FIRST DAY
MONDAY—MORNING SESSION

REPORT ON DAIRY PRODUCTS

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The past year has seen extensive efforts devoted to the development of methods for the detection of foreign fats in dairy products, the publication of a very important paper on cheese sampling, and renewed interest in the determination of the freezing point of milk.

Foreign Fats.—The development of methods for the detection of adulteration of butterfat can be approached in two ways. The first depends on measurements of the constants which are characteristic of butterfat and requires an extensive background of authentic data for the interpretation of the results of the analysis. The second approach attempts to detect the presence of an adulterant directly through the measurement, qualitative or quantitative, of the properties of the added substance.

The determination of the Reichert-Meissl value of the fat is an example of the first type of analytical method. This determination measures the water-soluble volatile fatty acids distilled under carefully controlled empirical conditions from the acidified solution of the saponified fat. The Kirschner value is somewhat more specific in approximating the butyric acid content of the Reichert-Meissl distillate. This procedure, however, has not been shown to have greater value in either precision or sensitivity over the Reichert-Meissl value for the detection of adulteration. During the past year two procedures for the specific estimation of butyric acid by chromatographic procedures have been proposed (1, 2). These methods have the advantage of eliminating the empirical distillation of the Reichert-Meissl determination and measuring directly the butyric acid component of butterfat by well established chromatographic technics. As with all new methods, they do not have the extensive background of authentic data necessary for the interpretation of results. On the other hand, the literature quoting the range of possible Reichert-Meissl values for butterfat is hardly satisfactory. Many of the unusually low values which have been quoted are based upon determinations using the butterfat from single animals, or from herds which have received a poor diet. The modern dairy industry, which utilizes mixed herd milk from well fed cows, at the present time undoubtedly supplies a food with a much narrower range of variation in chemical and physical constants than would be indicated by the repeatedly quoted "constants" which

have accumulated over the past five or six decades. For example, a Reichert-Meissl value of less than 27 has not been encountered by the Referee in normal commercial samples of butter within the past few years. The usual range is 28 to 31. Obviously, a thorough and extensive investigation of these constants is necessary. The newer technics would not be handicapped by a background of historical values which are not applicable to present day production. However, since many regulatory officials have accumulated data applicable to their jurisdictions by the official Reichert-Meissl procedure, an investigation of the chromatographic methods should also include comparisons with this older method.

Modern advances in fat and oil technology may seriously limit the applicability of this type of approach to the detection of butterfat adulteration. As pointed out by Kummerow (3), the production of "rearranged fats," which incorporate butyric acid into plant or animal fats to produce a "synthetic" butterfat with physical and chemical properties closely resembling those of butterfat, is a definite technological possibility. Adulteration with a skillfully compounded fat of this nature would not be detected by methods which depend upon the determination of butyric acid.

A modification of this type of method which depends upon measurements of butterfat constants has been suggested by Bhalerao and Kummerow (3). These investigators propose two methods based upon differences in triglyceride structure of fats. They state: "Even animal fats, such as lard and beef tallow, do not have the same proportion of mono-, di-, and trisaturated glycerides as butterfat." Their first method measures the solubility of the fat in absolute alcohol at 20°C. (22 ± 4 per cent insoluble) and then determines the difference in refractive index of the alcohol-soluble and alcohol-insoluble triglycerides. In the case of butterfat, these two fractions are reported to have approximately the same refractive index, while in the case of other fats the values varied appreciably. The second method subjects the alcohol-soluble and -insoluble fractions to "inter-esterification" in the presence of sodium methoxide to obtain a completely random distribution of the triglycerides. The difference in refractive indices before and after the rearrangement is again noted. It was reported that a mixture of 25 per cent coconut oil, 25 per cent beef tallow, and 50 per cent lard, which has approximately the same mixed fatty acid composition as butterfat, could be detected at the 10 per cent level in butterfat. If the results reported are confirmed on a wider variety of butterfat samples, this procedure could replace present methods because of its reported universal applicability.

The second approach to the detection of adulteration utilizes a property of the adulterant. The Associate Referee on foreign fats in butterfat is continuing his investigation of the detection of *vegetable* oils in butterfat through the detection of plant sterols by the sterol acetate melting-point

test. Although the vegetable sterols (phytosterols) differ from the characteristic animal sterol (cholesterol) only by the presence of an additional ethyl group on the sterol side chain, this difference is manifested by a 10° difference in the melting point of the sterol acetates. With such a distinct difference in melting point, differences in other properties, particularly chromatographic, would be expected. Chromatographic investigations in the Referee's laboratory, however, have failed to provide a separation technic, either on paper or on adsorption or partition columns, when the free sterols or some of their derivatives are used. Two by-products of this study were the development of a simple carbon column, which has already been applied by the Associate Referee to the purification of sterols (16) and a chemical method for the differentiation of phytosterol from cholesterol which utilizes the formation of propionic acid on drastic oxdiation of phytosterol (16). This chemical test may be useful to verify results obtained by the sterol acetate melting point test since the sensitivity is of the same order of magnitude in the case of the common domestic seed oils.

The sterol approach to this problem has the advantage of specificity. The phytosterols are characteristic plant products and their presence in butterfat would be proof of adulteration with a vegetable fat. Phytosterols have been reported to be incapable of being adsorbed from the intestinal tract (4) so it is extremely unlikely that they could be found in edible animal tissues.

Another possible approach utilizing the sterols, which might have even greater specificity, would be a fundamental study of the identity of the various sterols in foods. Bauman (5) has classified the plant sterols into two groups, 'fast acting" and "slow acting," according to the rate of reaction with the Lieberman-Burchard reagent. He has ascribed the faster rate of reaction to sterols which contain a double bond in the Δ_{22-23} position of the sterol nucleus. He has stated (6) that olive oil has a relatively large amount of "fast acting" sterols and suggests that this property may be the basis of a method for estimating the olive oil content of blends. With the widespread interest in sterol research, accentuated by the advent of cortisone and related products, the tools may soon be available for a fundamental study of sterols in foods. These may have a much wider applicability in food analysis than to the single problem of the detection of adulteration of butterfat.

A microbiological method for the differentiation of plant and animal sterols has also been investigated in the Referee's laboratory. A mycobacterium with potentialities for metabolizing cholesterol completely, but not metabolizing sitosterol, was obtained from Dr. Theresa Stadtman of the National Institutes of Health. This particular organism did exhibit a preferential metabolism of cholesterol. Unfortunately, in our laboratory it also metabolized phytosterols appreciably before completely utilizing cholesterol, and therefore it was not deemed suitable for regulatory pur-

poses. Further culturing of the organism under special conditions, or a search for other organisms, may result in obtaining one that will selectively perform the required cholesterol metabolism without attacking the sitosterol.

Vegetable oils contain relatively large amounts of tocopherols in comparison with animal fats (7). Furthermore, they may differ in the relative proportions of the tocopherol isomers. The addition of five per cent of corn oil containing 1000 mmg total tocopherol to a butterfat containing 30 mmg total tocopherol can be easily detected. Such an ideal situation, however, is rarely encountered by the regulatory chemist; butterfat contains a variable amount of tocopherols and the range has not yet been definitely established; the effect of tocopherol-rich feed on the tocopherol content of the butterfat must be known; tocopherols can be removed during refining of vegetable oils; tocopherols added to foods as antioxidants would give false positive results. Therefore, the tocopherol test at the present stage of development would appear to be valuable as confirmatory evidence only when supported by other tests.

An interesting test which has remained dormant for 30 years is Seidenberg's method for the detection of beef fat in butter fat (8). Ten grams of sample is dissolved in 100 ml of a mixture of alcohol and ether, and the solvent is aspirated off at constant temperature. The volume at which turbidity occurs is noted. Mixtures containing beef fat become turbid when approximately one-third of the solvent has been aspirated off, whereas butter fat solutions do not become turbid until more than half of the solvent has been eliminated. Results on a few samples indicate that the method will detect the presence of 10 per cent of beef fat in butter fat.

The recent Canadian Trade Information Letter No. 92 of August 10, 1953, from the Department of National Health and Welfare, advising of revision in regulations defining butterfat, will be of interest to all regulatory officials: "Milk Fat (Butter Fat) is the fat of cow's milk and shall have (a) a specific gravity of not less than 0.905 at a temperature of 40°C., (b) a tocopherol content of not more than 50 micrograms per gram as determined by the method employed by the Food and Drug Laboratories, (c) a Reichert-Meissl number of not less than 24, and (d) a Polenske number of not more than 3.5, and where the Polenske number exceeds 10 per cent of the Reichert-Meissl number there shall be deemed to have been an addition to the milk fat of fat other than that of cow's milk."

It may be that the detection of foreign fats in butterfat may require the application of the same multiple method philosophy that is necessary in the objective determination of decomposition by chemical indices. In the latter case, it has been repeatedly demonstrated that no single method is adequate to detect all forms of decomposition in a food.

Babcock Test.—The Associate Referee on Fat in Milk has submitted for action by the Association an extensive report entitled "Procedures for

Sampling and Testing Milk by the Babcock Method." As the Associate Referee has stated, this report obviously represents a tremendous amount of thought and discussion. It has been in committees of the American Dairy Science Association for almost five years and was finally approved by that Association at its last annual meeting.

Although the Referee is sympathetic with the aims and objectives of this report, it appears that the details require very careful consideration to determine if they comply with the policy of the A.O.A.C. This Association is devoted primarily to the development and testing of methods of analysis, a fact which has been interpreted to include directions to insure a representative analytical sample. It has never attempted to incorporate directions which might more properly be in the realm of statutes or regulations. Such items in the report from the American Dairy Science Association as "Weighing milk," "Abnormal milk," "Period of composite sampling," appear to be of such a nature. It is therefore recommended that this report be studied by Subcommittee C with regard to policy considerations. It is suggested that the report might be more properly considered by the Association of Food and Drug Officials of the United States in much the same way that that Association has recommended a Uniform State Food and Drug Bill. The objectives of the report on the Babcock Test and the model Food and Drug Bill are the same—to obtain uniform laws and regulations for products subject to state regulation.

The strictly analytical aspects of this report, however, should receive prompt attention by the A.O.A.C. The Referee believes that this can best be done in conjunction with collaborative tests of the new "detergent" Babcock test. The Associate Referee did not conduct such studies during the past year, since he was informed that in the opinion of the originators of the method, *viz.*, the Bureau of Dairy Industry, collaborative studies should be delayed until further work on some of the details had been completed. The test is now ready for collaborative study, and such studies should include changes recommended by the American Dairy Science Association. Among these are the temperature of tempering and pipetting, the volume of sample, the reading of the meniscus, etc. Since there are a large number of variables to be studied, the experiment should be statistically designed to obtain all the necessary information in the most economical fashion. More than the usual number of collaborators will be required so that they may be divided into groups for testing various phases of the problem. Such a study is also complicated by the fact that milk is not a satisfactory product for collaborative study because of fat separation and fat loss on the sides of the container. Therefore, comparison of analyses of the same milk by different methods or under different conditions will have to be conducted in each collaborating laboratory.

Reconstituted Milk.—A definite need has existed for many years for a

satisfactory test for the detection of reconstituted milk. Little progress has been made since one of the first methods was proposed by Evenson (9). The method proposed by Edwards (10) has not received further attention. Recently a method based upon the protein reducing value and the whey protein nitrogen has been developed by Dr. Choi of the American Dry Milk Institute. This method has been studied to some extent in the Louisiana State Laboratory, and satisfactory results have been reported (11). It is therefore recommended that an Associate Referee be appointed to study this method collaboratively.

Cheese Sampling.—The present directions for sampling cheese date back to before the first edition of *Official Methods of Analysis.* No satisfactory study that might form the basis for a recommendation for a revision of the present method had been published until the appearance of the paper by Price, Winder, Swanson, and Sommer (12).

The present A.O.A.C. directions suggest the use of a wedge sample, but in view of the impracticability of such a sample under commercial conditions, it also provides for a plug sample. Price, *et al.*, show that a plug sample which excludes the rind portion consistently overestimates the moisture content of the corresponding wedge. Practically, however, this point is not too important to the regulatory chemist who may be examining natural cheese for compliance with standards whose specifications have been determined by reliance upon the conventional plug samples. It is very important, however, to the commercial chemist whose analyses may have a direct bearing on the composition of processed cheese products with a maximum moisture specification. The samples of these types of products are not subject to the same type of sampling bias as the natural cheese.

The most important conclusion of this study is that the standard deviations (σ) of such widely different samples as a center core or an edge core do not differ by much more than 0.1 per cent, and the corresponding control limits (3σ) (measurements may be expected to fall beyond these control limits by chance alone only 3 times in 1000 samples) do not differ by more than 0.3 per cent. Furthermore, both the top and the bottom of a cheddar should be sampled to take into account a differential loss of moisture during storage, particularly when the cheeses are not turned. Therefore, in sampling a vat lot, for minimum variation combined with practicality, a center core sample from both the top and bottom is recommended. The advantages of this sample are minimum damage to the cheese and ease of location. The moisture values given by this sample do not differ appreciably from those given by other plug samples, and this difference is more in the direction of the true moisture content of the cheese than any other plug sample studied. As the authors point out, however, the moisture values obtained by any plug type of sample is only a "working" moisture content of the cheese, not the true moisture

content which, according to these authors, cannot be accurately estimated in practice since it varies with the conditions affecting rind formation. Even a plug sample centered at one inch from the edge of the cheese did not give a moisture value which differed significantly (statistically) from the average of a six-plug sample taken from both the top and bottom in a manner similar to that specified in *Officials Method of Analysis* for a single side.

If verified, it would be desirable to have the sampling directions derived from this extensive scientific study incorporated into the sampling procedure of *Official Methods of Analysis*. It is difficult to conceive of a pattern for a collaborative study that would supply the necessary information. One possible plan would be to compare the moisture values obtained from the center core sample, the present A.O.A.C. cores (single side), and the wedge sample on a large number of cheeses. Such an ambitious program could only be undertaken by, or in cooperation with, a large cheese processing operation in which the residual cheese from the sampling operation can be used during the manufacturing operations.

Preparation of Samples.—The Waring blendor has been shown to be an admirable piece of apparatus for the preparation of sample of semi-solid foods such as creamed cottage cheese, pressurized cream, and french dressing. Subcommittee C, however, has been reluctant to recommend the adoption of these manipulations as procedures without a demonstration that moisture losses during the preparation are negligible. Recently a surface cooling accessory for the Waring blendor has been announced[1] that is reported to maintain the contents of the container at only 1 or 2° above the temperature of the circulating water and thus to reduce surface evaporation. Such a device may overcome the objections to the Waring blendor, and the Associate Referee on Pressurized Cream has indicated that a study of this accessory will be made.

Cryoscopy of Milk.—This subject was originally reopened as the result of the request of the New York State Association of Milk Sanitarians. A study of the freezing point of fluid milk in the New York City area showed a peak of the frequency distribution curve at −0.531°C. (13). Only 0.65 per cent of the 1450 samples examined showed a freezing point of −0.550°C. or lower. It is suggested that present day milk supplies differ substantially from those of thirty years ago when the A.O.A.C. constant for the freezing point of milk was established, particularly with respect to the practice of pasteurization, almost universal in the present day, which retards lactic acid formation, protein and other constituent degradation, and enzymic action. Furthermore, the heat treatment of pasteurization and the pressure treatment of homogenization are suggested as factors that influence the freezing point of milk as well as experimental errors. Most important of all, the authors state: "In milk

[1] Central Scientific Co., Chicago 13, Ill.

production, handling and processing, small quantities of water find their way into the milk from wet surfaces of equipment. This results from approved washing and sterilizing procedures. The question then arises, how much variation, if any, in freezing points, results from normal farm and plant procedures?"

