	2.11
0-94636	9.04	5.78	22.44	46.80	2.09
0-9527	9.02	5.91	22.18	46.17	2.08
0-94634	9.04	5.92	22.27	48.29	2.17
0-9539	9.56	5.61	24.90	45.59	1.83
0-94638	9.68	5.47	23.01	47.61	2.07
0-9512	9.40	5.68	22.31	47.39	2.12
0-9526	9.66	5.75	22.40	47.16	2.11
0-94642	9.60	5.67	22.75	45.99	2.02
0-94619	9.50	5.92	23.19	47.25	2.04
0-94640	9.34	5.64	23.06	46.44	2.01
0-94641	9.88	5.55	23.32	46.31	1.99
0-94637	9.58	5.46	22.67	47.07	2.08
0-95192	9.54	5.31	22.44	46.04	2.05
0-94627	9.62	5.78	23.31	49.6	2.13
0-9513	10.08	5.28	21.88	50.2	2.29
0-94618	9.78	5.84	23.31	45.25	1.94
0-94635	9.82	5.60	22.45	49.2	2.19
0-94639	10.12	5.32	22.58	49.2	2.18
0-94631	9.70	4.12	22.49	49.1	2.18
0-94633	9.54	5.64	22.71	49.05	2.16
0-94626	9.70	6.13	22.18	50.02	2.26
0-94625	9.88	5.77	21.97	48.75	2.22
0-94628	10.00	6.05	22.45	51.0	2.27
0-9522	10.00	6.08	23.31	45.75	1.96
0-94632	9.72	5.75	22.58	50.6	2.24

were examined, and a significantly higher proportion of crude starch to protein was found than for any of the seed of sweet varieties. As illustrated in Figure 1 this relationship persisted in the canned product. Previous work indicated that both crude starch and protein increase with maturity. so that overlapping of very immature Alaska (smooth) peas and more mature, sweet, wrinkled peas was to be anticipated. However, when the canned peas are mature enough to show alcohol-insoluble solids of 20 per cent or more there is real likelihood that this ratio may have considerable diagnostic value.

The alcohol-insoluble solids content of the canned, soaked, dry Alaska peas is considerably higher than the writers have ever found in commercially canned, soaked, dry peas, the latter usually ranging from 22.5

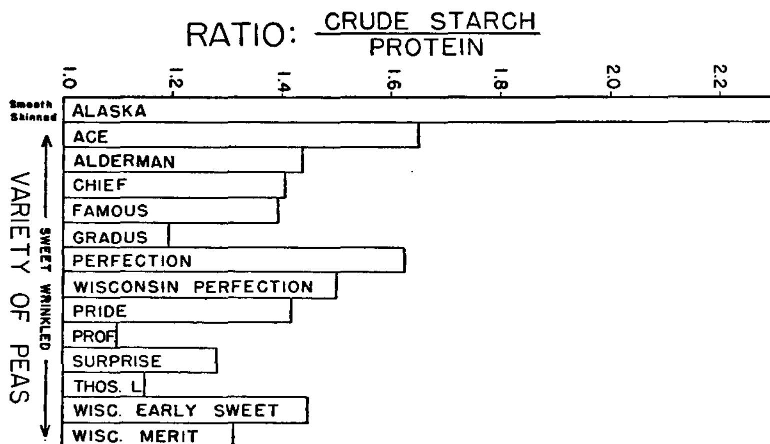

FIG. 1

to 26 per cent alcohol-insoluble solids. No previous alcohol-insoluble solids determinations on authentic canned, soaked, dry sweet peas have been noted.

From Figure 2 it appears that the crude dextrin (water soluble, alcohol insoluble, acid-hydrolyzable polysaccharides) of the single sample of

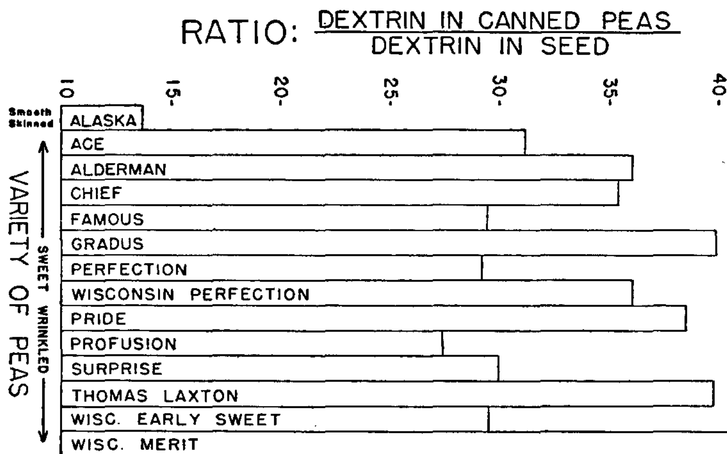

FIG. 2

canned Alaska peas is more soluble in the blanch water than the crude dextrin of the sweet, wrinkled varieties. Consequently less crude dextrin is observed in the drained canned Alaska peas. It has been noted in the

past by this laboratory that the filtration of the alcohol-insoluble solids of sweet peas was more difficult than that of the Alaska peas of the same maturity. This observation coupled with the results for crude dextrin ndicates that future investigation may reveal dissimilar polysaccharides n the wrinkled and smooth pea varieties.

SUMMARY

(1) No determinable invert sugar was found in the pea seed.

(2) All seed contained from 4.7 to 10.2 per cent of sucrose.

(3) Appreciable amounts of the sugar, ash, and crude dextrin of the dry peas were leached out prior to canning; *i.e.*, in the soaking and blanching phases.

(4) The drained liquor of the canned peas contained appreciable amounts of ash, protein, and sugar—more sugar than the drained peas.

(5) The pectic acid content of the drained canned peas was not sufficiently different, among the varieties examined, to furnish a valuable objective test for varietal discrimination.

(6) Alcohol-insoluble solids, and ratio of crude starch to protein, were much higher in the Alaska peas than in any of the sweet, wrinkled varieties.

PRESERVATIVE, CHEMICAL, AND BACTERIOLOGICAL EFFECT OF HYPOCHLORITE SOLUTION ADDED TO MILK*

By GEO. H. HAUSER and W. H. KING (Division of Laboratories, Louisiana State Health Department, New Orleans, La.)