During the past year there was published (14) a very important survey of market milk sponsored by the Committee on Milk Production, Distributions, and Quality of the Agricultural Board and the Food and Nutrition Board of the National Research Council. The average freezing point of 135 samples of market milk from 8 cities was −0.540°. The data include samples admitted by the authors to contain added water, although the exclusion of these particular samples would not materially affect the average. It is concluded: "It would appear, therefore, that doubt might be cast upon the A.O.A.C. standard of −0.550°C. for the freezing point of milk as applied to present day milk supplies. A recent study by Paley and Tzall on the freezing points of milk in the New York City market questions the A.O.A.C. standard and is in fairly good agreement with the present study."

It is interesting to note that the data from both of these studies on market milk show a frequency distribution skewed in the direction of a higher freezing point (indicating added water) in contrast with the normal distribution pattern usually exhibited by biological systems. A skewed frequency distribution usually indicates that forces other than chance alone are influencing the data, and this particular type of pattern is well known to regulatory authorities experienced in the detection of adulteration of foods with the universal adulterant—water.

The regulatory official has always based his interpretation of the results of analysis on a comparison with data from authentic samples. Such samples produced or manufactured under his immediate supervision provide a range of values for the pure substance. Further investigation of commercial products produced under his observation provides additional values for what constitutes good commercial practice. A survey of data from market samples merely supplies information with regard to what is actually being produced, not what should be produced.

Neither of the above two studies provide data on authentic samples, and until such data are supplied there is no scientific reason for revising the present constant. It is true that present day conditions are different from those of thirty years ago, and this factor certainly renders further study necessary. However, it would appear that sufficient consideration was not given to the equally plausible alternate conclusion that certain of the milk supplies examined contained added water. A re-examination of the freezing points of authentic milk samples and such milks processed under supervision should be conducted by the Associate Referee to determine the facts.

Milk By-products in Mixed Feeds.—The Referee on Feeding Stuffs recommends the adoption of a method for total solids in milk by-products in mixed feeds and suggests that such a method belongs in the Dairy Products Chapter. The Referee on Dairy Products concurs with the recommendation for adoption, but suggests that the choice of a final place for this method be left to the Committee on Revision of Methods of Analysis.

Critical Temperature of Dissolution.—The Referee has previously overlooked recommending this test for adoption for the purpose of distinguishing oleomargarine from butter. Collaborative work has been performed (15) and the test has been used for more than three years without adverse criticism.

RECOMMENDATIONS

It is recommended*—

(1) That work be continued on methods for preparation of sample, sucrose, and acidity of ice cream and frozen desserts.

(2) That further work be done with the mechanical shaking method for the preparation of butter samples.

(3) That work be continued on the Babcock method for the determination of fat in homogenized milk.

(4) That collaborative studies be performed on the official Babcock test, on the modification submitted by the Associate Referee, and on the detergent test.

(5) That work on the detection and estimation of foreign fats in dairy products be continued and expanded by the appointment of additional Associate Referees for the various aspects of the problem.

(6) That new studies be inaugurated on the detection of reconstituted milk.

(7) That the present procedure for sampling cheese, **15.123,** be reviewed and restudied.

(8) That studies on the freezing point of milk be conducted on authentic milk and on such milk pasteurized and homogenized.

(9) That the critical temperature of dissolution for distinguishing oleomargarine from butter (*This Journal,* **33,** 495 (1950)) be adopted, first action.

REFERENCES

(1) KEENEY, M., University of Maryland Misc. Publication 153, January 1953; *Ice Cream Trade J.*, **49,** 30 (May 1953).
(2) HARPER, W. J., and ARMSTRONG, T. V., Abstract in *J. Dairy Sci.*, **36,** 566 (1953).
(3) BHALERAO, V., and KUMMEROW, F. A., *ibid.*, **36,** 567 (1953).
(4) SCHOENHEIMER, R., *Science,* **74,** 579 (1931).
(5) MOORE, P. R., and BAUMANN, C. A., *J. Biol. Chem.*, **195,** 615 (1952).

* For report of Subcommittee C and action of the Association, see *This Journal,* **37,** 69 (1954).

(6) BAUMANN, C. A., *Personal communication.*
(7) LANGE, W., *J. Am. Oil Chemists Soc.*, **27**, 414 (1950).
(8) SEIDENBERG, A., *Ind. Eng. Chem.*, **10**, 617 (1918); *This Journal*, **5**, 512 (1922); **6**, 437 (1923).
(9) EVENSON, O. L., *J. Dairy Sci.*, **5**, 97 (1922).
(10) EDWARDS, G. F., *This Journal*, **33**, 855 (1950).
(11) CLAY, C. L., *Personal communication.*
(12) PRICE, W. V., WINDER, W. C., SWANSON, A. M., and SOMMER, H. H., *This Journal*, **36**, 524 (1953).
(13) PALEY, C., and TZALL, B., *24th Annual Report of N. Y. State Assn. of Milk Sanitarians*, 81 (1950).
(14) DAHLBERG, A. C., ADAMS, H. S., and HELD, M. E., *Sanitary Milk Control and Its Relation to the Sanitary, Nutritive, and Other Qualities of Milk*, Publication 250, National Research Council, Washington, D. C., 1953.
(15) FELMAN, H. A., and LEPPER, H. A., *This Journal*, **33**, 492 (1950).
(16) LADA, A., *ibid.*, **37**, 551 (1954).

REPORT ON CRYOSCOPY OF MILK

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Recent reports indicate a need for improvement in both the technics and the interpretation of results of the cryoscopic method. In a collaborative study reported by Shipe, Dahlberg, and Herrington (1), nine laboratories using the official method and a common sample reported a maximum variation of 0.0275°C. among the average freezing point values for one series of samples. Of eleven duplicate samples analyzed by both the New York City Department of Health and the New York State Department of Agriculture and Markets, there were three in which the variation between laboratories was sufficient to change the classification from "adulterated" to "non-adulterated" (2). Although concordant results by competent analysts on like samples is a criterion of acceptance of official methods, the several referee reports by Hortvet and Bailey offer no such proof of reproducibility. Of the possible factors which cause variations, the manner of tapping the thermometer appears most important. Shipe, *et al.* (1), found that continuous tapping gave more consistent results. At the Walter Reed Army Medical Center, it was shown that an individual determination could be altered as much as 0.013°C. by changing the manner of tapping, yet still comply with the official method. This factor could be eliminated by employing a thermistor as the temperature-sensitive element. Investigation of this possibility is under way and should be continued.

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In a study of milk supplies of eight cities for the National Research Council, Dahlberg, Adams, and Held (3) found the average freezing point of market milk to be −0.540°C. and they state: "It would appear, therefore, that doubt might be cast upon the A.O.A.C. standard of −0.550°C. for the freezing point of milk as applied to present day supplies." Of 1,372 samples of market milk analyzed by Paley and Tzall (4), only 40 per cent of the samples showed freezing points within the tolerance of the three per cent of the official method. Lampert, reporting on herd milk (5), found an average freezing point of −0.536°C. and recommended using −0.540°C. as the average freezing point. Lythgoe (6) advocates separate standards for individual cow, small herd, and market milk, and states with reference to market milk: "If such a sample freezes above −0.540°C., the analyst is probably justified in declaring the presence of added water." Shipe, Dahlberg, and Herrington (7) found the average freezing point of 594 samples from individual cows to be −0.539°C., and that several factors influenced the freezing point. They call for a "more extensive study to establish the correct range of values for the present milk supply in this country."

The findings of these and other competent analysts justify a reappraisal of the interpretation of results appearing in the method. The original collaborative study reported by Associate Referee Bailey (8) showed an average freezing point of −0.545°C. and concluded that "the minimum freezing point depression of −0.530°C. and maximum of −0.566°C. for milk from normal individual cows and the minimum of −0.530°C. and maximum of −0.562°C. for the milk from normal herds is reasonably substantiated by the experience of all collaborators." Hortvet (9) repeats these figures in his Referee report and adds the following: "Owing to these observed natural variations, it is advisable to adopt a tolerance figure in passing judgement on market samples. A tolerance of 3 per cent may be deducted from results for added water calculated on the basis of an average freezing point depression of −0.550°C. A thorough investigation of the cryoscopic properties of authentic samples in a given locality may justify a smaller, but scarcely a larger, tolerance figure. Owing to the narrow variations actually found among market milks of genuine character, it is not necessary in practice to deduct the tolerance figure from results showing added water in amounts above 3 per cent." This is apparently the source of the last paragraph in **15.32** of the present method. The average freezing point of −0.550°C. proposed therein by Hortvet (he reported finding an average freezing point of −0.548°C. for 75 samples in his original paper (10)) was based on data published prior to the development of the official method, just as table **41.28** uses −0.550°C. as the average freezing point and is based on Winter's table published in 1905 (11). In view of the question raised as to the applicability to the present day milk supply of the average freezing point of −0.550°C., and

pending the results of a new and extensive collaborative study to establish a more valid average and normal range, it is recommended that the interpretation be changed by omitting table **41.28** and the reference to it, changing T in the formula to the minimum freezing point of -0.530°C., and omitting any tolerance. Such a change would be compatible with the original collaborative work reported by Bailey (8) and would permit continued acceptance of the method.

RECOMMENDATIONS

It is recommended*—

(1) That a collaborative study be made of an improved manner of tapping the thermometer.

(2) That investigation be continued of improved methods of milk cryoscopy.

(3) That subsequent to the findings of the collaborative study on tapping, an extensive survey should be undertaken to establish the valid average and normal range of the freezing point for milk.

(4) That Paragraph **15.32** be amended by omitting "Ascertain percentage . . . excess of 3%," and substituting: "Calculate percentage of added H_2O (W) as follows:

$$W = \frac{100(T - T')}{T}, \quad \text{in which}$$

T = minimum freezing point of normal milk (-0.530°), and T' = true freezing point of given sample."

(5) That Table **41.28** be omitted.

REFERENCES

(1) SHIPE, W. F., DAHLBERG, A. C., and HERRINGTON, B. L., *J. Dairy Sci.*, **36**, 916 (1953)
(2) CORASH, P., 19th Annual Report of the New York State Association of Milk Sanitarians, 53 (1945).
(3) DAHLBERG, A. C., ADAMS, H. S., and HELD, M. E., National Research Council Publication **No. 250** (1953).
(4) PALEY, C., and TZALL, B., 24th Annual Report of the New York State Association of Milk Sanitarians, 81 (1950).
(5) LAMPERT, L. M., *This Journal*, **22**, 768 (1939).
(6) LYTHGOE, H. C., *ibid.*, **35**, 442 (1952).
(7) SHIPE, W. F., DAHLBERG, A. C., and HERRINGTON, B. L., *J. Dairy Sci.*, **35**, 924 (1953).
(8) BAILEY, E. M., *This Journal*, **5**, 484 (1922).
(9) HORTVET, J., *ibid.*, **6**, 424 (1923).
(10) ———, *J. Ind. Eng. Chem.*, **13**, 198 (1921).
(11) HENDERSON, J. B., and MESTON, L. A., *Chem. News*, **110**, 283 (1914).

* For report of Subcommittee C and action of the Association, see *This Journal*, **37**, 69 (1954).

REPORT ON FAT IN DAIRY PRODUCTS

STANDARDIZED PROCEDURES FOR THE BABCOCK TEST FOR MILK

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A study of *Official Methods of Analysis* of the Association of Official Agricultural Chemists reveals no significant refinements in procedures for the Babcock test during the past 35 years. In fact, procedures for all phases of sampling and testing milk have never been stated in detail by the Association. This is not intended as a criticism; the dairy industry has not, until recent years, demanded more refined and detailed technics.

The Babcock test is unique among chemical tests in that it is subjected to variable technics in different states. The most important of these include the following: (*a*) Methods of sampling milk from weighing tanks; (*b*) temperatures for preparing, handling, and storing fresh and preserved composite samples; (*c*) sampling and pipetting temperatures for fresh and preserved composite samples; (*d*) temperatures in centrifuges and in water baths; and (*e*) reading the fat columns. It is unlikely that any other important chemical test is subjected to as many variable technics as have been imposed on the Babcock test by statutes and traditional practices. These variables provide grounds for disputes that may involve court litigation and thereby disrupt good relations among producer, dealer, and consumer groups.

The desirability of standardizing the test is obvious in the light of these facts:

(1) Dealers and handlers use the test as a basis for paying producers or marketing organizations for their milk.

(2) They also use the test as a basis for selling milk, cream, and other products to other dealers.

(3) Operators of milk plants need to know the fat content of their products in order to check the efficiency of their operations.

(4) Variations in methods and technics make it difficult or almost impossible for any agency, public or private, to verify the accuracy of tests of milk fat and other milk products on a legal basis.

(5) Milk and cream are shipped in interstate commerce.

At the annual meeting of the American Dairy Science Association in 1946, a subcommittee was appointed to evaluate the status of the Babcock test and to determine whether it had been kept abreast of other technological advances in the dairy industry. At the suggestion of industry

* The following members of the subcommittee of the American Dairy Science Association assisted in the preparation of this report: B. Heinemann, Producers Creamery Company, Springfield, Mo., *Chairman;* W. A. Cordes, National Dairy Products Company, Inc., New York, N. Y.; L. M. Lambert, State Department of Agriculture, Sacramento, Calif.; J. E. Edmondson, University of Missouri, Columbia, Mo.; T. I. Hedrick, U. S. Department of Agriculture, Chicago, Ill.; and J. H. Willingham, Texas Technological College, Lubbock, Texas.

groups and regulatory agencies, similar action was taken by the Association in 1938, but the study was suspended during the war. The subcommittee which was appointed in 1946 immediately began to obtain the facts from the proper agencies. The Department of Agriculture, Board of Health, or other responsible regulatory agency in each state was asked to send to the committee a copy of its laws pertaining to the testing of milk. In some states the agency sent agricultural college publications which served as state regulations. The committee tabulated the technics used in sampling and testing milk in each state and returned them to the state for verification. An abbreviated summary of this study was reported in 1947 (1), and revealed much variation in sampling and testing technics for milk. There was lack of uniformity in the use of the Babcock test among different states; some states approved technics which were prohibited by others. It was the opinion of the committee that some of the procedures were wrong and others were unnecessary. Recently (2) the study of 1947 (1) was brought up to date with a discussion of technics of the test as conducted in the different states.

As a result of its study, the subcommittee (1) recommended that standardized procedures for all phases of the Babcock test be formulated and submitted to the American Dairy Science Association for consideration. In 1950, Dr. G. H. Wilster of Oregon State College was appointed chairman of a committee to formulate detailed procedures for the test, and he continued as chairman until his retirement, because of illness, in 1952. During his chairmanship, much work was done which provided a basis for the final report. In 1952, Mr. Burdet Heineman assumed chairmanship of this committee and is responsible for the final draft of procedures which are based on the best experimental evidence and experience at the present time. These procedures were approved by the executive committee of the American Dairy Science Association at the University of Wisconsin in June, 1953. This is the first time a unified set of procedures for all phases of the Babcock test has ever been formulated on a national basis (3).

The recommended procedures give explicit directions (and precautions) for weighing and sampling of bulk milk, the care of composite samples, the preparation of samples for testing, and for the test itself. The complete procedures will be published in the *Journal of Dairy Science* and they will be incorporated in properly edited form in *Official Methods of Analysis*, 8th Ed.