On occasions, routine examinations of market milk in the State of Louisiana have disclosed lots which exhibited abnormally low bacteria counts and occasionally sterile plates. This led to suspicion of the use of preservatives in these lots. In some instances chemical tests indicated the presence of substances which oxidize hydrogen iodide to free iodine when heated in acid solution. This suggested that hypochlorites, such as are allowed by the State Sanitary Code for sterilizing utensils and equipment around milk-producing and handling establishments, may have been deliberately added to milk as a preservative and/or to reduce bacteria counts. In some instances the samples possessed a distinct, disagreeable chemical taste.

There seems to be some doubt in the minds of dairy specialists as to the efficacy of chlorine-generating compounds as preservatives in milk. Johns,[1] studying the effect of various chlorine compounds, found that liquid

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 25, 26, 1944.

[1] *Sci. Agr.*, **10**, 553–563 (1930).

hypochlorite preparations acted rapidly and effectively against *Esch. coli*, *A. aerogenes*, and *Str. lactis*, while the spores of *B. subtilis* were not destroyed. Further studies by Johns,[2] comparing the relative effectiveness of hypochlorites and formaldehyde as preservatives of milk, showed that formaldehyde is much more effective. Furthermore he found considerable variation in the effect of various chlorine compounds on the taste of the milk.

Davis,[3] experimenting with sodium hypochlorite solutions added to milk, stated that these preparations will not materially reduce the bacterial count in milk in concentrations as high as 1:5000, and when used in dilutions of 1:20,000, their taste and odor may be detected (and are even noticeable in concentrations of 1:10,000). His observations, presumably confined to raw milk, were made at 2, 16, and 18 hour intervals after addition of hypochlorite. He therefore concluded that these preservatives were impracticable.

Strell,[4] working with aqueous solutions (or suspensions) of lactose, lactic acid, casein, and butterfat in concentrations normally found in milk added free chlorine solutions in the amount of 300–470 p.p.m. His findings are briefly summarized below:

Lactose was oxidized by the chlorine to lactic acid, then to d-gluconic acid and ultimately to d-saccharic acid, the *p*H of the solution dropping from 6.5 to 2–3. This drop in *p*H indicated (to Strell) that the action of the chlorine was one of straight oxidation and did not involve the formation of chlorine addition or substitution products. The chlorine reacted very rapidly with the *lactic acid* solutions, although an appreciable residual (20–40 p.p.m.) remained after 23 hours. The chlorinated solutions developed an aromatic odor owing to the formation of pyruvic acid. The chlorine reacted comparatively rapidly with the *casein* solutions, the major portion of it having reacted in the first 3 hours, with some residual remaining after 24 hours. *Butterfat* was attacked most rapidly of all.

Rupp[5] found that when dilute solutions of hypochlorites and chloramines were added to milk to the extent of 20 p.p.m. or more of available chlorine, their presence can be detected even after standing 48 hours in the refrigerator. He believed that when small amounts of free chlorine are added to milk in the above vehicles, such amounts combine loosely with the protein of the milk, necessitating a rather drastic procedure of acidifying and heating the milk to obtain a positive test with potassium iodide and starch.

EXPERIMENTAL WORK

In order to obtain first-hand evidence regarding the germicidal and preservative effects of the addition of "free chlorine compounds" to milk, the following experiment was made:

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[2] *Can. Pub. Health J.*, **21**, 162–168 (1930).
[3] *Am. J. Pub. Health*, **20**, 308–309 (1930).
[4] *Wasser*, **14**, 187–215 (1939–40), (Pub. 1940).
[5] U.S.D.A. Bull. No. 1114 (1922).

One gallon of fresh milk was obtained from a private herd under known conditions. All the containers used for the milk were known to be free from chlorine compounds. Within a few hours the milk was divided into twenty-four 100 ml. portions and placed in sterilized, all-glass jars. A 10,000 p.p.m. chlorine solution was prepared from one of the powdered chlorine compounds available on the New Orleans market for dairy use. It is stated by the manufacturer that this powder gives a clean solution, free from suspended solid matter; in any water at a level 100 p.p.m. of free chlorine, in hard water at 200 p.p.m., and in salt water at 300 p.p.m. The 10,000 p.p.m. solution had a small amount of white sediment. The supernatant liquid was alkaline to phenolphthalein, and *p*Hydrion paper indicated a *p*H of approximately 10. The Beckman *p*H meter gave a *p*H of 11.6 uncorrected for presence of sodium ion. Titration of this alkalinity with hydrochloric acid gave an electrometric titration curve expected for hypochlorite solution between a *p*H of 8 and one of 4 (Beckman meter). Calculated in terms of neutralizing value the amounts added to the milk had an alkalinity capable of neutralizing 0.002–0.02 percent of lactic acid. A 1–10 dilution of this solution was also prepared. One or the other of these two solutions was added to 18 of the 100 ml. portions of milk to give three groups of samples containing, respectively, 10, 50, and 100 p.p.m. of free chlorine. The 6 control milk samples were divided equally among the 3 groups. In each group there were therefore two series of 4 samples each, containing, respectively, 0, 10, 50, and 100 p.p.m. of free chlorine. One series was carefully pasteurized at once by heating to 143°F. for 30 minutes. It was then cooled and subjected to a standard plate count† within an hour, appropriate dilutions being made to determine accurately the low counts. The companion series of raw milks in the first group were counted at the same time and in the same manner. The other two groups were held at 50°F., and at the end of 1 day the second group was treated the same way as the first. The third group was held for 2 days and again treated as was group 1.

Table 1 shows triplicate bacterial counts on the raw milk series, and on the pasteurized milk series, of the 3 groups (zero time, 1 day, and 2 days). In Figures 1 to 4 the same data are so presented as to show graphically the relationship between plate count and time (Figs. 1 and 2), and between plate count and free chlorine (Figs. 3 and 4).

Previous experiments in this laboratory indicate that the Rupp test may show greater sensitivity after the milk has been in contact with free chlorine for at least one day. Therefore, Rupp tests were made only after the first and second days. Positive Rupp tests were obtained only on those samples containing 50 or 100 p.p.m. of free chlorine. Organoleptic tests were made on the first day and at various intervals thereafter. A chemical

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† Standard Methods for the Examination of Dairy Products—American Public Health Association.