REFERENCES

(1) HEINEMANN, B., HERREID, E. O., JOSEPHSON, D. V., ENGLAND, C. W., and SWOPE, W. D., *J. Dairy Sci.*, **30**, 963 (1947).
(2) HERREID, E. O., and HEINEMANN, B., *Ill. Agr. Exp. Sta. Cir.*, **709** (1953).
(3) ———, BURGWALD, L. H., HERRINGTON, B. L., and JACK, E. L., *J. Dairy Sci.*, **33**, 685 (1950).

REPORT ON FROZEN DESSERTS

PREPARATION OF SAMPLE, ACIDITY, AND SUCROSE

By HUGH M. BOGGS (Food and Drug Administration, Department of Health, Education, and Welfare, Philadelphia 6, Pa)., *Associate Referee*

PREPARATION OF SAMPLE

An alternate method for the preparation of samples of frozen desserts which contain insoluble particles was proposed in the 1951 and 1952 reports of the Associate Referee (1, 2). Since the last report, two additional pint samples of both cherry-vanilla and butter-pecan ice cream were examined by this method. The recoveries are given in Table 1. In the case of both flavors, the fruit or nut flavoring ingredient was added by injecting the material into the almost frozen mix at the end of the mixing period (common commercial practice). Also, it is believed that these flavors containing a quite solid fruit and a nut, would give as favorable recoveries as could be expected. The only probable factors influencing the variable recovery of solid particles would seem to be variation in the individual pint packages, the degree of washing, and the dryness of the particles at weighing.

An attempt to use this method on peppermint-stick ice cream was unsuccessful because the peppermint candy dissolved in the melting mix.

The drained portions of the two flavors listed in Table 1 were also analyzed for sucrose and total acidity. The results for sucrose indicated fairly complete separation of the solid constituents.

TOTAL ACIDITY IN FROZEN DESSERTS

Total acidity was determined in triplicate on five flavors of ice cream by weighing 20 g of melted, mixed sample into a 200 ml Erlenmeyer flask, diluting with 20 ml of recently boiled distilled water, and titrating with 0.1 *N* alkali, using 1 ml of 1 per cent phenolphthalein as indicator. Titra-

TABLE 1.—*Determination of drained solids*

FLAVOR	FRUIT OR NUTS ADDED	PINT NO.	FRUIT OR NUTS RECOVERED	RECOVERY
	per cent		*per cent*	*per cent*
Cherry-vanilla	16.13	1	18.54	112
		2	15.17	94
Butter-pecan	8.04	1	5.85	73
		2	5.71	71

tion was to the "first perceptible color" and magnetic stirring was employed. The addition of 1 ml. of 0.00024 per cent rosaniline hydrochloride as advocated for like samples by Johnson and King (3) did not produce a detectable color, even in the vanilla ice cream sample. Results for total acidity on these five samples are shown in Table 2.

SUCROSE IN ICE CREAM

The five samples of ice cream were analyzed for sucrose by the method outlined in the 1952 report of the Associate Referee (2). Results are given in Table 3.

TABLE 2.—*Determination of total acidity*

FLAVOR	TOTAL ACIDITY (ML 0.1 *N* ALKALI/100 G)		
	(1)	(2)	(3)
Peppermint-stick	21.5	21.8	22.0
Vanilla	23.5	22.5	22.5
Chocolate	42.0	42.0	43.0
Cherry-vanilla	24.0	23.8	25.5
Butter-pecan	19.5	21.3	21.0

TABLE 3.—*Determination of sucrose*

FLAVOR	SUCROSE	
	per cent	
Peppermint-stick	16.68	17.34[a]
Vanilla	13.59	13.79[b]
Chocolate	19.07	19.30
Cherry-vanilla	14.14	14.00[b]
Butter-pecan	17.00	16.89

[a] Not duplicates.
[b] Approximately 14% sucrose; more sucrose was added to other samples.

Results for sucrose were about as expected, except that results with the vanilla ice cream were slightly lower than those called for by the formula.

RECOMMENDATIONS

It is recommended*—

(1) That the methods for sucrose in ice cream and total acidity in ice cream be submitted to collaborative work during the coming year.

(2) That work on the alternate method for preparation of samples of frozen desserts containing insoluble particles be discontinued.

* For report of Subcommittee C and action of the Association, see *This Journal*, **37**, 69 (1954).

REFERENCES

(1) BOGGS, H. M., *This Journal*, **35**, 212 (1952).
(2) ———, *ibid.*, **36**, 190 (1953).
(3) JOHNSON, E. I., and KING, J., *Analyst*, **76**, 504 (1951).

REPORT ON PREPARATION OF SAMPLE OF PRESSURIZED CREAM

By CATHERINE G. CUNNINGHAM (Food and Drug Administration, Department of Health, Education, and Welfare, Boston 10, Mass.), *Associate Referee*

At the suggestion of the Referee on Dairy Products, experiments were performed to evaluate possible loss of weight of sample during preparation in the Waring blendor. The results showed a fairly constant loss of approximately 0.3 per cent. It was thought advisable to include in the method a provision for actual determination of loss of weight and to apply the correction to results for fat, solids, or other determinations. The Associate Referee has done some preliminary work, incorporating this provision into the proposed method.

A new device is now available for controlling the temperature of the contents of a blendor during mixing. The Associate Referee proposes to study the use of this device and to submit samples for collaborative study.

RECOMMENDATION

It is recommended*—

That the method described in *This Journal*, **36**, 128 (1953), be subjected to collaborative study.

REPORT ON FOREIGN FATS IN DAIRY PRODUCTS

By J. H. CANNON (Food and Drug Administration, Department of Health, Education, and Welfare, St. Louis, Mo.), *Associate Referee*

Last year the Associate Referee reported a procedure for the detection of vegetable fats in milk fat (1). It was stated that the procedure had been successfully used to detect 5 per cent of coconut oil in 95 per cent of butter fat. Continued study of the proposed method revealed, first, that repeated runs with 5 per cent coconut fat and 95 per cent butter fat did not always

* For report of Subcommittee C and action of the Association, see *This Journal*, **37**, 69 (1954).

yield the same good results. Sometimes the mixed acetates seemed to be contaminated with some waxy or greasy material which caused the melting point to be indistinct and unsatisfactory. (This contamination was occasionally obtained even with pure coconut oil.) No reason for these failures could be found, since the determinations were made in the same manner each time. Second, the physical properties of the plant sterols and of butter sterols are so similar that the ordinary processes of fractional precipitation or extraction will not separate them satisfactorily.

The Associate Referee was unwilling to submit for collaborative study the method proposed last year because of the unexplained failures with authentic mixtures. The General Referee suggested an additional step, devised by Lada (2), which consists of a carbon column purification. It was found that preliminary purification of the sterols in this manner made subsequent handling of the sterol residues more satisfactory and yielded higher melting acetates from coconut oil and coconut oil–butter mixtures than were obtained without this step.

On the basis of work done this year, the Associate Referee now believes that collaborative work is in order, using the method submitted last year, re-written to include the carbon column purification suggested by Lada. It is so recommended.*

REFERENCES

(1) CANNON, J. H., *This Journal*, **36**, 181 (1953).
(2) LADA, A., *ibid.*, **37**, 550 (1954).

No reports were given on fat in homogenized milk; phosphatase test, preparation of butter samples; sampling and preparation of sample of soft cheeses; or sampling, fat, and moisture in hard cheeses.

REPORT ON FEEDING STUFFS

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

RECOMMENDATIONS

It is recommended†—

(1) That the study of the following subjects be discontinued:
   (a) Ash in feeding stuffs

* For report of Subcommittee C and action of the Association, see *This Journal*, **37**, 69 (1954).
† For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 60, 61 (1954).

(b) Milk by-products in mixed feeds

(2) That Associate Referees be appointed for the following:

(a) Molasses in feeds, *This Journal*, **36**, 457 (1953).

(b) Gossypol in feeds, *This Journal*, **36**, 1108 (1953).

(3) That work on the following be continued:

(a) Crude fat or ether extract

(b) Crude protein in feeding stuffs

(c) Drugs in feeds

(d) Fat in fish meal

(e) Microscopic examination

(f) Mineral constituents of mixed feeds

(4) That the method for crude fat in baked dog food, as revised this year by the Associate Referee, replace the proposed method of last year and be adopted, first action.

(5) That the method for crude protein (**22.10**) be changed to read:

"Det. N as directed under **2.24**, using mercury as the preferable catalyst in case of material digestible with difficulty. Multiply results by 6.25, or in the case of wheat grains, by 5.70."

(6) That the Enheptin ® (2-amino-5-nitrothiazole) method be adopted, first action.

(7) That the revised method for *p*-arsanilic acid be adopted, first action.

(8) That under "Total Solids" (Dairy Products, p. 231), and after **15.15**, the following be added, and adopted, first action:

Method III (Neutralization with Zinc Oxide for High Acid Products)

"Add ca 2 g ZnO, A.C.S. (freshly ignited or oven dried) to flat-bottom dish not less than 5 cm in diam. Weigh. Add ca 1 g sample and weigh quickly. Add ca 5 ml distd H_2O and distribute sample evenly on the bottom of the dish. Heat on steam bath, exposing max. surface of dish bottom to live steam, until apparently dry. Heat at 98–100° in an air oven 3 hrs or until constant in wt. Cool in desiccator; then weigh quickly. Calc. wt of the residue. Titrate acidity of the sample and calc. as lactic acid (**15.4**). To compensate for the moisture formed when the acid is neutralized by ZnO, add 0.1 g to the residue wt for each g of acid (as lactic) in the weighed sample. Report % residue (corrected) as total solids."

REPORT ON NON-PROTEIN NITROGEN AND CRUDE PROTEIN IN FEEDING STUFFS

By FRANCES L. BONNER and E. A. EPPS, JR., *Associate Referee* (Louisiana Agricultural Experiment Station, Baton Rouge, La.)

DETERMINATION OF UREA

Although it has been known for years that urea and ammonium salts may replace a portion of the protein in diets of ruminants, it is only in the

past few years that urea has been used in quantity in commercial feedstuffs. A more recent development has been the ammoniation of molasses and plant materials such as the residue from the manufacture of furfural. Both urea and ammoniated carbohydrates are permissible ingredients of feeds for ruminants, but because of their low cost they might be substituted for protein without being named in the ingredient statement. For this reason the regulatory chemist must have some means for their detection and measurement. The official method (1) for determination of urea depends upon conversion of urea to ammonia by urease. Perkins, *et al.*, (3) have reported that the official method does not give consistent results and have proposed a modified method in which a buffer is used. Griem (2) has found that ammoniated carbohydrates are not decomposed by magnesium oxide or stronger alkalies, or by acid or urease. The present work consisted largely of checking the work of Perkins and of Griem.

The buffer employed by Perkins, *et al.*, was made by dissolving 140 grams of $Na_4P_2O_7 \cdot 10H_2O$ and 12 ml of 85 per cent phosphoric acid in 1 liter of water. The buffer, as prepared in this laboratory, gave a *p*H of 7 when 2 ml was added to 200 ml of water. Although it was not so stated by the authors, the buffer was presumably used to obtain a *p*H of approximately 7, which is generally agreed to be about optimum for urease activity. The effect of this buffer on suspensions of 2 grams of various feeds in 200 ml of water is shown in Table 1. The *p*H measurements were made a few minutes after the feed was added to the water and the mixture was stirred during the determination. After the initial reading, 2 ml of buffer was added and the *p*H was redetermined. Standing overnight had little

TABLE 1.—*Effect of pyrophosphate buffer on pH of feed suspensions and on the determination of added urea*

SAMPLE	INITIAL *p*H	*p*H WITH BUFFER	PER CENT UREA NITROGEN			
			I	II	III	IV
Bagasse	4.8	6.8	0.03	0.01	0.25	0.23
Shrimp meal	8.0	7.0	0.16	0.16	0.40	0.39
Meat and bone scraps	5.7	6.7	0.16	0.15	0.37	0.38
Alfalfa meal	6.0	6.5	0.02	0.02	0.24	0.26
Calf kit	5.4	6.0	0.11	0.09	0.36	0.34
Malt house feed	5.4	6.7	0.07	0.05	0.28	0.28
Poultry worming mix	6.0	6.8	0.04	0.03	0.27	0.27
Cottonseed meal	6.0	6.8	0.04	0.04	0.28	0.28
Range nuggets	6.0	6.8	0.03	0.02	0.26	0.25
Dairy feed	5.8	6.6	0.03	0.02	0.25	0.24
Dehydrated sweet potatoes	6.0	6.9	0.00	0.00	0.23	0.23
Broiler mash	5.4	6.6	0.03	0.07	0.26	0.27
Ground wheat	5.2	6.8	0.00	0.01	0.22	0.24
Mineral mix	7.7	7.2	0.01	0.00	0.23	0.23

effect on the action of the buffer. The data show that use of the buffer brings the suspensions near *p*H 7.

In Table 1 are also shown averages of duplicate determinations of urea and added urea in feeds as follows:

Column I: Urea nitrogen determined by A.O.A.C. method.
Column II: Urea nitrogen determined by A.O.A.C. method with addition of 2 ml of buffer.
Column III: 0.23% urea nitrogen added, A.O.A.C. method.
Column IV: 0.23% urea nitrogen added, A.O.A.C. method with addition of 2 ml buffer.

These data indicate no advantage in use of a buffer, as equally good results were obtained by both methods and recovery of added urea was satisfactory. There seems to be no reason for change in the A.O.A.C. method for determining urea.

NITROGEN IN AMMONIATED PRODUCTS

Determination of ammonia nitrogen, urea nitrogen, and total nitrogen on several ammoniated products is shown in Table 2.

TABLE 2.—*Determination of nitrogen in ammoniated products*

SAMPLE	AMMONIA NITROGEN	UREA AND AMMONIA NITROGEN	TOTAL NITROGEN
	per cent	*per cent*	*per cent*
Molasses	0.01	0.03	0.45
Ammoniated condensed distiller's molasses solubles	0.94	1.00	2.32
10% protein equivalent ammoniated molasses	0.22	0.23	1.68
16% protein equivalent ammoniated molasses	0.42	0.44	2.41
Molatein 15% protein equivalent	0.24	0.22	2.28
Ammoniated furfural residue	1.79	2.66	4.97

All the materials show a small amount of ammonia nitrogen and, except for the ammoniated furfural residue, no urea nitrogen. The latter shows more ammonia nitrogen, which would be expected because furfural is prepared by acid hydrolysis of pentosans and some salts would be formed with residual acid. Attempts to liberate ammonia by treatment with sulfuric acid and reducing agents gave results similar to those in Table 2. Apparently nitrogen is very firmly bound in these materials. Anhydrous ammonia will react with reducing sugars to form glycosylamines but attempts to isolate pure compounds were unsuccessful. It is possible that a number of different stable nitrogen compounds are formed as the

result of ammoniating molasses. It is very important that efforts be made to find a method for analyzing such materials.

KJELDAHL METHODS

In spite of the great amount of study which has been devoted to the Kjeldahl method for determining nitrogen there is still difference of opinion as to the most satisfactory manner of carrying out the procedure. Variables such as temperature, time of digestion, and catalyst may greatly influence the results obtained. Although mercury is generally believed to be the best catalyst, many analysts prefer to use copper. The official method calls for collection of evolved ammonia in standard acid; many laboratories use boric acid instead of standard acid in the receiver and are interested in gaining acceptance of this practice. Collaborative work this year consisted of comparing the effectiveness of mercury and copper as catalysts and of comparing use of standard acid and boric acid in the receiver.

Closely related to the problem of crude protein analysis is the determination of urea and ammoniated carbohydrates which are being used in increasing amounts in feeding stuffs. The necessity for distinguishing between true protein and these protein substitutes is obvious. Some exploratory work on this subject was carried out in the Associate Referee's laboratory and is described above.

Samples of poultry feed, cottonseed meal and nicotinamide (theoretical, 22.94% N; assay, 22.64% N) were submitted to nine collaborators for analysis in triplicate by the following three methods: I, *Official Methods of Analysis*, **2.24,** using copper as catalyst; II, **2.24,** using mercury as the catalyst; III, **2.24,** using mercury as catalyst and replacing the standard acid in the receiver with 25 ml of 4 per cent boric acid. All collaborators were instructed to use 18 grams of potassium sulfate and to use methyl purple as indicator.

Analysis was also made by a fourth method which sought to eliminate the need for sulfide or thiosulfate when mercury is used as the catalyst. Zinc will displace mercury from solution and thus permit satisfactory recovery of ammonia without the necessity of precipitating mercury in the usual manner. Some collaborators, however, obtained low results by this method and for this reason the data are not reported.

RESULTS AND DISCUSSION

The analytical data are summarized in Table 3. Collaborators are not identified and only the average results for each collaborator are given. The per cent nitrogen is reported instead of per cent protein because there is no advantage in magnifying differences by use of the protein conversion

TABLE 3.—*Collaborative results*[a] *for nitrogen in feeding stuffs*

COLLABORATOR	POULTRY FEED			COTTONSEED MEAL			NICOTINAMIDE		
	I	II	III	I	II	III	I	II	III
A	3.08	3.06	3.06	6.49	6.55	6.51	22.16	22.52	22.36
B	3.11	3.09	3.05	6.63	6.67	6.63	22.55	22.62	22.11
C	3.00	3.09	3.03	6.55	6.56	6.48	22.44	22.53	22.56
D	3.01	3.04	3.06	6.52	6.52	6.56	20.85	22.41	22.36
E	3.06	3.03	3.08	6.54	6.49	6.56	21.28	22.49	22.29
F	3.08	3.05	3.11	6.56	6.56	6.60	22.59	22.69	22.66
G	3.00	3.05	2.92	6.37	6.43	6.18	21.87	22.29	21.81
H	3.08	3.09	3.09	6.61	6.60	6.58	22.95	23.15	22.87
I	3.01	3.02	3.07	6.45	6.47	6.58	21.39	22.62	23.03
Average	3.05	3.06	3.05	6.52	6.54	6.52	22.00	22.58	22.44
s	0.045	0.029	0.057	0.091	0.084	0.154	0.681	0.254	0.729

[a] I = *Official Methods of Analysis*, **2.24**, copper catalyst.
II = Same, mercury catalyst.
III = Same, mercury catalyst, 25 ml 4% boric acid in receiver.

factor. No significant differences were found in the analysis of the poultry feed and cottonseed meal by the three methods. For nicotimanide, *t* values obtained by comparing the analyses obtained by the use of copper catalyst with the assay of the sample and with the results obtained with mercury catalyst show that use of copper leads to significantly lower values. Use of mercury catalyst and of boric acid in the receiver show no bias. One of the collaborators felt that a larger volume of boric acid should be used in the receiver to prevent possible loss of ammonia.

The writers have also found that the present official method for urea, **22.22,** gives satisfactory recovery of urea. Ammoniated carbohydrates are not decomposed by magnesium oxide or urease. At this time there is no method for distinguishing these materials from true protein.

RECOMMENDATIONS

It is recommended*—

(1) That the A.O.A.C. method **2.24** be modified by adding ". . . When the presence of refractory nitrogen compounds is suspected, use mercury as the catalyst."

(2) That work on catalysts for the Kjeldahl method be discontinued.

(3) That use of 50 ml of 4 per cent boric acid in the receiver be made an alternative procedure, first action.

(4) That in the interest of uniformity and simplicity, the Kjeldahl method **(2.22)** and the Gunning method **(2.23)** be dropped from the official methods.

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 61 (1954).

(5) That no further work on the determination of urea **(22.22)** be performed.

(6) That work on determination of ammoniated carbohydrates in feeding stuffs be initiated.

COLLABORATORS

Archie C. Wark, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Everard G. Baker, Mississippi State Chemical Laboratory, State College, Miss.

C. H. Perrin, Canada Packers, Ltd., Toronto, Canada.

Clyde L. Ogg, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa.

H. A. Davis, New Hampshire Agricultural Experiment Station, Durham, N. H.

Vesta F. Staab, New Hampshire Agricultural Experiment Station, Durham, N. H.

E. D. Schall, Department of Agricultural Chemistry, Purdue University, Lafayette, Ind.

Frank P. Yoon, Feed Laboratory, California Department of Agriculture.

Frances L. Bonner, Louisiana Agricultural Experiment Station, Baton Rouge, La.

REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Wash. 4, D. C., 1950, Par. **22.22.**

(2) GRIEM, W. B., private communication.

(3) PERKINS, A. T., MERRILL, J. F., and ROGERS, S. N., *This Journal*, **34**, 450 (1951).

REPORT ON MINERAL CONSTITUENTS OF MIXED FEEDS

COPPER DETERMINATION IN MINERAL FEEDS

By J. C. EDWARDS (Florida Department of Agriculture, Chemical Division, Tallahassee, Fla.), *Associate Referee*

This study of the determination of copper in mineral feeds follows the recommendation made by the Associate Referee last year (1). The colorimetric reagent te raethylene pentamine was used (2). The sample distributed by the Associate Referee was a trace-mineralized salt, and contained in addition to copper the approximate percentages of the following: P, 0.084; Co, 0.009; MnO, 0.181; Fe, 0.13; NaCl, 99.4. Twenty-five collaborators reported (Table 1). The results of each collaborator were averaged to derive a single value.

The sample contained a theoretical percentage of 0.033 per cent copper. The average of results closely approaches this, but the spread is a little

wider than desirable. It is believed that this spread would be narrowed considerably if two slight modifications of the method are applied: (*a*) Making the standards to a volume of approximately 50 ml with water before the addition of the tetraethylene-pentamine; and (*b*) filtering the standards before reading. These points were not made clear in the method supplied to collaborators.

It was felt that if the method delivered good results with this trace amount of copper, it would surely be adaptable to other mineral feeds which generally contain much more. Its facility suggests its use for control work, and the Secondary Plant Food Laboratory of the Fertilizer Section of the Florida State Laboratory has adopted it for the routine analysis of all copper samples.

TABLE 1.—*Results of collaborators*

COLLABORATOR NUMBER	PER CENT Cu		COLLABORTAOR NUMBER	PER CENT Cu	
	PROPOSED METHOD	OTHER METHODS		PROPOSED METHOD	OTHER METHODS
1	0.037		14	0.049	
2	0.024		15	0.025	
3	0.058		16	0.070	
4	0.025		17	0.033	
5	0.045		18	0.032	
6	0.037	0.039[a]	19	0.040	
7	0.015	0.027[b]	20	0.049	
8	0.034		21	0.033	
9	0.043		22	0.035	0.028[d]
10	0.045		23	0.045	0.043[e]
11	0.033	0.032[c]	24	0.045	
12	0.023		25	0.037[f]	
13	0.030		26	0.085[f]	

Average: 0.038% Cu.
Mean Deviation: 0.0095%
Standard Deviation: 0.012%

[a] Spectrograph. [b] A.O.A.C. 2.62. [c] *Anal. Chem.*, **24**, 371 (1952). [d] Carbamate. [e] Ammonium hydroxide. [f] Received too late to be included in the average.

COPPER DETERMINATION IN MINERAL FEEDS

REAGENTS

(a) *Tetraethylene pentamine.*
(b) *Copper sulfate.*—$CuSO_4 \cdot 5H_2O$; A.C.S.

STANDARDS

Dissolve 1.9645 g $CuSO_4 \cdot 5H_2O$ in H_2O and dil. to 500 ml; each ml is equivalent to 1 mg Cu. Use 1–10 ml of this soln in making up standards. Prepare standards in 100 ml Pyrex glass-stoppered volumetric flasks. Add 4 ml concd HCl and make to

vol. of ca 50 ml. Add 5 ml tetraethylene pentamine. Make to vol. with H_2O, stopper, and mix thoroly. Prepare a blank, using all reagents except Cu. Filter blank and standards before reading color.

DETERMINATION

Ash 8 g sample 2 hrs at 600°C.; transfer to 200 ml volumetric flask with 20 ml HCl and 50 ml H_2O. Boil 5 min., make to vol., and mix thoroly. Allow soln to settle. Aliquots may be taken from this soln for detn of Ca, P, Co, and Cu. Pipet 50 ml aliquot into 100 ml glass-stoppered volumetric flask and add 5 ml tetraethylene pentamine. Make to vol. with H_2O and mix thoroly. Filter soln before comparing with standards in colorimeter or spectrophotometer. Color readings should be made within 30 min. Compare color with standard Cu soln in colorimeter, using a red or No. 66 filter. A wavelength of 620 mμ is used with a spectrophotometer.

Report % Cu to third place to right of decimal (.000%).

COMMENTS OF COLLABORATORS

In order to avoid spattering and over-heating, many of the collaborators pointed out the necessity of diluting the blank and standard copper solutions before adding the amine. This precaution has now been incorporated into the procedure. Other collaborators spoke of the necessity of filtering standard and sample solutions before the colorimetric reading, and this precaution has also been added. Several mentioned the difficulty of handling the viscous amine and suggested that it be diluted before use. One collaborator expressed concern about the stability of the the amine reagent and stated that a fresh bottle should be used if some time had elapsed since the development of the standard curve. Another collaborator attempted purification of the reagent, but concluded that varying degrees of purity were not responsible for significant deviations in results. Still another complained of its fluorescent and turbid nature. Other collaborators expressed themselves as being entirely satisfied with the method, although one collaborator speculated about the possible interference of cobalt in certain types of mineral feed.

SUMMARY

There was some conflict in the statements of collaborators, but most considered the method favorably. This Associate Referee has been using the method for several months and finds it reliable. Checks are very good. The color is stable for at least two hours and the method is fast and simple. None of the elements encountered in ordinary mineral feeds have offered any serious interference.

Seventy per cent of the reported results fell within a standard deviation of 0.012 per cent. It is felt that this is as good as could be done with most of the methods now in use, and since the method is so much faster

and easier than others known to the writer, it is recommended that another study be made of the method with the slight modifications incorporated. It is believed that the method will prove adaptable to control work on a mass production basis, and results will come within an accepted tolerance for accuracy.

The Associate Referee wishes to thank the following collaborators for their interest and helpful comments. The order of listing of results has no bearing on the order of listing of the collaborators.

E. F. Budde, Research Laboratories, The Quaker Oats Co., Chicago, Ill.

Albert B. Heagy, and Cecil Pinkerton, Inspection and Regulatory Service, College Park, Md.

J. C. Edwards, Florida Department of Agriculture, Chemical Division, Tallahassee, Fla.

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Earl S. Packard, Agricultural Experiment Station, Orono, Me.

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John L. Clough, State Board of Agriculture, Dover, Del.

Harry J. Fisher, and Sherman Squires, Connecticut Agricultural Experiment Station, New Haven, Conn.

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Loyd L. Nesbitt, Lime Crest Research Laboratory, Limestone Products Corporation of America, Newton, N. J.

Glenn C. Mowery, Department of Agriculture, Nashville, Tenn.

Willis Richerson, Oklahoma State Board of Agriculture, Oklahoma City, Okla.

Marvin H. Snyder, and Mrs. Timmerman, Department of Agriculture, Charleston, W. Va.

REFERENCES

(1) EDWARDS, J. C., *This Journal*, **36**, 198 (1953).
(2) CRUMPLER, T. B., *Anal. Chem.*, **19**, 325 (1947).

REPORT ON CRUDE FAT IN BAKED DOG FOOD

By HAROLD H. HOFFMAN (Florida Department of Agriculture, Tallahassee, Fla.), *Associate Referee*

The collaborative study made in 1952 was continued with minor changes in the procedure. The method was published in *This Journal*, **37**, 98 (1954). Three dog foods, having different levels of fat and typical of commercial products, were used.

COLLABORATIVE WORK

Ground portions of three baked dog foods (AB, BB, and CB) and their respective premixes (AM, BM, and CM) were sent to eleven collaborators.[1] Analyses for moisture by **22.3** or **22.7** and for fat by **22.25** were requested on all samples. Analyses for fat by the proposed method were requested on the baked samples.

Average moisture values reported by each of nine collaborators appear in Table 1. These values were used to convert the averages of the fat values to a dry matter basis as shown in Table 2.

TABLE 1.—*Per cent moisture*

COLLABORATOR	TRIALS	AM	AB	BM	BB	CM	CB
1	3	11.67	8.10	10.40	6.43	9.40	5.07
2	3	11.95	6.36	10.20	6.20	8.79	4.76
3	3	11.63	6.67	9.98	6.54	9.01	5.39
4	1	11.1	6.5	10.1	6.4	8.7	4.9
5	1	11.14	6.01	9.78	6.07	8.37	4.61
6	3	12.01	6.12	10.31	6.05	8.44	4.77
7	3	11.50	6.80	10.13	6.57	8.33	5.50
8	3	11.9	6.4	10.5	6.3	9.2	4.9
9	2	10.93	5.84	9.56	5.83	8.30	4.47

Collaborators 2 and 9 used Röhrig in place of Mojonnier tubes.

Collaborators 4 and 7 also made triplicate determinations on the

[1] Grateful acknowledgment is made to Mr. E. F. Budde, The Quaker Oats Research Laboratories, for preparing and distributing samples.

TABLE 2.—*Per cent crude fat (dry basis)*

COLLABORATOR	TRIALS	AM PREMIX	AB BAKED		BM PREMIX	BB BAKED		CM PREMIX	CB BAKED	
		22.25	22.25	PROPOSED	22.25	22.25	PROPOSED	22.25	22.25	PROPOSED
1	3	3.25	1.45	4.24	4.43	1.71	5.59	5.63	3.55	6.82
2	3	3.01	1.52	4.92	4.33	1.55	5.54	5.72	3.45	6.80
3	3	3.27	1.76	4.10	4.08	1.83	5.62	5.32	3.65	6.82
4	3[a]	3.33	1.60	3.87	4.51	1.78	5.31	5.87	3.79	6.80
5	2	3.42	1.33	4.57	4.59	1.44	5.62	5.77	3.57	6.90
6	3	3.70	1.22	2.95	5.42	1.75	4.34	6.53	3.74	6.29
7	3	2.71	1.18	3.25	3.93	1.42	5.00	5.38	3.21	5.96
8	4	3.61	1.60	3.31	4.73	1.76	4.43	5.95	3.76	5.76
9	3[b]	3.39	1.35	3.88	4.52	1.54	4.72	5.76	3.51	5.89
Average		3.30	1.45	3.90	4.50	1.64	5.13	5.77	3.58	6.45
Standard Deviation		0.30	0.19	0.64	0.42	0.16	0.52	0.35	0.18	0.47
Coefficient of Variation, %		9.1	13.1	16.4	9.3	9.8	10.1	6.1	5.0	7.3

[a] Except single trials on AB, BB, and CB by 22.25.
[b] Except dual trials on all samples by 22.25.

premixes by the proposed method. The average values for AM, BM, and CM, respectively, after conversion to dry basis, were as follows: For Collaborator 4, 4.30, 5.48, and 6.97 per cent; for Collaborator 7, 2.71, 5.01, and 5.53 per cent.

Collaborator 5 subtracted a reagent blank of 0.70 per cent from fat obtained by the proposed method. No other collaborator referred to the existence of a significance blank.