TABLE 1.—*Effect of addition of hypochlorite solution on bacteria count of one lot of milk*

FREE Cl, P.P.M.	STANDARD PLATE COUNT			ARITHMETIC AV.	LOGARITHMIC AV.†
*0 Days**	1	2	3		
Raw 0	3,800[a]	4,600[a]	3,800[a]	4,067[a]	4,100[a]
10	3,200[a]	460[a]	480[a]	1,380[a]	890[a]
50	290[a]	80[a]	103[a]	158[a]	132[a]
100	80[a]	120[a]	196[a]	132[a]	123[a]
Past.					
0	1,150	500	TNTC	800+	750+
10	Spr.§	Spr.	Spr.	—	—
50	400	300	Spr.	350	350
100	300	100	Spr.	200	170
1 Day					
Raw					
0	9,400[a]	8,400[a]	5,500[a]	7,770[a]	7,500[a]
10	16,800[a]	3,700[a]	5,300[a]	8,600[a]	6,900[a]
50	560[a]	980[a]	120[a]	553[a]	410[a]
100	50[a]	99[a]	89[a]	79[a]	75[a]
Past.					
0	4,600	3,000	1,400	3,000	2,600
10	5,000	600	1,400	2,000	1,620
50	600	600	500	600	560
100	420	300	800	500	460
2 Days					
Raw					
0	18,200[a]	TNTC	7,800[a]	13,000+[a]	12,000+[a]
10	TNTC	TNTC	39,200[a]	39,000+[a]	39,000+[a]
50	16,200[a]	24,400[a]	TNTC	20,300+[a]	20,000+[a]
100	1,040[a]	860[a]	860[a]	920[a]	915[a]
Past.					
0	11,200	Spr.	2,200	6,700	5,000
10	18,000	17,600	13,800	16,500	16,700
50	1,820	1,200	1,260	1,400	1,400
100	480	1,800	800	1,000	800

* Samples for plate count taken within 1 hour after addition of hypochlorite.
† Public Health Bull. No. 220, p. 34 (1939), U.S.P.H.S.
§ Spreaders prevented accurate counts.
[a] Multiply by 1000.

flavor was not detected unless 100 p.p.m. of chlorine had been added. None of the pasteurized samples had soured after 8 days. After 4 days the 0 chlorine raw milks were sour; after 7 days the 10 p.p.m. raw milks were sour; and after 8 days the 50 p.p.m. raw milks and one out of three 100 p.p.m. milks were sour.