DISCUSSION

Assuming the fat found in the premixes to be the true fat in the corresponding baked samples, method **22.25** permitted only 47 per cent recovery after baking while the proposed method gave 115 per cent recovery. The lower-numbered collaborators tended to find more fat by the proposed method in the baked samples than did other collaborators. This was particularly noticeable in Sample CB.

Collaborator 6 submitted the following comments:

"Moisture by **22.3** and fat by **22.25** were easily reproducible. The same was not true of the proposed method. The percentage of fat found, using the proposed method, was about twice the amount found by using **22.25**. The results by the proposed method also were not reproducible with any reasonable accuracy. The acid digestion of the proposed method appeared to render part of the starches soluble in the ether. Thus the starches passed through the cotton and paper filters about as readily as the fats. The procedure was easy to carry out and no problem of settling out was experienced as in using the Röhrig apparatus in the previous tests."

RECOMMENDATION

It is recommended*—

That the method herein described be adopted, first action, and replace the procedure recommended last year.

COLLABORATORS

Grateful acknowledgment is made to the following collaborators (not listed in the same order as in the tables):

E. F. Budde, The Quaker Oats Research Laboratories, Chicago, Ill.

R. B. Carson, and I. Hoffman, Canada Department of Agriculture, Ottawa, Ontario, Canada.

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Leo J. Faneuf, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

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Arthur L. Haskins, Frear Laboratories, State College, Pa.

Willis Richerson, Oklahoma Department of Agriculture, Oklahoma City, Okla.

* For report of Subcommittee A, and action of the Association, see *This Journal*, 37, 61 (1954).

E. D. Schall, Indiana Agricultural Experiment Station, Lafayette, Ind.
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REPORT ON MILK BY-PRODUCTS IN MIXED FEEDS

DETERMINATION OF TOTAL SOLIDS

By ARA O. CALL (Western Condensing Company, Appleton, Wis.),
Associate Referee

The 1953 collaborative study was a continuation of that carried on last year (1). The study was, however, limited to a comparison of the official method for solids (2) with a modification using zinc oxide as a neutralizing agent. Two typical, commercial samples were used.

DESCRIPTION OF METHODS

The following instructions were sent to all collaborators:

Method A.—(A.O.A.C. **15.14**) "Weigh 2.5–3 g prepd sample, **15.2**, into weighed flat bottom dish not less than 5 cm in diam., using ca 5 g and Pt dish if ash is to be detd on same portion. Heat on steam bath 10–15 min., exposing max. surface of dish bottom to live steam; then heat 3 hrs in air oven at 98–100°. Cool in desiccator, weigh quickly, and report % residue as total solids."

Note: The above sample weight is for milk. Inasmuch as the test samples are considerably higher in solids it is suggested that ca 1 g be used and that sufficient distilled water (ca 5 ml) be added to distribute the sample evenly on the bottom of the dish.

Method B.—Same as Method A except that ca 2 g of zinc oxide, A.C.S. (freshly ignited or oven dried) is to be added to each sample dish before weighing and the calculation is modified to take into account the moisture formed in the neutralization of any acid present by the zinc oxide. E.g.:

$$ZnO + 2\ R{-}COOH \rightarrow (R{-}COO)_2Zn + H_2O.$$

Titrate the acidity of the sample and calculate as lactic acid. For each two moles of lactic acid, one mole of water will be lost.

$$\text{Mol. wt. lactic acid} = 90;\ (2 \times 90 = 180)$$
$$\text{Mol. wt. water} = 18;\ 18/180 = 0.1.$$

Thus for each gram of acid (as lactic) in the weighed sample, add 0.1 g for lost water. Sample calculation:

$$\text{Net wt. "as is" sample} = 1.0382$$
$$\text{Net wt. dried sample} = .3746$$

4.5 g of sample required 22.0 ml 0.1 *N* NaOH for neutralization; then $22.0 \times 0.090 \times 0.1 \times 100 \div 4.5 = 4.4\%$ acidity, as lactic.

To correct for water lost in neutralization:

$$1.0382 \times 0.1 \times 0.044 = 0.0046$$
$$0.3746 + 0.0046 = 0.3792$$
$$\frac{0.3792}{1.0382} \times 100 = 36.52\% \text{ solids (corrected)}$$

The neutralization of high acid milk by-products for solids determination is not new. Harrisson reported the use of zinc oxide for this purpose as early as 1934 (3); the American Butter Institute recommends its use in the determination of total solids in condensed buttermilk (4); Bergman as Associate Referee reported its use in a collaborative study (5); Call and Van Poucke reported the use of zinc oxide and sodium hydroxide for the neutralization of samples (6); and the British workers Muers and Murphy (7) also recommended neutralization. The latter, comparing several neutralizing agents, chose calcium carbonate. However, they state: "Zinc oxide was an equally effective reagent under these conditions, but calcium carbonate was finally chosen chiefly because it could be obtained easily in a pure and very finely divided form as precipitated chalk."

In general, the results of this collaborative study are in good agreement with last year's study. Table 1 shows the average values reported by the fourteen collaborators. It is interesting to note there was an improvement in the precision this year as shown by lower standard deviation values. As in all previous work, the modification employing neutralization resulted in significantly higher solids values than those obtained with the official method which employs no neutralization. Values obtained by the

TABLE 1.—*Total solids in condensed milk by-product feeds—average values reported by collaborators (1953)*

COLLABORATOR	SAMPLE 1		SAMPLE 2	
	METHOD A	METHOD B	METHOD A	METHOD B
	per cent	*per cent*	*per cent*	*per cent*
1	41.58	42.04	59.18	60.03
2	38.81	40.44	57.19	58.47
3	41.17	42.51	57.11	59.04
4	42.02	42.26	58.57	58.81
5	43.02	43.58	58.44	60.39
6	41.19	41.59	58.19	57.89
7	40.91	42.08	57.17	58.75
8	43.17	43.29	58.23	59.08
9	41.18	41.71	58.05	58.44
10	41.99	41.83	58.82	58.27
11	41.48	41.94	57.77	59.18
12	41.18	43.24	57.19	59.96
13	41.26	42.50	57.78	59.16
14	41.05	43.19	57.63	59.02
Mean	41.43	42.40	57.95	59.04
Standard Deviation	0.275	0.336	0.324	0.234
95% Confidence limits of the mean	±.327	±.399	±.385	±.278

official method were only 97.7 and 98.2 per cent as great as those obtained by the neutralization modification for samples 1 and 2, respectively.

Two collaborators also analyzed the samples by the Mojonnier method. Their average results are shown in Table 2.

TABLE 2.—*Results by Mojonnier method*

	WITHOUT NEUTRALIZATION		WITH NEUTRALIZATION	
	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2
	per cent	*per cent*	*per cent*	*per cent*
Collaborator 3	42.09	58.61	42.28	59.16
Collaborator 12	41.35	58.73		

The rather wide variation in the ability of the various collaborators to replicate their own results is somewhat disheartening. Some probable explanations for this variation may be: (*a*) The drying out of the samples due to exposure to the air may not be kept to a uniform minimum; (*b*) it has been observed in the Referee's laboratory that a sample size giving a dried residue of from 0.3 to 0.6 gram is most desirable. Despite the instruction that approximately 1 gram samples should be used, it was noted that the sample size varied from 0.67 to 2.40 grams; (*c*) it has also been observed that many of the drying ovens in use not only may lack thermostat sensitivity, but often the temperature in various parts of the oven may vary widely.

SUMMARY

As a sequel to last year's collaborative study, two commercial condensed milk by-product feeds were used for comparing the official total solids method **(15.14)** with a modification employing zinc oxide as a neutralizing agent. The results show: (*a*) The use of zinc oxide as a neutralizing agent in the total solids determination gives significantly higher results. This is in agreement with earlier work; (*b*) the precision of the zinc oxide modification was slightly, but not significantly, greater than the official method; (*c*) there was a wide variation in the ability of the fourteen collaborators to replicate their own results.

RECOMMENDATIONS

The Associate Referee recommends*—

(1) That under "Total Solids" (Dairy Products, **15.14–15.15**, the following be added: "*Method III (Neutralization with Zinc Oxide for High*

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 61 (1954).

Acid Products). Add ca 2 g of ZnO, A.C.S. (freshly ignited or oven dried) to flat-bottom dish not less than 5 cm in diam. Weigh. Add ca 1 g sample and weigh quickly. Add ca 5 ml distd H_2O and distribute sample evenly on bottom of dish. Heat on steam bath, exposing max. surface of dish bottom to live steam, until apparently dry. Heat at 98–100° in an air oven 3 hrs or until constant in wt. Cool in desiccator, then weigh quickly. Calc. wt. of residue. Titrate the acidity of the sample and calc. as lactic acid (**15.4**). To compensate for the moisture formed when the acid is neutralized by ZnO, add 0.1 g to the residue wt for each g of acid (as lactic) in the weighed sample. Report % residue (corrected) as total solids."

(2) That further collaborative work on the total solids determination of Milk By-Products in Mixed Feeds be discontinued.

COLLABORATORS

The Associate Referee wishes to thank the following collaborators:

Hugh M. Boggs, Food and Drug Administration, Department of Health, Education and Welfare, Philadelphia, Pa.

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W. S. Thompson, and D. M. Stalter, Ohio Dept. of Agriculture, Section of Feeds and Fertilizers, Columbus, Ohio.

REFERENCES

(1) CALL, ARA O., *This Journal*, **36**, 213 (1953).

(2) *Official Methods of Analysis*, 7th ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950, p. 231.

(3) HARRISSON, J. W. E., *This Journal*, **18**, 645 (1935).

(4) Laboratory Manual, *Methods of Analysis for the Butter Industry*, American Butter Institute, 110 N. Franklin, Chicago 6, Ill.

(5) BERGMAN, R. E., *This Journal*, **30**, 613 (1947).

(6) CALL, ARA O., and VAN POUCKE, R. F., *ibid.*, 35, 785 (1952).
(7) MUERS, M. M., and MURPHY, F. E., XIIth International Dairy Congress, p. 872 (1949).

REPORT ON DRUGS IN FEEDS

ASSAY OF ENHEPTIN ® AND ARSANILIC ACID

By RICHARD T. MERWIN (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

In accordance with last year's recommendation, the Associate Referee re-submitted the modified method for Enheptin ® (1) to collaborative study and, during the year, developed a new method for *p*-arsanilic acid which was studied collaboratively. He also undertook original research on amino acid coupling interference in certain types of feed assays. It was hoped that a new method for nitrophenide would be included, and one was actually developed, but it proved unsuitable on some feeds because of the amino acid interference.

No change was made in the modified method for Enheptin (1). Enheptin (2-amino-5-nitrothiazole) is easily extracted from the feed with acetone. When reduced with sodium hydrosulfite, it loses its characteristic yellow color. The difference in the spectrophotometric absorbance between the reduced and the unreduced compound is used as a basis of measurement of concentration. It was expected that the slight re-wording of the method to emphasize use of a wide-mouthed 50 ml volumetric extraction flask and the use of freshly-prepared phosphate buffer solution of *p*H 9.0 would yield better results.

The method for *p*-arsanilic acid is short and simple. The drug is dissolved in citric acid solution, the feed protein is removed with hydrochloric acid, an aliquot of the filtrate is diazotized and coupled with N-1-naphthylethylenediamine dihydrochloride, and the color complex is read spectrophotometrically at 545 mμ. Because critical technic is not required, the Associate Referee believed that collaborators would have no difficulty with the method.

METHOD FOR ENHEPTIN

REAGENTS

(a) *Ammonium chloride soln.*—5% aq. soln.

(b) *Boric acid buffer, pH* 9.0.—Prep. the following solns: **(1)** 6.203 g boric acid and 7.456 g KCl made up to 500 ml with H_2O. **(2)** 0.2 *M* soln of NaOH. Take 50.0 ml of **(1)** and 21.40 ml of **(2)** and make up to 200 ml with H_2O.

(c) *Sodium hydrosulfite soln.*—Prep. 1% soln of Na hydrosulfite in boric acid buffer soln **(b)** and use not later than 5 min. after prepn.

DETERMINATION

Transfer 2 g ground feed to a 50 ml wide-mouthed volumetric flask, add 10 ml acetone and let stand 2 min., swirling occasionally. Make to vol. with H_2O, mix, and filter immediately thru *coarse* paper. Transfer 25 ml aliquot to 50 ml volumetric flask, add 15 ml of NH_4Cl soln and mix. Make to vol. with H_2O, mix, and filter thru Whatman No. 42 paper (or equivalent), discarding first 10 ml of filtrate.

Place a 4 ml aliquot in each of 2 small beakers. To the first, add 0.5 ml of the freshly prepd 1% Na hydrosulfite soln in boric acid buffer. Make both volumes to 10 ml and read immediately on a spectrophotometer against H_2O at 388.5 mμ. Subtract the reading of the reduced soln from that of the unreduced and compare resulting absorbance to standard curve.

Prep. standard soln by dissolving 100 mg recrystallized 2-amino-5-nitrothiazole in 100 ml acetone and make to vol. of 1 l with H_2O. Transfer aliquots of 4, 8, 12, 16, and 20 ml to 100 ml volumetric flasks and dil. to vol. with H_2O. Treat 5 ml aliquots of each diln as in assay procedure, reading the absorbance of the unreduced soln against the reduced soln as a blank, obtaining readings at 20, 40, 60, 80, and 100 mmg concn.

METHOD FOR *P*-ARSANILIC ACID

(Applicable in absence of drugs such as sulfonamides)

REAGENTS

(a) *Citric acid soln.*—2.00% aq. soln.

(b) *Sodium nitrite soln.*—Freshly prepd 0.10% aq. soln.

(c) *Ammonium sulfamate soln.*—0.50% aq. soln.

(d) *N-1-naphthylethylenediamine dihydrochloride soln.*—0.10% aq. soln. Store in dark bottle.

DETERMINATION

Transfer 4 g ground feed to 200 ml volumetric flask, add 40 ml of citric acid soln, and let stand 10 min., swirling occasionally. Add 80 ml of H_2O, then 20 ml of HCl (1+1), mix well, and make to vol. with H_2O. Shake thoroly and filter thru Whatman #42 paper (or equivalent), discarding first few ml if turbid.

To two 50 ml beakers add 5 ml of filtrate and 1 ml of $NaNO_2$ soln. Mix and let stand 5 min. Add 1 ml of ammonium sulfamate soln and let stand 2 min. Then add to the first beaker only, 1 ml of coupling reagent (d), mix, and wait 10 min. before making both solns to vol. of 15 ml. Read both against H_2O blank, at 538 mμ in spectrophotometer, subtracting absorbance of feed blank from sample absorbance. Refer to standard graph for concn of drug.

To prep. standard, transfer 0.100 g pure *p*-arsanilic acid to 100 ml volumetric flask, add 0.5 g anhyd. Na_2CO_3 and 20 ml H_2O, and dissolve. Make to vol. with H_2O. Transfer 10 ml to 100 ml volumetric flask and make to vol. with H_2O. Aliquot 2 ml of second soln to 100 ml volumetric flask and add H_2O to mark. Treat aliquots of 2, 4, 6, and 8 ml in 50 ml beakers with 1 ml of 1+1 HCl, proceeding as in assay, making to final vol. of 15 ml after coupling, and reading at 538 mμ in spectrophotometer, using H_2O blank. Plot absorbance points representing concns of 4, 8, 12 and 16 mmg *p*-arsanilic acid.

Two samples of feed, with vials of drugs from the same lots used to prepare the samples, were sent to each collaborator. Unknown to them, the samples contained: (*a*) 0.100 per cent Enheptin ; and (*b*) 0.0100 per cent *p*-arsanilic acid and 0.0125 per cent nitrophenide. The bottles were identified by their drug labels only, omitting mention of nitrophenide.