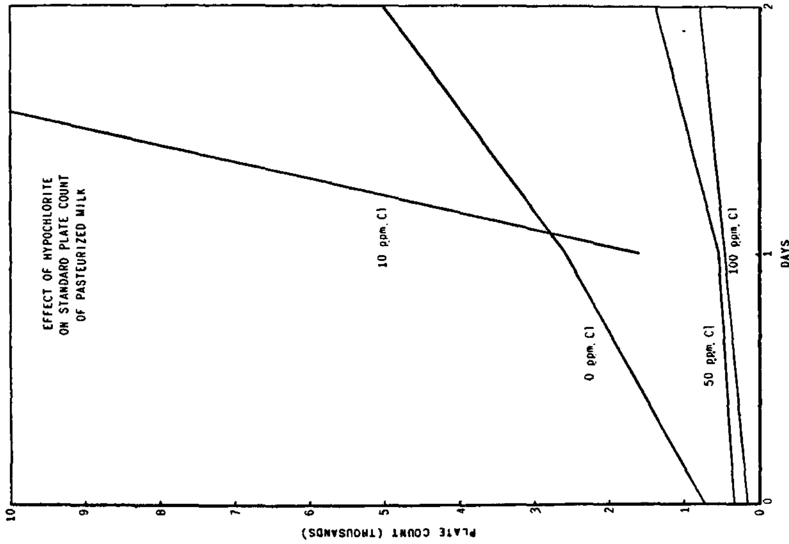

FIG. 2

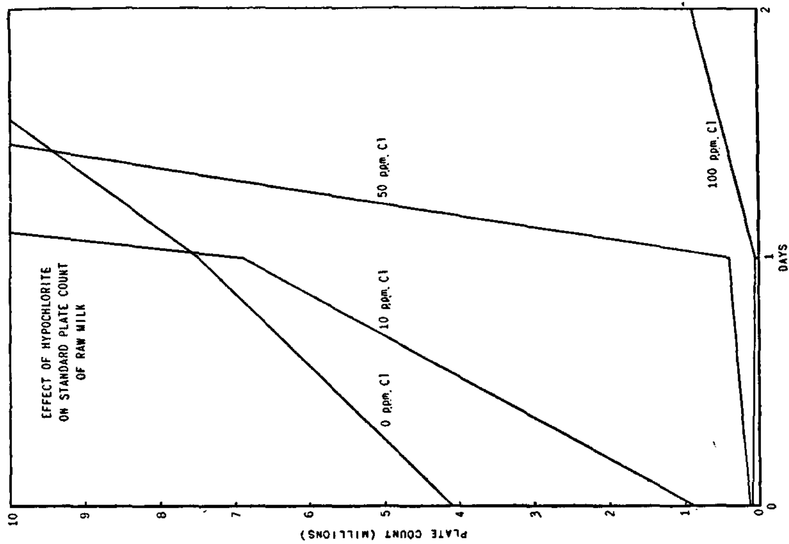

FIG. 1

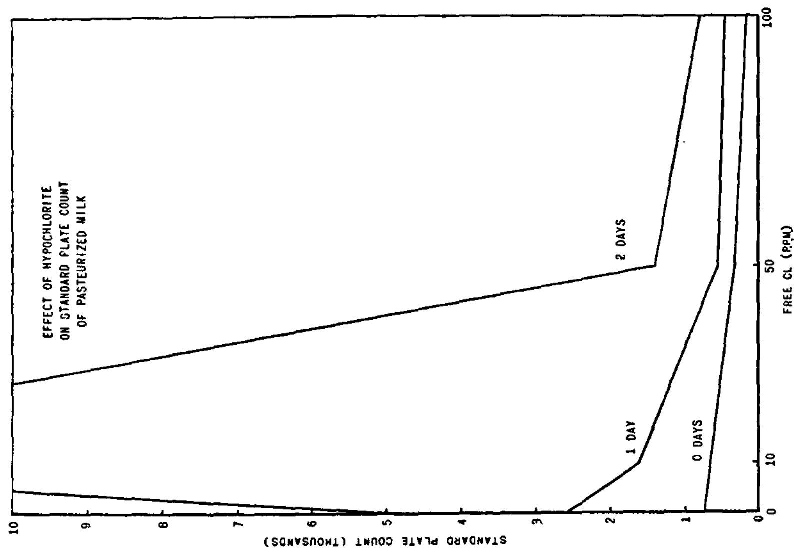

FIG. 4

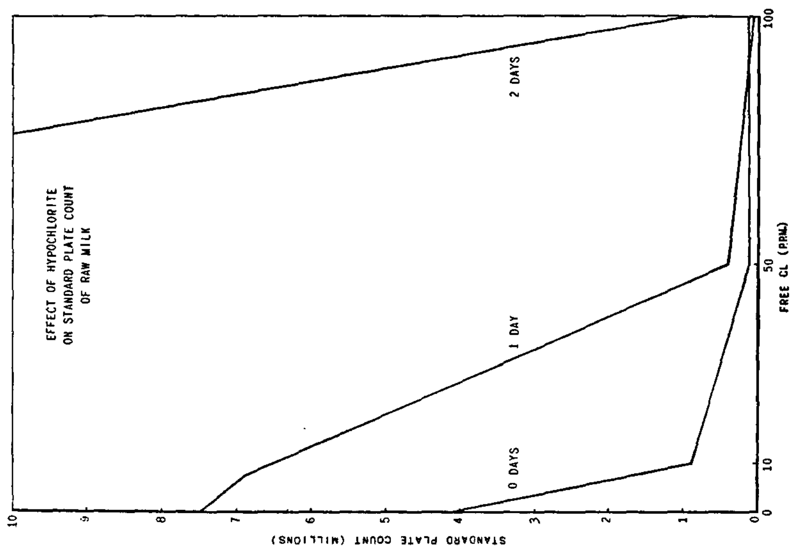

FIG. 3

DETECTION OF FREE OR SEMI-COMBINED CHLORINE IN MILK

Previous experience with the Rupp test confirms statements in the literature that this test is reliable in that no false reaction is given by chlorine-free milk. Keister[6] does say that milk containing more than 2.5 p.p.m. of copper may give a false Rupp test for free chlorine. Since milk naturally contains less than that amount of copper, the Rupp test was assumed by him to be reliable for testing fresh milk for chlorine. While stainless steel pasteurizing equipment is displacing older types, some chromel metal (which contains copper) and tinned copper equipment are still occasionally used. Milk cans are tinned-iron. Our experience confirms that of Rupp and Davis that the test is sensitive to 20–50 p.p.m. or more. In no instance has it been found to be more sensitive, and up to 20 p.p.m. may be present without showing up in the test. Efforts were made to devise a method to remove free or loosely combined chlorine from milk containing added hypochlorites, in order to apply the ortho-tolidine test. Both steam distillation and aeration with carbon dioxide gas, while applicable to acidified water solutions of hypochlorites, failed to separate detectable quantities of chlorine from milk containing up to 100 p.p.m. of free chlorine. There is, however, little necessity for a more sensitive method to differentiate between those faint traces of chlorine added to milk by the proper use of sterilizing solutions on containers and pasteurizing equipment, and amounts of chlorine resulting from illegal use of such solutions. The usual concentration of sterilizing solutions recommended is 100–200 p.p.m. Thus, in order to add 20 p.p.m. of chlorine through careless use of sterilizing solution, one must add 10–20 per cent of water to the milk.

THE RUPP TESTS*

REAGENTS

(a) *Potassium iodide solution.*—Dissolve 7 grams of KI in 100 ml. of distilled water. Prepare fresh.

(b) *Hydrochloric acid.*—To 100 ml. of concentrated HCl add 200 ml. of distilled water.

(c) *Starch solution.*—Boil 1 gram of starch in 100 ml. of distilled water. Cool before using. (In our work 1 gram of potato starch was ground with a little cold water in a mortar and added to boiling water.)

TEST

(1) To 5 ml. of milk or cream in a medium-sized test tube add 1.5 ml. of the iodide solution, mix thoroughly by shaking, and observe the color of the milk.

(2) If the color is unaltered, add 4 ml. of HCl, mix thoroughly by means of a glass rod flattened at one end, and note the color of the curd.

(3) Next place the tubes in a large water bath, previously heated to 85°C., and allow to remain for 10 minutes. (During this interval the curd will have risen to the surface.)

(4) Then cool the tubes rapidly by placing in cold water, and when cold examine for any color change that may have taken place, either in the curd or in the liquid below.

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[6] *Am. J. Pub. Health*, **15**, 781 (1925).
* Described by Rupp (U.S.D.A. Bull. No. 1114).

(5) Finally, add 0.5–1 ml. of starch solution to the liquid below the curd and note the result.

If a yellow color is obtained after step 4 of the Rupp test, or if a light purple to dark blue color is obtained after addition of the starch, more than 10 p.p.m. of free chlorine has been added to the milk. If 200 p.p.m. or more has been added the sample will be colored yellow on the addition of the KI solution. If the milk has been processed in contact with chromel metal or copper equipment and a positive Rupp test is obtained, determination of copper in the milk should be made.[7] If present in an amount greater than 2.5 p.p.m. a positive Rupp test is not reliable. (Compounds containing chloramine T also give positive Rupp tests.)

SUMMARY AND CONCLUSIONS

Experiments were made to determine the germicidal and preservative effect of various amounts of a commercial chlorine compound added to one lot of milk of known history. Under the conditions of the experiments the addition of hypochlorite to fresh milk may appreciably reduce the bacteria count whether or not there is subsequent pasteurization. It was also shown that hypochlorites, when added to milk in amounts not readily detectable by taste, may produce a mild but definite preservative effect on the milk. The Rupp test for free or semi-combined chlorine in milk was studied and found to be reliable for detecting preservative quantities. Since over 10 p.p.m. of free chlorine must be added to milk before a positive Rupp test can be obtained, it is concluded that these faint traces of chlorine which might find their way into market milk by proper use of sterilizing solutions would not give a positive Rupp test.

ACKNOWLEDGMENT

Appreciation of the authors is expressed to Miss Lucille Godelfer of Dr. Hauser's laboratory for making the plate counts reported here.

ANILIN OIL-METHYLENE BLUE STAIN FOR THE DIRECT MICROSCOPIC COUNT OF BACTERIA IN DRIED MILK AND DRIED EGGS

By W. R. North (Food and Drug Administration, Federal Security Agency, Washington, D. C.)