Copies of the methods were sent to each collaborator with requests to report four results on each feed.

Nitrophenide was incorporated in the *p*-arsanilic acid sample, unknown to any collaborator, to prove that it did not interfere in the analysis of *p*-arsanilic acid. In last year's study of the nitrophenide method it was proved that *p*-arsanilic acid interfered when using Lederle Method No. 1806 for nitrophenide (1). Complex drug mixtures in feeds marketed during the past two years, which incorporate anti-coccidials and organic arsenicals, call for extensive research in developing methods and a more meticulous analytical technic.

Collaborators' results on Enheptin are given in Table 1, and those on *p*-arsanilic acid are given in Table 3.

Because the assay of medicated feeds is a new field of analysis, new factors that influence results are being evaluated as their effects are revealed by research. Among them is drug deterioration. For some time it has been known that certain drugs are not stable when mixed with feeds.

TABLE 1.—*Assays of feed containing 0.100 per cent Enheptin* ®

COLLABORATOR	ENHEPTIN FOUND	STANDARD DEVIATION	COLLABORATOR	ENHEPTIN FOUND	STANDARD DEVIATION
	per cent			*per cent*	
1	.062, .065. .060, .062; *av.* = *.062*	.001%	7	.106, .095, .105, .107, *av.* = *.103*	.006%
2	.084, .090, .085, .085, .081, .095, .085, .097, .087, .086. .085; *av.* = *.087*	.005%	8	.125, .098, .102, .094, 120, .110; *av.* = *.108*	.012%
			9	.080, .081, .081, .080; *av.* = *.081*	.001%
3	.102, .102, .090, .091; *av.* = *.096*	.007%	10	.082, .075, .030, .030, .040, .040; *av.* = *.050*	.023%
4	.093, .092, .092, .094; *av.* = *.093*	.001%	11	.093, .097, .093, .097; *av.* = *.095*	.002%
5	.075, .078, .074, .074, .076 *av.* = *.075*	.002%	12	.088, .091, .080, .083; *av.* = *.085*	.005%
6	.085, .089, .091, .089; *av.* = *.089*	.003%	13	.093, .083, .090, .086; *av.* = *.088*	.005%

Averages of all, omitting Nos. 1 and 10, = .091 per cent
Standard deviation of laboratory averages from the group mean, omitting Nos. 1 and 10, = .009 per cent

Storage studies have revealed that with present methods, sulfaquinoxaline and nitrophenide are not fully recoverable from old feeds. To this list must now be added Enheptin. Extensive research on methods for sulfaquinoxaline and nitrophenide within the past two years has not resulted in development of methods that will recover 100 per cent of the originally added quantities of these drugs from old feeds.

In the current study, one test lot of Enheptin indicated a loss of 35.5 per cent of the drug over a 13.5 month period when it was assayed by the present collaborative method. The 1952 collaborative sample showed an average recovery of 96 per cent when it was assayed by the Associate Referee in June, 1952 (1). In July, 1953, the same feed yielded an average of only 62 per cent, as shown in Table 2. Each annual group of figures was checked against concurrent standards. The feed had been stored in a gallon jar with metal screw cap, without precautions as to over-exposure to air and light.

TABLE 2.—*Loss of Enheptin ® in 0.100% feed*

DATE PREPARED	DATE OF ASSAY	AMOUNT RECOVERED, AVERAGE OF 4
		per cent
6/2/52	6/2/52	0.096
	7/15/53	0.062 (35.5% loss)
4/6/53	4/6/53	0.098
	7/15/53	0.089 (9.2% loss)

Table 2 also indicates the apparent percentage loss of Enheptin in the collaborative sample. The loss is approximately 9 per cent in three months. Because most of the collaborators assayed the feed from two to three months after its preparation, due allowance for loss from decomposition should be taken into consideration in interpreting results.

Thus, since a 3 months delay in analyzing the sample might produce a loss of 9 per cent of the drug, the average of .091 per cent for the results of all except two of the collaborators indicates that the method determines 100 per cent of the Enheptin in the feed (Table 1). The collaborators' average is only .002 per cent higher than .089 per cent, the amount found present experimentally after 3 months.

In general, the Enheptin method shows very good reproducibility. The standard deviation from their own means for all except two collaborators ranges from .001 to .007 per cent. The standard deviation of the laboratory averages from the group mean, for all except two, is .009 per cent. The standard deviation from the group mean calculated from last year's

figures (1) for the same Enheptin method is .010. The group average last year was .086 per cent on a .100 per cent feed. The fact that the drug deteriorates was not then known and the group average was probably close to the actual percentage of Enheptin.

The troubles experienced by a few of the collaborators, evidenced by their wide deviations from the general average, must be attributed to technic. It should be emphasized that the first filtration of the acetone-water solution of feed should take place rapidly. For this purpose, a coarse filter paper is essential, preferably one with a crêpe surface. Slow filtration causes some slight paper absorption of the drug. The method is a meticulous one but it is capable of good precision and a high degree of accuracy.

COMMENTS OF COLLABORATORS

Enheptin Method

No. 1.—The recrystallized drug "seemed to have a potency 10 to 15 per cent lower than that sent by Lederle Laboratory a couple of years ago."

(It became known to the Associate Referee, only after his collaborative reports were received, that the pure drug also deteriorates.)

No. 3.—Larger samples (10 grams) and a longer acetone standing period—"at least one hour"—were suggested.

(Larger samples were tried on the collaborative feed with the same results. Enheptin is soluble in acetone on contact. No. 3 also found that adding 1 to 2 mg of sodium hydrosulfite to the cell for reduction of the drug worked well. He observed that too much or too little gave erratic results. The amount is critical and this modification must be applied with caution.)

No. 4.—"In routine assays, 2.5 grams of feed is extracted with 75 ml of acetone and diluted to 100 ml with water. By this method the laboratory obtained a value of 0.106 compared to .0926 per cent as given in the report."

(The lower volume of acetone in the collaborative method was obtained experimentally and proved to be sufficient to maintain solubility. Quantities from 5 to 25 ml were tried. It was noticed that with quantities larger than 10 ml, more turbidity resulted in the final aliquot unless the excess acetone was evaporated. The present extraction procedure was devised to maintain a low but constant acetone-water relationship. Blanks are also lower because less feed color appears in the filtrate.

The high value of .106 per cent is against the trend of diminishing recovery with age.)

No. 5.—"The Enheptin extract still appeared cloudy after discarding the first 10 ml of filtrate."

(Slight turbidities occur with older feeds but they do not affect the accuracy of the method. In general, however, the fresher the feed, the lower the turbidity.)

No. 6.—"Three drops of 'Foamex' were added after the water and acetone were mixed," to good advantage. Solutions proved less cloudy. "The method specified use of a 4 ml aliquot, but we assumed, according to our calculations, that 5 ml was meant."

(The 4 ml aliquot on a .100 per cent feed places the absorbance reading on a more accurately readable portion of the Beckman Model DU scale.)

No. 9.—"Checks are nice and the results agree with those obtained by our old method." (2).

Due to lack of uniformity of the sample, results with the *p*-arsanilic acid method, where stability of the drug is not a factor, show only fair reproducibility. Variations cannot be attributed to incomplete solubility; if this were so, the average of all results would be less than theoretical. The procedure used to dissolve the drug has proved very effective, even at much higher levels of concentration. Experimentally, 100 milligrams was thus dissolved—500 times the amount present in the 2 grams of collaborative feed. The procedure should be followed in the order specified; the sequence was devised to minimize acid hydrolysis of the feed protein.

During development of the method, standard drug solutions added to blank feeds consistently yielded ±2 per cent of the theoretical .0100 per cent. The collaborators' general average of .0102 per cent in comparison with the theoretical amount is considered excellent. Seven of the individual averages are satisfactorily close to the known amount of drug. A perfect mixture of drug and feed would have better demonstrated the high degree of accuracy and excellent reproducibility of the method.

In practice, uniform distribution of minute amounts of drug is difficult to achieve when varying particle size is a factor. The author has been

TABLE 3.—*Assays of feed containing 0.0100 per cent p-arsanilic acid*

COLLABORATOR	p-ARSANILIC ACID FOUND	STANDARD DEVIATION	COLLABORATOR	p-ARSANILIC ACID FOUND	STANDARD DEVIATION
1	*per cent* .0084, .0094, .0124, .0126, .0091, .0094, .0106, .0118 *av.* = *.0105*	.0016	7	*per cent* .0092, .0099, .0094, .0100; *av.* = *.0096*	.0004
2	.0098, .0125, .0089, .0114, .0101, .0100, .0094, .0097, .0099, .0098; *av.* = *.0101*	.0010	8	.0132, .0128, .0110, .0120, .0088, .0104, .0124, .0098; *av.* = *.0113*	.0016
			9	.0098, .0097, .0099, .0097; *av.* = *.0098*	.0001
3	.0015, .0014, .0088, .0123; *av.* = *.0110*	.0015	10	.0085, .0087; *av.* = *.0086*	.0001
4	.0097, .0109, .0106, .0100; *av.* = *.0103*	.0005	11	.0118, .0116, .0123, .0126; *av.* = *.0121*	.0005
5	.0090, .0074, .0076, .0108, .0098; *av.* = *.0089*	.0014	12	.0015, .0015, .0016, .0016; *av.* = *.0016*	.0001
6	.0105, .0096, .0108, .0112; *av.* = *.0105*	.0007	13	.0082, .0093, .0100, .0095; *av.* = *.0093*	.0008

Averages of all, omitting No. 12, = .0102 per cent.
Standard deviation of laboratory averages from the group mean, omitting No. 12 = .0010 per cent.

informed that Lederle Laboratories has each lot of nitrophenide micronized before mixing with soya bean meal to form its commercial premix. The *p*-arsanilic acid used in the collaborative sample was Eastman Kodak Company's No. 1369. It proved to be of varying particle size and was not micronized before mixing with feed. The Associate Referee regrets this oversight.

To check for non-uniformity of mixture, 4 gram samples were substituted for the 2 grams called for in the method. A 200 ml volumetric flask was used and double the quantity of solutions were employed. The results of this comparison of sample size are shown in Table 4. Much better reproducibility was obtained, although accuracy remained the same.

TABLE 4.—*Effect of sample size on recovery of p-arsanilic acid (.0100 per cent added)*

SAMPLE SIZE	2 GRAMS	4 GRAMS
p-Arsanilic acid found, %	.0115 .0082 .0105 .0090	.0102 .0102 .0092 .0096
Average	.0098	.0098
Standard Deviation	.0015	.0005

Because non-micronized *p*-arsanilic acid is undoubtedly being mixed with commercial feeds, with resulting non-uniformity, cognizance of this fact is acknowledged with the following necessary alterations of the method:[1]

Transfer 4 g of ground feed to 200 ml volumetric flask, add 40 ml citric acid soln and let stand 10 min., swirling occasionally. Add 80 ml H_2O, then 20 ml HCl (1+1), mix well, and make to vol. with H_2O. Shake thoroly and filter thru Whatman #42 paper (or equivalent), discarding first few ml if turbid.

The *p*-arsanilic acid method is applicable only in the absence of other drugs such as sulfaquinoxaline and similar sulfonamides. The sulfa drugs, since they are readily diazotized and coupled, must first be separated from *p*-arsanilic acid. Methods for such drug mixtures are being considered. The collaborators have proved that nitrophenide does not interfere.

COMMENTS OF COLLABORATORS

p-Arsanilic Acid Method

No. 1.—"Because of wide variation in results, uniformity of the sample was questioned."

[1] *This Journal*, 37, 106 (1954).

No. 2.—"Variability of results is apparently due to extraction procedure."

No. 6.—"The method was found to be clear, concise, and easy to follow."

No. 8.—"Slight turbidity in the filtrates may have caused the wide spread in results."

(The method usually produces very clear filtrates. Slight turbidities have no detectable effect on results.)

No. 9.—"The figures check with our own method." (3). "Your method, however, is much simpler and faster and gives very low blanks, no doubt because the extraction is carried out in the cold. We are using this new modification routinely now and like it very well."

No. 10.—The method is "very acceptable." "The standard solution does not keep and should be prepared fresh each day."

(It is an axiom that fresh solutions should always be used in preparing standards. In checking stability, the Associate Referee obtained good reproducibility of curves from a month-old standard stock solution. After 3 months, the same solution showed a 20 per cent loss.)

AMINO ACID COUPLING INTERFERENCE

In an attempt to develop a short method for nitrophenide (m,m'-dinitrodiphenyl-disulfide) the Associate Referee tried various reductions of the drug with zinc dust in 60 ml of 1.33 per cent NaOH solution. (Reduction of the drug to two amino groups is necessary for diazotization and coupling to form a suitable color for spectrophotometric measurement.) Alkaline solutions of the medicated feed were kept in a boiling water bath for forty minutes, made acid with 10 ml of HCl (1+1), cooled, brought to a volume of 100 ml, and filtered.

Excellent reproducibility of varying quantities of drug was thus obtained by readings of coupled solutions at 545 mμ.

It was noticed, however, that standards had to be prepared in the presence of the same stock feed. Standards run without feeds exhibited much lower absorbance readings. It was concluded that something in the feed, and in quantity varying from feed to feed, was being diazotized and coupled to produce, at times, a substantial amount of color.

It was decided to run blanks on feeds and on common feed components. The components chosen were those present in the A.O.A.C. basal rachitic chick ration, namely, yellow corn, wheat middlings, and casein, as well as wheat red dog flour. Two typical unmedicated commercial feeds, a growing ration and a laying mash, were also chosen. All were treated by alkaline hydrolysis of the feed protein for forty minutes. The resultant coupling produced moderate to considerable depths of color. The colors were slow to develop, but became quite pronounced within ten minutes. After thirty minutes, their absorbances were much higher. The colors showed maximum absorption at 545 mμ and were of the same purplish-red shade as the coupled nitrophenide.

L-Tryptophane.—Since mixtures of amino acids result from both alkaline and acid hydrolysis of proteins, a study of the naturally occurring

amino acids suggested that tryptophane (β-indole-α-amino-propionic acid) might be the interference. Nitrous acid treatment of the α-amino group adjacent to the carboxyl group, through deamination, would form a hydroxy acid. Nitrous acid treatment of the secondary amino group in the pyrrole nucleus, however, might lead to diazotization. This reasoning proved correct. Tryptophane coupled readily with N-1-naphthylethylenediamine in acid solution showed a maximum absorption at 545 mμ, and produced the same color as coupled nitrophenide.

To rule out the possibility of chance, dl-tryptophane, as well as several lots of l-tryptophane, isolated and purified from natural sources, were also similarly coupled. Tryptophane coupling was verified by Mr. W. J. Mader, Analytical Research Department, Chemical Control Division, Merck & Co., Rahway, N. J., who kindly offered co-operation when the possibility of amino acid interference was suggested. Mr. Mader also reported that investigation of 18 other common amino acids resulted in no coupling.

In reviewing the literature, the Associate Referee found that Eckert (4), using N-1-naphthylethylenediamine as reagent, had published a colorimetric method for tryptophane. After studying hydrolyzates of albumin and gelatin, he concluded that only this one of the common amino acids would couple.

Because the development of color seemed to be a function of time, readings of coupled tryptophane (5 mg per 25 ml) were taken at intervals of one minute for thirty minutes. The absorbance after seven minutes from the time of adding the reagent was .060; at ten minutes, .066; at fifteen minutes, .075; and at thirty minutes, .089. The plateau of constant absorbance was not determined, but is believed to be a matter of hours. Evidence was obtained that a *p*H lower than .95 gave lower readings and that more $NaNO_2$ for diazotization slightly increased the absorbance.

In the light of this study, methods for sulfaquinoxaline and nitrophenide must be re-appraised. Both methods may cause partial hydrolysis of feed protein, whether alkaline or acidic. Proof has been obtained that the boiling water bath treatment of blank feeds in mildly alkaline or mildly acid solution can produce tryptophane coupling. Types of feeds with higher than normal tryptophane content account for higher than theoretical drug recovery. Prolonged water bath treatment increases the error. One control feed of .0120 per cent sulfaquinoxaline, prepared by adding a standard solution to wheat middlings, gave a recovery of .0140 per cent with the A.O.A.C. official method. This is an exaggerated case, because wheat middlings prove to be the richest source of tryptophane of the feed materials examined.

During the quest for causes of error in the analysis of sulfaquinoxaline and nitrophenide feeds (aside from their deterioration with storage), measurements of tryptophane interference revealed interesting facts.

When quantities of 5 mg of tryptophane, 25 mmg of sulfaquinoxaline, and 5 mg of tryptophane plus 25 mmg of sulfaquinoxaline were coupled under exactly controlled conditions, theoretical readings for the drug and the mixture were obtained seven minutes after adding the last reagent. At ten minutes, the absorbance of the drug solution was unchanged, but the density of the mixture increased to indicate 26 mmg of sulfaquinoxaline. During the next thirty minutes, the absorbance of the drug solution remained constant, but the other solutions kept increasing in absorbance. A comparison of the absorbance readings obtained is given in Table 5.

Attempts to evaluate sulfaquinoxaline by subtracting a corresponding tryptophane absorbance at any period beyond seven minutes resulted in low values. However, it was found that a valid correction could be made if the difference between the tryptophane readings at seven minutes and at thirty minutes was subtracted from the absorbance reading of the mixture at thirty minutes. At any time beyond the seven minute reading, similar calculations were also valid.

TABLE 5.—*Effect of tryptophane coupling on absorbance*

TIME (MIN.)	SULFAQUINOXALINE (25 mmg)	TRYPTOPHANE (5 mg)	MIXTURE (25 mmg+5 mg)
7	.178	.060	.178
8	.177	.064	.180
9	.177	.065	.184
10	.177	.066	.185
12	.177	.067	.186
14	.177	.073	.191
15	.177	.075	.190
24	.177	.085	.202
25	.177	.086	.203
30	.177	.089	.206

It is unlikely that such serious interferences will be experienced in routine medicated feed analysis if precautions are taken to avoid prolonged water bath treatment. An indication of tryptophane interference will be apparent if absorbance readings increase slowly beyond the ten-minute period usually specified for the color development.

In developing the *p*-arsanilic acid method, some compensating factors were observed, even though a slight tryptophane coupling took place in tests on the blank feed used to prepare the collaborative sample. In the coupling reaction, competition between tryptophane and *p*-arsanilic acid in complexing with the N-1-naphthylethylenediamine seems to take place. It appears that coupling of the later is accomplished so rapidly that tryptophane coupling is suppressed or prevented when the amount is small, as it is in the cold-extraction process of the *p*-arsanilic acid method.

The Associate Referee intends to continue studies of tryptophane interference, to re-investigate the sulfaquinoxaline method, especially with respect to the possibility of higher recovery from old feeds, to continue work on development of a short nitrophenide method, to improve methods for mixed drug feeds, and to apply the Cassil-Wichmann procedure (5) to the assay of arsenical feeds.

ACKNOWLEDGMENTS

Appreciation of the helpful cooperation of the following collaborators is acknowledged. Their names appear in the order in which their work is tabulated in the reports:

Harold H. Hoffman, Feed Laboratory, State Department of Agriculture, Tallahassee, Fla.

W. J. Mader, Analytical Research Dept., Chemical Control Div., Merck & Co., Rahway, N. J.

Sidney Williams, Department of Health, Education, and Welfare, Food and Drug Administration, Boston, Mass.

John Reid, Wirthmore Research Laboratory, Malden, Mass.

Roland W. Gilbert, University of Rhode Island, Agricultural Experiment Station, Kingston, R. I.

Charles Weber, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

W. R. Flach, Eastern States Farmers' Exchange, Buffalo, N. Y.

C. A. Luhman, State Department of Agriculture, Sacramento, Calif.

Sigmund W. Senn, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

Charles V. Marshall, Department of Agriculture, Ottawa, Ontario, Canada.

James N. Turner, The Park & Pollard Co., Buffalo, N. Y.

Earl S. Packard, University of Maine, Agricultural Experiment Station, Orono, Me.

Mrs. Janice Lewis, Connecticut Agricultural Experiment Station, New Haven, Conn.

RECOMMENDATIONS

It is recommended*—

(1) That the Enheptin ® method be adopted, first action.

(2) That the method for *p*-arsanilic acid be adopted as altered, first action.

(3) That studies of methods for mixed drug feeds be undertaken.

(4) That studies of methods for nitrophenide be continued.

REFERENCES

(1) MERWIN, R. T., *This Journal*, **36**, 219 (1953).

(2) *Method for Assaying Feeds for Enheptin* ®, Lederle Laboratories Div., American Cyanamid Co., New York, N. Y.

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 60 (1954).

(3) *Analysis of Poultry Feeds for Arsanilic Acid and Nitrophenide*, Lederle Laboratories Div., American Cyanamid Co., New York, N. Y.
(4) ECKERT, H. W., *J. Biol. Chem.*, **148**, 205 (1943).
(5) CASSIL, C. C., and WICHMANN, H. J., *This Journal*, **22**, 436 (1939).

No reports were given on ash in feeding stuffs, fat in fish meal, or microscopic examination.

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MACINTIRE (University of Tennessee Agricultural Experiment Station, Knoxville 16, Tenn.), *Referee*

During the past year, the Associate Referees have conducted studies upon the determination of exchangeable hydrogen, replaceable potassium, hydrogen-ion concentration, molybdenum, and fluorine. In conformity with their suggestions and recommendations, it is recommended*—

(1) That the exchangeable hydrogen values indicated by the calcium-acetate official procedure be checked further against liming experiments on different types of soil exposed to natural weather conditions and be analyzed for calcium sorptions and *p*H values.

(2) That the use of the flame photometer be further studied as a means for the determination of replaceable potassium.

(3) That studies on soil *p*H be continued.

(4) That the colorimetric determination of molybdenum be studied by the Associate Referee and that the collaborators be requested to make determinations by means of the methods used in their respective laboratories and by the procedure reported recently by Will and Yoe, *Anal. Chem.*, **25**, 1363 (1953).

(5) That a proposed "automatic" titration device, or devices, be made the objective of collaborative study of the determination of fluorine.

(6) That studies on the "combination dithizone–spectrographic method" and on the polarographic procedure for the determination of zinc in soils be continued.

(7) That the study of the determination of copper in soils be continued.

(8) That the utilization of carmin as an indicator in the determination of the boron content of soils be studied further, and that *p*-nitrobenzene-azo-1,8-dihydroxynaphthalene-3,6-disulfonic acid, or "chromotrope-B," be studied as a suitable reagent for that determination.

(9) That the survey and comparison of methods for the determination of phosphorus **(a)** fraction in "available" state, and **(b)** proportion of or-

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1954).

ganic–inorganic forms therein (*This Journal*, **30**, 43 (1947)), be continued.

(10) That the Associate Referee on exchangeable calcium and magnesium continue his work.

REPORT ON HYDROGEN ION CONCENTRATION OF SOILS

By LANNES E. DAVIS (Dept. of Soils, University of California, Davis, Calif.), *Associate Referee*

Recent theoretical and experimental studies of certain aspects of the electrochemical behavior of ion exchange materials by Jenny and coworkers (2) has cast some doubt upon the significance of electrometric measurements of *p*H in colloidal materials. By inference, this doubt is extended to soils.

Jenny, *et al.*, found evidence that the salt bridge error may be of considerable importance in studies with ion exchange resins and soil clays under certain conditions. A preliminary report to the Association was made by Davis (1) in 1950.

It would appear that if an appreciable salt bridge error is present in normal soils, the measured *p*H vaues would be sensitively affected by moderate variations in the concentration of KCl in the salt bridge. Experimental studies by Davis and Rible (3) have demonstrated that there was no significant effect of variation in the KCl concentration between saturation and 1/64 of saturation. The work was done with 28 California soils, varying in *p*H between 5.57 and 8.05. It is concluded tentatively that the salt bridge error is probably of no greater significance in the case of soils than in that of other colloids, and undoubtedly of less significance than in the case of ion exchange resins.

RECOMMENDATIONS*

It is recommended that, for the present, studies of soil *p*H be continued without regard to recent theoretical difficulties. Particular attention may well be given to consideration of correlation between soil *p*H measurements and the behavior of crop plants.

REFERENCES

(1) DAVIS, L. E., *This Journal*, **34**, 588 (1951).

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1954).

(2) JENNY, H., *et al.*, *Science*, **112**, 164 (1950).
(3) DAVIS, L. E., and RIBLE, J. M., *This Journal*, **36**, 1146 (1953).

REPORT ON EXCHANGEABLE HYDROGEN IN SOILS

*p*H VALUES RELATED TO DEGREE OF CALCIUM SATURATION UNDER VARYING CONDITIONS

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville 16, Tenn.), *Associate Referee*

The work of the Associate Referee during the past year was directed towards the establishment of a clearer relationship between soils limed according to exchangeable hydrogen content (calcium-acetate procedure) and their *p*H values that were determined under various conditions.

In the 1952 report (14) the calcium ($CaCO_3$) requirements, as indicated by the calcium-acetate procedure, were tested through the reaction of soils with finely divided calcium carbonate in a six-hour wet digestion on the steam bath. The resultant *p*H values at calcium sorptions corresponding to exchangeable hydrogen values were from 6.6 to 7.1, which were considered as corroborating the calcium-acetate procedure for the exchangeable hydrogen determination. Because many of the investigations of soil buffer curves (4–8, 10, 11) were carried out on soil suspensions ranging in ratio from 1:2.5 to 1:10, it was considered of interest to compare certain characteristics of such curves with those obtained by the soil–$CaCO_3$ steam bath reaction. The $Ca(OH)_2$–CO_2–air equilibration procedure proposed by Jensen (8) appeared most desirable for such study, because it has been employed by a number of investigators and has found special application in the determination of lime requirement (4, 6, 8, 10, 15).

The points of particular emphasis in this report will be the determined *p*H values at levels of replacement of the exchangeable hydrogen content, and the apparent calcium requirement indications at *p*H 7 in the equilibrated suspensions, in relation to previous findings from soil–$CaCO_3$ reactions (14).

EXPERIMENTAL

For the study of the calcium sorption–air equilibration and *p*H relationship, the soils selected were chiefly those used in the previous experiment on soil reactivities with $CaCO_3$ (14). These soils represent extreme ranges of chemical, physical, and mineralogical composition. Multiple charges of each soil were weighed and placed in 125 ml fat-extraction flasks. The

weight taken for the different soils varied from 1 to 10 grams, according to the sorption capacity of the particular soil and the capacity of the flask to accommodate the required volume of $Ca(OH)_2$ solution. To each soil were added several different portions of a $Ca(OH)_2$ stock solution that was measured through an attached buret and calculated to supply the near-equivalent of its determined exchangeable hydrogen content and to provide equilibrium *p*H values of near 7. Where the $Ca(OH)_2$ required was less than 50 ml, sufficient water was added to make to that volume. The maximal volume of $Ca(OH)_2$ accommodated in the container was 75 ml. The flasks were fitted with inlet and outlet tubes for the required bubbling of CO_2 and air through the soil suspensions, and were placed on a Ross-Kershaw type shaker and connected in series. Equilibration was attained through successive circulations of (*a*) air, one hour; (*b*) CO_2 gas, thirty minutes; and (*c*) water-washed outside air, sixteen hours. The air circulation was obtained by means of an electric vacuum pump. The rate of flow of the air and of CO_2 gas was adjusted to one liter per minute. Immediately after the air equilibration, the *p*H values of the soil suspensions were determined by means of the glass electrode, using either the battery or the line model *p*H meter. In the case of the line model, the electrodes were dipped directly into the suspension; for *p*H measurements with the battery model, however, a small portion of the suspension was withdrawn for that purpose into a small beaker.

For the carbonate analysis, which is often necessary to check completeness of carbonate reaction, the soil supensions were evaporated to dryness on the steam bath and the analysis was carried out according to the steam distillation procedure (12). The metal cation contents of the soil were determined by the titration procedure given in *Official Methods of Analysis* (1, **3.34**). The organic matter contents were determined by the Walkley-Black spontaneous heat procedure (16).

*p*H VALUES OF AIR-EQUILIBRATED SOIL SUSPENSIONS

The *p*H values of 19 soils after equilibration with four different quantities of $Ca(OH)_2$ are given in the middle columns of Table 1. The *p*H values corresponding to points of complete exchangeable hydrogen replacements, as indicated by the calcium-acetate procedure (13), were obtained by interpolation and are given in the second column from the right of the same table. The calcium sorptions corresponding to *p*H 7 of the equilibrated suspensions were obtained by interpolation and are given in the right hand column of Table 1. The *p*H of the equilibrated suspensions at exchange neutrality ranged from 6.6 to 7.8. With the exception of the highly organic soils, the soil suspensions indicated a *p*H of about 7.6, and in all cases the values were about 0.6 above those obtained with the same soil at exchangeable hydrogen neutralization obtained through reaction

TABLE 1.—*Reaction values of $Ca(OH)_2$–air equilibrated soil suspensions with interpolations of pH values at levels of exchangeable hydrogen replacement, and of calcium requirement for pH 7*