Previous to the assembly of the methods incorporated in *Standard Methods for the Examination of Dairy Products*, Eighth Edition, 1941, published by the American Public Health Association, an anilin oil-methylene blue stain (developed in the Bacteriological Division of the Food and Drug Administration several years earlier) was recommended by personal communication (1938) to the committee on "standard methods for analyzing frozen desserts and ingredients." The recommendation was made on the virtue of the stain for staining dead bacteria, without detailed comparative studies. Evaluation of its merits at the time was

[7] *Methods of Analysis, A.O.A.C.*, 1940, 414.

based on random unrecorded observations from which the conclusion was reached that it offered definite advantage over the plain methylene blue stain for the examination of dried milk and kindred products.

Since that time considerable work has been done, especially on dried eggs, and this stain is now recommended for use in the microscopic examination of both liquid and dried eggs.[1] Although little work has been done with these products which affords a direct comparison of this stain with the plain methylene blue stain, examination of authentic samples by several different workers has resulted in exceptionally close agreement. Such agreement apparently is not possible with the plain methylene blue stain.[2] However, a limited series of comparative examinations has been made on dried milk, and the results recorded in Table 1 are sufficiently impressive to warrant attention.

Thirty-four such comparative counts were made. The counts are based on dried milk smears prepared as follows: (1) A smear was stained with the standard Breed stain for liquid milk (0.3 gram of certified methylene blue powder to 30 ml. of 95 per cent ethyl alcohol); (2) a duplicate smear was prepared with the anilin oil stain (anilin oil 3 ml., ethyl alcohol 95% 10 ml., 1.5 ml. of concentrated HCl added slowly to the anilin oil and ethyl alcohol, 30 ml. of saturated alcohol solution of methylene blue, sufficient water to equal total volume of 100.0 ml.;* and (3) the film prepared with the standard stain was counted, decolorized, and restained with the anilin oil methylene blue.

It will be noted that in all instances higher counts were obtained on duplicate smears with the anilin oil stain than with the standard (plain methylene blue) stain. In 30 out of 34 samples, or 88.2 per cent, the counts were at least twice as high; in 12, or 35 per cent, the counts at least 5 times as high; and in 3, or 8.8 per cent, they were at least 10 times as high as the counts obtained with the plain methylene blue stain. When the same plain methylene blue prepared smears were decolorized and restained with the anilin oil methylene blue, the counts in 26, or 76 per cent, were more than doubled; in 11, or 33 per cent, they were at least 5 times greater; while 3, or 8.8 per cent, had counts 10 times greater than were obtained with plain methylene blue. These check closely with the figures obtained on the duplicate smears stained with the anilin oil stain.

Comparative examination of a limited number of samples of fluid raw milk has not revealed significant differences between the counts obtained with the standard plain methylene blue stain and the anilin oil methylene blue stain.

The anilin oil stain has the definite advantage of producing a very pale blue background against which the organisms are deeply stained. This

[1] *This Journal*, **28**, 61 (1945).
[2] Roy Schneiter, *This Journal*, **25**, 740–745 (1942).
* Eighth Edition "Standard Methods for the Examination of Dairy Products" p. 175—5th line from bottom erroneously states 55.5 ml.

facilitates counting, both for the experienced person and the novice. There is little or no chance of overstaining (smears have been left in the stain for 24 hours without adversely affecting the result), which eliminates the necessity for destaining in alcohol. Also the smears require no special handling but may be transferred from xylol to alcohol and then to the stain without any intermediate steps. The necessity for special fixation, required of smears of dried milk to be stained with plain methylene blue, is eliminated.

TABLE 1.—*Comparative direct microscopic counts per gram of dried milk*

NO.	1 STANDARD (BREED) STAIN	2 ANILIN OIL STAIN	RATIO 1:2	3 STANDARD BREED STAIN DESTAINED AND RESTAINED WITH ANILIN OIL STAIN	RATIO 1:3
1	5,400*	16,200*	1:3.2	10,000*	1:1.8
2	5,120	12,400	1:2	16,200	1:3.1
3	540	2,700	1:5	2,500	1:4.6
4	1,280	1,890	1:1.4	1,600	1:1.2
5	2,700	32,400	1:12.0	24,840	1:9.1
6	9,170	45,360	1.5	34,500	1:3.7
7	78,200	334,000	1:4.2	448,000	1:5.7
8	47,500	156,000	1:3.3	60,400	1:1.2
9	19,500	167,000	1:8.5	180,000	1:9.2
10	18,900	97,000	1:5.1	86,400	1:4.5
11	29,700	162,000	1:5.4	510,000	1:16.7
12	36,720	804,000	1:21.9	848,000	1:23
13	244,000	907,000	1:3.7	1,080,000	1:4.4
14	562,000	2,160,000	1:3.8	1,684,000	1:3
15	208,000	640,000	1:3.0	938,000	1:4.4
16	130,000	972,000	1:7.4	1,673,000	1:12
17	97,200	408,000	1:4.2	490,000	1:5
18	200,000	285,000	1:1.4	826,000	1:4.1
19	16,000	163,000	1:10	21,600†	1:1.4
20	9,700	41,500	1:4.2	22,000	1:2.2
21	21,400	97,000	1:4.6	97,000	1:4.6
22	43,000	124,000	1:2.9	116,000	1:2.7
23	8,100	19,400	1:2.3	12,400	1:1.5
24	10,000	265,000	1:26	52,300	1:5
25	58,400	183,000	1:3.1	98,000	1:1.6
26	755,000	1,220,000	1:1.6	1,600,000	1:2.1
27	19,400	204,000	1:10.7	307,000	1:16
28	8,600	37,800	1:4.3	29,000	1:3.3
29	1,620	6,400	1:3.9	5,900	1:3.6
30	9,200	38,000	1:4.1	21,000	1:2.2
31	27,000	86,000	1:3.1	85,000	1:3.1
32	41,500	265,000	1:6.4	226,000	1:5.5
33	4,300	29,000	1:6.7	27,500	1:6.3
34	25,300	—	—	27,000	1:1.06

*Multiply results by 1000 in each case. † Poorly stained.

NOTES

Color Test for Certain Foreign Resins in Vanilla Extract*

The A.O.A.C. tentative qualitative tests for vanilla resins[1] are useful in differentiating these resins from some types of foreign resins. Since the last A.O.A.C. report by Sale,[2] on the application of these tests to certain resins, two plant extracts not previously encountered have been used as adulterants for vanilla extract. These plant materials, Wild Cherry Bark and St. John's Wort, contain alcohol-soluble resins which resemble vanilla resins superficially. However, adulterated extracts containing these foreign plant resins behaved abnormally in the A.O.A.C. tests for vanilla resins. Removal of alcohol produced only a slight turbidity, but subsequent addition of acid resulted in the formation of considerable precipitate. Parts of the dried resin films exhibited distinct red colors in contrast to the yellow or brown colors of true vanilla resins. These films were brittle and could be removed from the beakers quite readily, whereas vanilla resins form oily, adhering films. The adulterated dried resins were much less soluble in cold alcohol than vanilla resins, and the addition of of $FeCl_3$ to these alcoholic solutions produced transient greenish colors. Although these tests indicated the presence of foreign resins, a more sensitive and distinctive test was desired. Such a test was developed in the course of this work, and it has proved very useful for laboratory and court demonstration purposes.

In the examination of these foreign resins, it was observed that a terpene-like odor was emitted upon ignition. Since it was known that the Komarowsky test for amyl and other higher alcohols[3] was also given by turpentine[4] and terpenes,[5] this test was applied to these plant resins. When the dried resins of Wild Cherry Bark and St. John's Wort were examined by a slightly modified Komarowsky test, intense red colors were produced, whereas vanilla resins yielded only light brown colors. Mixtures of vanilla resins with these foreign resins also gave distinct red colors in this test. These color reactions were verified by testing the dried resins from several hundred vanilla extracts, including 20 authentic extracts, and several commercial and laboratory-prepared extracts of Wild Cherry Bark and St. John's Wort. The modified Komarowsky test is performed as follows:

Mix 5–10 mg. of dried resins with 10 ml. of 0.1% solution of p-dimethyl-amino benzaldehyde in alcohol. Cautiously add 25 ml. of H_2SO_4, mix, and heat on steam bath 5 minutes. Cool, and add 150 ml. of water. A light brown color is produced by vanilla resins while a red color is given by some foreign resins.

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* By J. Fitelson and T. Riggs (U. S. Food and Drug Administration, Federal Security Agency, New York, N. Y.).

[1] *Methods of Analysis, A.O.A.C.*, 1940, 322.
[2] *This Journal*, 9, 446 (1926).
[3] *Chem. Ztg.*, 27, 1086 (1903).
[4] H. P. Basset, *J. Ind. Eng. Chem.*, 2, 389 (1910).
[5] H. Kreis, *Chem. Ztg.*, 34, 470 (1910).

Identification of Monochloracetic Acid as Barium Monochloracetate*

In a recent paper Wilson and Keenan[1] describe a method for the identification of monochloracetic acid, based on the formation of barium monochloracetate monohydrate and the determination of the optical-crystallographic properties of this salt. It has been the author's experience with this method that, on evaporation of the barium solution, the salt occasionally crystallizes out as anhydrous barium monochloracetate. However, this caused no difficulty in the identification but served rather as an additional check, since it was found possible under such conditions to determine both the optical-crystallographic properties of the anhydrous salt and

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* By William V. Eisenberg (Food and Drug Administration, Federal Security Agency, Washington, D. C.).

[1] *This Journal*, 27, 446 (1944).

those of the monohydrate. The anhydrous salt is readily converted to the monohydrate by dissolving a small amount in a drop of water on a microscope slide and allowing the solution to evaporate at room temperature. The monohydrate is then suitable for the determination of the optical properties by the immersion method.

The optical properties of the anhydrous salt, as determined by the immersion method, are compared with those of the monohydrate in the following table:

Substance	*Crystal Habit*	*Extinction*	*Elongation*	$n\alpha$	$n\gamma$
Barium monochloracetate anhydrous	six-sided plates	parallel	positive	1.512	1.638
Barium monochloracetate monohydrate	six-sided plates	parallel	negative	1.582	1.611

The anhydrous salt recovered from a determination on a sample by the Wilson-Keenan method was heated at a temperature of 100°–105°C. for four hours and showed no change in the optical properties. It is obvious from this that barium monochloracetate may, under certain conditions, crystallize out as the anhydrous salt. The foregoing data on the optical properties of the anhydrous salt supplement those given for the monohydrate in the method, thus serving as a check as well as making the identification possible under conditions where either salt may crystallize out from solution.

Precautionary Note on Volatility of C.I. No. 17 (Aminoazotoluene) and Other Colors*

In the detection and identification of dyes, significant losses in dye strength usually occur during the stripping and redyeing operations, especially in low concentrations of water-soluble dyes. Instances of complete loss of dye in some food samples from exposure to sunlight, or through the biological reductive processes of fermentation, have also been noted. A third source of loss is recorded below.

The commercial use of the non-certifiable oil soluble colors C.I. Nos. 17 and 19 (suspected of being carcinogenic) called for the examination of a large number of cosmetic preparations for the presence of these dyes. In one sample, in which C.I. No. 17 was found, a puzzling and very marked difference in the amount of color present was observed on check samples of the same preparation. Investigation revealed that this discrepancy was due to the variations in the length of time the residue from the alcoholic extract remained on the steam bath.

This was shown experimentally by heating 1 ml. portions of pure C.I. No. 17† in alcoholic solution (.04% and .002%) for 30 minutes on the steam bath, and then diluting to 10 ml. with alcohol. When compared with a 1:10 dilution of the original dye solutions, a loss of approximately 70 per cent in color intensity was observed with the Duboscq colorimeter.

The presence of volatile yellow material may be demonstrated in the alcoholic washings from the underside of a watch glass covering the evaporating dish in even a five-minute heating period on the steam bath, after removal of alcohol.

The "Colour Index" (C.I.) mentions that No. 15 has been abandoned as a dyestuff because of its volatility, and there are doubtless others. Prompt removal from the steam bath after solvent evaporation would seem to be indicated as safe practice with dyes suspected of being volatile, especially when such dyes are present in small amounts.

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† Obtained from Associate Referee C. F. Jablonski.

Gravimetric Determination of Phenothiazine*

Phenothiazine has come into quite general use as an antihelmintic in the treatment of sheep and swine for round worms.[1] In some cases the drug is administered in capsule or pellet form; in others the drug is introduced into the feed, much as a mineral supplement is used. For example, one prepared vermicide sold on the Canadian market has the following composition:

Oilcake meal or bone meal Iron Salt Laxative material	90%
Phenothiazine	10%

Phenothiazine is easily oxidized by many agents, and colorimetric methods of estimation are based upon these reactions.[2] However, the authors wished to study the precipitation of phenothiazine and its determination gravimetrically since Bernthsen[3] observed that platinum chloride gives a green precipitate with phenothiazine and suggested its use as a qualitative reagent.

TABLE 1.—*Composition of the precipitate*

ELEMENTS	CALCULATED	FOUND
	per cent	*per cent*
Carbon	39.16	40.33
Hydrogen	2.46	2.76
Chlorine	19.28	18.88
Nitrogen	3.81	3.20
Sulfur	8.72	8.52
Platinum	26.55	26.86
	99.98	100.55

TABLE 2.—*Practical results*

SAMPLE	THEORETICAL	FOUND
	per cent	*per cent*
A	100.0 Tech.	96.5
B commercial	5.0	4.83
C commercial	10.0	9.59

The most common solvents for phenothiazine are 95% alcohol and anhydrous ether. Accordingly, the antihelmintic preparation was ground fine and extracted with alcohol in a Soxhlet until a colorless solution passed through.

The alcoholic solution of phenothiazine was made up to 200 ml., mixed well, and allowed to settle until clear. In this manner colloidal material which passed

* By R. Payfer and Charles V. Marshall (Plant Products, Department of Agriculture, Ottawa, Canada).

[1] Smith, Munger, and Siegler, *J. Econ. Entomology*, **28**, 727 (1935).
[2] Eddy and De Eds, *Food Research*, **2**, 305 (1937).
[3] *Ann. Chem.*, **230**, 73 (1885).

through the extraction thimble was eliminated. An aliquot of 2 ml. was placed in a 10 ml. beaker, and 1 ml. of chloroplatinic acid solution (40 grams per liter)† was added. The precipitate was allowed to settle one hour and then transferred to a tared sintered glass crucible. Washing with alcohol was continued until the washings were colorless. The precipitate was dried at 100°C. to constant weight.

$$\text{Wt. of precipitate} \times 0.5416 = \text{wt. of phenothiazine in the aliquot.}$$

The above factor assumes that the green compound is an addition product of phenothiazine and platinum, formed according to the equation:

$$2\ C_{12}H_9NS + H_2PtCl_6{,}6H_2O \rightarrow Pt(C_{12}H_9NS)_2Cl_4 + \cdots$$

The actual composition is shown in Table 1.

This method is believed to be both rapid and accurate over the range studied. Its application to the determination of small amounts would require further study.

† This platinic chloride solution is regularly used in this laboratory as a reagent in the Lindo-Gladding official method for determination of potash in fertilizers.

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Subcommittee B: F. H. Wiley (1945), U. S. Food and Drug Administration, Washington, D. C.; Harry J. Fisher (1947), and Dan Dahle (1949).

Subcommittee C: J. O. Clarke (1945), U. S. Food and Drug Administration, Chicago 7, Ill.; C. S. Ladd (1947), and Joseph Callaway (1945).

Subcommittee D: J. Walter Sale (1949), U. S. Food and Drug Administration, Washington 25, D. C.; Kenneth L. Milstead (1949), and C. S. Ferguson (1947).

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METHODS OF ANALYSIS, 6th edition, 1945

It is expected that the 6th edition of "Official and Tentative Methods of Analysis, A.O.A.C." will be available for distribution by the fall of this year. The list price of the new edition will be $6.25, domestic postpaid.

ANNUAL MEETING CANCELLED

In response to the request for curtailed travel during the war emergency the Executive Committee has voted that no annual meeting of the Association be held in 1945.

Charles William Dabney, 1855–1945

CHARLES WILLIAM DABNEY

1855–1945

The death of Dr. Charles W. Dabney at Asheville, N. C., on June 15, 1945, just four days before his ninetieth birthday, removed the last of the founders of the Association of Official Agricultural Chemists. Although not an active participant in the work of the Association for over fifty years and known only to a few of its older members, he was a most potent influence in the movement that led to its foundation.

Charles William Dabney was born at Hampden-Sydney, Va., on June 19, 1855, the son of Robert L. Dabney, Chief of Staff to General "Stonewall" Jackson in the Civil War. After receiving his B.A. degree at Hampden-Sydney College in 1873, he attended the University of Virginia, where in his chemical studies he came under the inspiring influence of Professor John W. Mallet. For additional training in chemistry he went next to Göttingen University in Germany, where he was among the last group of American students to receive instruction in the famous school of Wöhler. To participate more fully in the spirit of German university life, he became a member of the student corps Hannovera, but not caring to indulge in the barbaric practices of some of his German associates he held aloof from the fighting of duels.

Having obtained his Ph.D. degree at Göttingen in 1880, Dabney returned to the United States, where the State experiment station movement was then at the commencement of its upward swing. He accepted a call to the directorship of the North Carolina Experiment Station, with which were coupled also for a time the duties of State Chemist. During this period he took an active part in the work of the Conventions of Agricultural Commissioners and Chemists which preceded and finally led up to the founding of the A.O.A.C. The first of these Conventions met at Washington, D. C. on July 28, 1880, as the result of a circular letter sent by Judge J. T. Henderson, Commissioner of the Georgia Department of Agriculture, in which he invited various State Commissioners, State Chemists, and Professors of Chemistry in State Universities and Agricultural Colleges to meet "for the purpose of adopting a uniform system for the analysis of commercial fertilizers." The first Washington Convention was largely an organization meeting in which committees were appointed to propose methods for the determination of phosphoric acid, nitrogen and potash in fertilizers. An adjourned meeting of this Convention was held a month later on August 27, in Boston, at which the recommendations of the previously appointed committees were adopted provisionally for a limited period of one year in order to give time for testing the accuracy of the proposed methods by collaborative research. Meantime, plans for a permanent organization of agricultural chemists were to be elaborated and presented at the next Convention which was scheduled to take place in Cincinnati, on August 18, 1881, in connection with the meeting of the American Association for the Advancement of Science.

It was at the Cincinnati meeting of Agricultural Chemists that Dr. Dabney first took part in the work of the Conventions, the previous meetings in Washington and Boston having been held before his appointment as State Chemist of North Carolina. The exceedingly wide discrepancy between the results of different chemists in determining reverted phosphoric acid in fertilizers was the chief topic of consideration at the Cincinnati meeting. In opening the discussion on this long debated problem, Dabney and others called attention to the dissatisfaction which existed with the methods then in use and showed the difficulties in the way of arriving at any method which would be universally accepted.

The subject continued to bother fertilizer analysts for many years and debate upon it was renewed at the next Fourth Convention of Agricultural Chemists, which was held at Atlanta on May 15 and 16, 1884. Dabney was the secretary of this Convention and the now exceedingly rare 60-page bulletin of its Proceedings was edited by him. His masterful way of getting at the heart of any difficulty that confronted him is well illustrated by his analysis of the phosphoric acid problem at the Atlanta Convention. Dabney stated that "reverted phosphoric acid" was only a scientific myth. The theory that the reverted phosphates are all dibasic phosphates was not supported by facts. He held that this mythical substance was only to be defined by the method used for its determination. All of the conditions necessary to describe the method must enter into the definition. In addition to the prescribed specifications of weight of fertilizer, specific gravity and volume of ammonium citrate solution, and temperature and time of digestion, it should be stated how much time was to be consumed in the filtration and washing. The slightest change in the details of the method meant a change in the definition of the so-called Reverted Phosphoric Acid. If chemists make even a slight modification in any of these details no comparison of analyses could be made. Dabney made a plea for a detailed study of the chemistry of the compounds that were removed by the standard citrate reagent with special reference to their solubility in the various types of ordinary soil solutions, and until this was done he declared that reverted phosphoric acid must remain not a substance, or several substances, but a conventional name for the result of a stipulated process.

A very important resolution introduced by Dabney at the Atlanta Convention was to the effect that only the agricultural chemists and officers of experiment stations and agricultural bureaus be considered as members. Hitherto any practical analytical chemist was entitled to vote at the Conventions, and as the opinions of some trade analysts were influenced by the mercenary interests of their clients it was deemed necessary for the sake of impartiality to limit the voting privilege to agricultural chemists who held official State or Federal positions. Dabney's resolution was in fact incorporated in Article II of the first Constitution of the A.O.A.C.

The Atlanta Convention of Agricultural Chemists was the last meeting of this precursor of the present Association of Official Agricultural Chemists, which was organized four months later at Philadelphia on September 9, 1884, in the Hall of the Utopian Club, in accordance with the concluding resolution of the Atlanta Convention that steps be taken "looking to the permanent organization of this body of agricultural chemists." This transition from a Convention to an Association of Agricultural Chemists was actually only a change in name, for the operations in the old organization went on unchanged into the new; Dabney continued to act as secretary, the same committees continued to make their reports, and the same methods of analysis continued to be tested. It has been too often forgotten that the Conventions of Agricultural Chemists that preceded the Association of Official Agricultural Chemists are as much a part of our organization's history as any of the regular annual meetings held after the year 1884.

In 1887 Dabney was elected to the presidency of the University of Tennessee and from this time on educational matters demanded more and more of his attention. He resigned his offices as Professor of Agricultural Chemistry and as Director of the Tennessee Agricultural Experiment Station in 1890, and he attended his last meeting of the Association of Official Agricultural Chemists in 1891. This did not mean, however, a complete cessation of the services which he rendered to the work of our organization. In 1893 he was appointed Assistant Secretary of Agriculture by President Cleveland, who was then entering upon his second term of office. The trustees of the University of Tennessee gave their consent to his accepting this appointment with considerable reluctance, but Dabney maintained close advisory relations with the trustees during his period of absence in Washington, and there was no interruption in the execution of his policies as President of the University.

As Assistant Secretary of Agriculture, Dabney had general supervision of the scientific work of the Department and this brought him into close relationship with the investigations on food adulteration which at that time were being actively pushed by Dr. Wiley. Considerable political pressure was brought to bear upon Secretary of Agriculture J. Sterling Morton by certain powerful commercial interests to have Dr. Wiley removed from office. The letters of protest finally increased so in number that Secretary Morton, on showing a huge pack of them to Dabney one day, remarked that as supervisor of the scientific work of the Department he must eliminate Wiley from his position as Chief of the Division of Chemistry. Dabney, who was in complete sympathy with Wiley's crusade against food adulteration, refused to carry out this order for his discharge and then looking Morton squarely in the eye replied: "If you discharge Wiley, my own resignation immediately follows and then *you* will have to go." Morton did not care to press the matter and Dr. Wiley went on with his crusade. In later years, after Dr. Wiley had secured the passage of the Federal Food and Drugs Act he expressed his appreciation to Dabney for the support which he had given him at this critical time.

Dr. Dabney, during his long presidency of the University of Tennessee from 1887 to 1904, became widely known for his advocacy of free public schools throughout the South. His educational interests were stressed not only in his book "Universal Education in the South," but in his numerous public addresses and in the objects which he installed as Chief of Government and States Exhibits at the New Orleans Exposition of 1883–4, as Chairman of the Board of Managers of Government Exhibits at the Atlanta Exposition of 1895, and at the Tennessee Centennial Exposition of 1897. The prominence thus gained resulted in his call in 1904 to the presidency of the University of Cincinnati, in which position he remained until his retirement in 1920. In late years he made Cincinnati his home, his winters being usually spent in Florida. It was while travelling from Florida to Cincinnati for the celebration of his ninetieth birthday that he was stricken with the attack which carried him away.

At the 50th Anniversary of the Association of Official Agricultural Chemists an invitation to the Memorial Dinner, which was held on October 30, 1934, was sent to Dr. Dabney as one of the founders and the first Secretary-Treasurer of the organization. His attendance at this banquet was one of the great events of this Golden Anniversary Meeting. At the head of the receiving line he greeted each of the nearly two hundred attending members and was heard to remark that the infant organization of a mere dozen which he had helped to foster had grown to one of almost incredible size. Those who were so fortunate that evening to hear Dr. Dabney's reminiscences of the early days of the Association enjoyed a treat that will never be forgotten.

Many honors came to Dr. Dabney in the course of his long career. He was awarded the degree of LL.D. by Davidson College and by the Universities of Washington and Lee, Yale, Johns Hopkins, and Cincinnati. He was also made a Chevalier, Legion d'Honneur, by the Republic of France. His Doctor's dissertation at Göttingen "Über eine Isopikraminsäure" has no apparent connection with his later grand scale investigations on the agricultural resources and the phosphates, potash deposits, lignites, and other mineral supplies of the Southern States, but it was owing to the discipline and powers of observation acquired in his early laboratory studies that his subsequent survey work was made possible. It is not, however, in the field of material resources but in that of education that Dabney's name will live the longest. His love for the natural sciences was always strong, but in later years the science of human relations became his dominating interest. He personified the saying of the Latin poet Terence:

"I am a man and I think nothing appertaining to mankind foreign to me."

C. A. Browne