SOILS	ORGANIC MATTER	EXCHANGE CATION CONTENT			MEQ. $Ca(OH)_2$ ADDED PER 100 G, AND pH AFTER EQUILIBRATION				pH AT EXCH. H REPLACEMENT	Ca REQUIRED FOR pH 7
		METAL	H	CAPACITY						
	per cent	*meq.*	*meq.*	*meq.*						*meq.*
Hartsells sandy loam, 1950 sample	6.76	1.6	11.8	13.4	9 *6.7*	10 *7.0*	12 *7.3*	14 *7.6*	*7.3*	10.0
Hartsells sandy loam, 1953 sample	4.31	1.4	8.1	9.5		5 *7.0*	8 *7.5*	10 *7.8*	*7.5*	5.0
Baxter silt loam, L 319	2.06	2.0	4.4	6.4	2 *6.9*	3 *7.2*	4 *7.5*	5 *7.7*	*7.6*	2.5
Bolton silt loam, 1940	2.14	7.7	4.4	12.1	2 *7.2*	3 *7.5*	4 *7.7*	5 *7.9*	*7.8*	2.0
Claiborne silt loam, 1941	3.35	5.3	5.6	10.9	3 *7.2*	4 *7.5*	5 *7.6*	6 *7.7*	*7.7*	2.5
Dickson silt loam, 1940	1.86	1.6	4.2	5.8	2 *6.7*	3 *7.3*	4 *7.6*	5 *7.8*	*7.6*	2.5
Fullerton silt loam, 1941	2.40	2.2	3.8	6.0	2 *7.2*	3 *7.5*	4 *7.7*	5 *7.9*	*7.7*	1.5
Montevallo silt loam, 1940	2.28	2.8	5.1	7.9	3 *7.0*	4 *7.2*	5 *7.4*	6 *7.6*	*7.4*	3.0
Sequoia silt loam, 1941	2.48	3.7	5.4	9.1	3 *7.1*	4 *7.3*	5 *7.5*	6 *7.7*	*7.6*	2.7
Tellico sandy loam, 1940	2.62	6.4	6.1	12.5	4 *7.2*	5 *7.5*	6 *7.6*	7 *7.8*	*7.6*	3.5
Cumberland clay subsoil, 1950	.17	.9	7.0	7.9	3 *5.6*	5 *6.0*	7 *6.3*	9 *7.4*	*6.3*	8.0
Leonardtown silt loam	3.02	3.1	4.7	7.8	2 *7.1*	3 *7.3*	4 *7.5*	5 *7.7*	*7.6*	1.8
Norfolk sandy loam, N.C.	1.69	.8	3.4	4.2	1.4 *6.7*	2 *7.3*	3 *7.6*	4 *7.9*	*7.7*	1.7
Susquehanna clay subsoil, Alabama	.10	2.5	27.5	30.0	15 *5.5*	20 *5.9*	25 *6.8*	27.5 *7.8*	*7.8*	26.5
Talladega clay loam, Georgia	3.90	1.4	8.4	9.8	5 *6.7*	6 *7.1*	8 *7.4*	10 *7.6*	*7.5*	5.8
Volusia silt loam, N. Y.	3.57	7.3	6.2	13.5	4 *7.2*	5 *7.5*	6 *7.6*	7 *7.8*	*7.6*	3.5
Wooster silt loam, Ohio	1.67	2.4	6.0	8.4	4 *7.0*	5 *7.5*	6 *7.7*	7 *7.8*	*7.7*	4.0
Portsmouth muck, Florida	28.0	2.6	60.8	63.4	20 *5.5*	40 *6.3*	60 *6.8*	70 *7.9*	*6.6*	62.0
Peat	89.0	19.0	128.0	147.0	48 *5.0*	78 *5.7*	99 *6.2*	140 *7.5*	*7.0*	128.0

with $CaCO_3$ on the steam bath (14). If the *p*H of the equilibrated suspensions are taken as true reactions, the calcium requirements indicated by the calcium-acetate procedure would prove excessive in the great majority of the soils tested. The same conclusion could be drawn more directly through a comparison of the calcium required for *p*H 7 in the equilibrated suspension (right-hand column, Table 1) with corresponding values of the determined exchangeable hydrogen of the same soils (fourth column from left of same table). The calcium required for *p*H 7 in the equilibrated suspensions is one-half to two-thirds the exchangeable hydrogen

indicated by the calcium-acetate procedure. The question arises as to the cause of such divergence in the indicated calcium requirement for *p*H 7, and as to which of these indications should be taken as more nearly representing the real *p*H of the soil under average conditions in field or greenhouse.

The most plausible explanation of these divergences lies in the well-known dilution effect upon *p*H. It was shown by Bradfield (3) that the 8-fold dilution of colloidal clay induced a change of *p*H from 4.02 to 4.30. A particularly impressive feature in Bradfield's experiment is that the dilution has an accelerative effect in raising the *p*H value; thus, upon an additional eight-fold dilution, the clay colloid had risen from *p*H 4.3 to 6.26. Since the colloid concentration in a given ratio of soil to solution will vary from soil to soil, it can be expected that the dilution effect will vary also with the type of soil. Furthermore, Keaton (9) has shown that increase in *p*H with dilution is greater with soils of above 50 per cent base saturation than with those of lower degrees of base saturation. The effect of dilution may be increased through air drying, which may induce decreases in *p*H of field soils ranging from 0.02 to 0.63 units, according to Baver (2).

As to the choice between the two procedures (the $Ca(OH)_2$–CO_2–air equilibration titration and the calcium-acetate hydrogen replacement) for indicating the calcium requirement, there are plausible reasons for accepting the indication by the calcium-acetate procedure as representing more nearly the effective liming needs of a soil in attaining a condition of approximate neutrality. The inadequacies of the titration curves, including the $Ca(OH)_2$–air equilibration, are attested by the universal use of "liming factors" (4, 7, 11). In the assignment of such factors, some investigators have made allowances for the slow reactivity of the coarser grades of limestone, for losses through leaching, and for plant uptake. Such allowances are quite proper considerations in liming practice, but should be considered as items apart from the prior and less-involved considerations of chemical procedure that indicate the most effective lime requirement for soil neutralization under field or greenhouse conditions.

Upon the basis of *p*H results on 80 soils that received inputs of precipitated $CaCO_3$ and were set aside in the greenhouse for four months, Pierre and Worley (11) found that their $Ca(OH)_2$ titration procedure required an average liming factor of 1.5. Davis (6) showed that a Hammond silt loam that had been air-equilibrated after additions of varying quantities of $Ca(OH)_2$ had attained *p*H values of 0.6 to 1.0 *p*H units lower than the corresponding values of the equilibrated suspensions after twelve months' air-drying storage.

The Associate Referee has found that a Hartsells sandy loam of rather high organic matter content (6.76 per cent), which has been limed with 12,000 pounds of 35–40 mesh limestone and exposed one year to outdoor

uncropped lysimeter leaching, has decomposed 95 per cent of the carbonate input. The losses of bases (Ca, Mg, and K) through leaching were 1,400 $CaCO_3$ equivalent. The net gain by the soil was $0.95 \times 12{,}000 - 1{,}400$, or 10,000 pounds $CaCO_3$ equivalent. That, plus an initial base content of 1,200 pounds gives a total base content of 11,200 pounds or 86 per cent base saturation. The *p*H value of the exposed soil was 6.5. On the curve of *p*H values plotted against reaction with $CaCO_3$ on the steam bath, the same soil at 85 per cent calcium saturation indicated a *p*H of 6.3 (14, p. 235). In *p*H measurements of soils, such a difference may be considered as not very significant. Although it would be desirable to have observations on a number of different types of soils that have been similarly exposed in lysimeters under controlled conditions, it is believed that the cumulative evidence indicates that the calcium-acetate procedure for exchangeable hydrogen gives a correct indication of the lime needs for soil neutrality.

REACTION VALUES OF SOILS AFTER DEHYDRATION

It has been shown that at equal degrees of calcium saturation, those soils that were equilibrated ($Ca(OH)_2$–CO_2–air) in suspension showed *p*H values 0.6 to 0.8 units higher than the soils that were reacted with $CaCO_3$ on the steam bath. Determinations of the *p*H values of the dehydrated calcium-soils were made after the soils were wetted to a paste, stirred, and allowed to stand one hour. Several investigators (8, 15, 17) have shown that the CO_2 partial pressure of the ambient atmosphere has a considerable effect upon the *p*H values of soil suspensions. It was thought that longer exposing of the dehydrated calcium-soils for equilibration with the CO_2 of the atmosphere might alter their *p*H values to the extent that they would approximate the values registered by the equilibrated suspensions. Accordingly, five soils were equilibrated with varying quantities of $Ca(OH)_2$ and the *p*H first determined in suspension. The suspensions then were evaporated on the steam bath, the residues were wetted to pasty consistency, and in the original reaction flask, outdoor air was circulated over the pastes for eighteen hours. The *p*H values were then re-determined.

The results (Table 2) indicate that the prolonged equilibration of the soil paste with the CO_2 of the atmosphere failed to alter the observed lower *p*H values of the dehydrated calcium-soils. The only exception to the general trend was found in the behavior of the Cumberland clay subsoil which yielded practically identical *p*H values in the 1:5 and in the 1:1 suspensions. It is possible that in this instance the clay concentration was so high that the higher dilution did not greatly affect the *p*H value.

From these results it may be concluded that the dehydrated soil suspensions, after reaction with $Ca(OH)_2$ and air equilibration or after reac-

tion with $CaCO_3$, can be depended upon to give proper *p*H values when wetted to a paste and exposed to outdoor atmosphere at least one hour before *p*H determination.

TABLE 2.—*Calcium sorptions and pH values of soils equilibrated with $Ca(OH)_2$-CO_2-outdoor air*

SOIL	$Ca(OH)_2$ (.0405 *N*) ADDED		$CaCO_3$ AFTER EQUILIBRATION		CALCIUM SORPTION PER 100 g.	*p*H OF SOILS	
	PER EXPERIMENT	PER 100 g	PER EXPERIMENT	PER 100 g		IN EQUILIBRATED SUSPENSION	IN PASTE
	ml	*meq.*	*grams*	*meq.*	*meq.*		
Hartsells fine sandy	0	0	——	——	——	5.2	4.8
loam,	12.5	5.1	.0000	.0	5.1	7.0	5.8
1953 sample	20.0	8.1	.0005	.1	8.0	7.5	6.5
10 gram	25.0	10.1	.0000	.0	10.1	7.7	6.9
	37.5	15.2	.0040	.8	14.4	8.4	7.7
	50.0	20.2	.0220	4.4	15.8	8.4	7.8
	62.5	25.3	.0420	8.4	16.9	8.4	7.8
Cumberland clay	0	0	——	——	——	5.0	4.7
subsoil,	7.5	3.0	.0000	.0	3.0	5.6	5.1
1950 sample	12.5	5.1	.0005	.1	5.0	6.0	5.9
10 gram	17.5	7.1	.0000	.0	7.1	6.3	6.3
	22.5	9.1	.0000	.0	9.1	7.4	7.3
	27.5	11.1	.0015	.3	10.8	7.5	7.7
	32.5	13.2	.0050	1.0	12.2	7.9	7.9
Susquehanna clay	0	0	——	——	——	5.0	4.1
subsoil,	18.8	15.2	.0000	.0	15.2	5.5	4.7
5 gram	25.0	20.2	.0005	.1	20.1	5.9	5.0
	31.3	25.4	.0000	.0	25.4	6.8	6.4
	33.8	27.4	.0000	.0	27.4	7.8	6.8
	37.5	30.4	.0010	.4	30.0	8.1	7.3
	40.0	32.4	.0030	1.2	31.2	8.2	7.4
Portsmouth muck	0	0	——	——	——	4.5	3.7
2 gram	10.0	20.2	.0005	.5	19.7	5.5	4.9
	20.0	40.5	.0005	.5	40.0	6.3	5.8
	30.0	60.8	.0010	1.0	59.8	6.8	6.7
	35.0	70.9	.0015	1.5	69.4	7.9	7.1
	40.0	81.0	.0030	3.0	78.0	8.4	7.4
	43.0	87.8	.0055	5.5	82.3	8.5	7.8
Peat	0	0	——	——	——	4.1	3.4
1 gram	12.5	50.6	.0015	3.0	47.6	5.0	4.4
	20.0	81.0	.0015	3.0	78.0	5.7	5.0
	25.0	101.2	.0010	2.0	99.2	6.2	5.7
	35.0	141.8	.0010	2.0	139.8	7.5	6.2
	45.0	182.2	.0030	6.0	176.2	8.1	7.0
	52.0	210.6	.0045	9.0	201.6	8.4	7.7

SUMMARY

Because of the wide use of titration or buffer curves in conjunction with studies on lime requirement and exchangeable hydrogen determinations, it was deemed pertinent to investigate the relationship between

the findings by one of these procedures with the indications by the calcium-acetate procedure on a number of different soils.

Determinations of *p*H values of 19 different soils that had been equilibrated with varying quantities of $Ca(OH)_2$, CO_2, and air have shown that at various levels of exchangeable hydrogen replacement, most soils in 1:5 suspensions showed *p*H values of 7.5 to 7.8, whereas the *p*H values of pastes, after reaction with $CaCO_3$ and dehydration, were 6.6 to 7.1. At *p*H 7, the equilibrated suspensions indicated a calcium requirement of one-third to one-half that indicated by the calcium-acetate procedure.

The universal employment of "lime factors" in connection with titration curves is taken as proof that these procedures fail to give proper indication of the lime necessary to produce neutrality. On the other hand, a soil limed according to its exchangeable hydrogen indication and exposed one year to lysimeter leaching gave a *p*H value which checked with the laboratory-determined value based on reaction with $CaCO_3$ of corresponding degree of base saturation.

RECOMMENDATIONS*

It is recommended that the exchangeable hydrogen values indicated by the official calcium-acetate procedure be checked further against liming experiments on different types of soils exposed to natural weather conditions and analyzed for calcium sorption and *p*H values.

ACKNOWLEDGMENT

Mr. Brooks Robinson analyzed the soils for organic matter and Miss Claire Veal determined their cation content.

REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Wash. 4, D. C., 1950.
(2) BAVER, L. D., *J. Am. Soc. Agron.*, **23**, 587 (1931).
(3) BRADFIELD, R., *J. Phys. Chem.*, **28**, 170 (1924).
(4) CHRISTENSEN, H. R., and JENSEN, S. T., *Trans. Second Com. Int. Soc. Soil Science, A*, 94 (1926).
(5) CROWTER, E. M., *J. Agr. Science*, **15**, 201 (1925).
(6) DAVIS, F. L., *Soil Sci.*, **59**, 175 (1945).
(7) DUNN, L. E., *ibid.*, **56**, 341 (1943).
(8) JENSEN, S. T., *Intern. Mitt. Bodenk.*, **14**, 112 (1924).
(9) KEATON, C. M., *Soil Sci.*, **46**, 259 (1938).
(10) NAFTEL, J. A., *J. Am. Soc. Agron.*, **28**, 609 (1936).
(11) PIERRE, W. H., and WORLEY, S. L., *Soil Sci.*, **26**, 363 (1928).
(12) SHAW, W. M., and MACINTIRE, W. H., *This Journal*, **26**, 357 (1943).

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1954).

(13) ———, *ibid.*, **35**, 597 (1952).
(14) ———, *ibid.*, **36**, 230 (1953).
(15) SIMMONS, C. F., *J. Am. Soc. Agron.*, **31**, 638 (1939).
(16) WALKLEY, A., *J. Agri. Science*, **25**, 598 (1935).
(17) WHITNEY, R. S., and GARDNER, R., *Soil Sci.*, **55**, 127 (1943).

REPORT ON THE DETERMINATION OF FLUORINE IN SOILS

By L. J. HARDIN (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

In previous collaborative studies of the determination of fluorine in soils, results showed that the direct double distillation procedure gives results higher than those obtained from perchloric acid distillation of the charge after incineration with calcium oxide fixative. A limited comparison of results obtained by the double distillation procedure with those obtained after alkali fusion of the charge prior to distillation indicated no advantage from the preliminary fusion (1). Additional collaborative studies of the titration step, in which the same soil distillate was titrated with thorium nitrate, showed excellent agreement in the values reported by the majority of the collaborators, but with notable exceptions among a few. This led to the conclusion that failure of two or more analysts to obtain comparable results could be due to the titration step rather than to the incomplete distillation of the fluoride from the sample (2).

No additional work on the method has been done by the Associate Referee this year. However, a large number of identical soil samples, of various types and fluorine content, have been analyzed by means of the double distillation procedure in two laboratories. Results for soils of three groups, based on fluorine content, are shown in Table 1.

TABLE 1.—*Comparison of averaged results on three soil groups*

	GROUP I		GROUP II		GROUP III	
	NO. OF SAMPLES	AV. FLUORINE	NO. OF SAMPLES	AV. SAMPLES	NO. OF SAMPLES	AV. FLUORINE
		(p.p.m.)		(p.p.m.)		(p.p.m.)
Lab. 1	34	1273	95	2633	66	981
Lab. 2	34	1326	95	2758	66	972

The average of the results indicates good over-all agreement, and individual values also were usually in the range of tolerance.

In the 1952 recommendations of the Associate Referee (2) reference was made to the possible advent of an "automatic" titration device.

When this or some similar instrument or technic is available at several laboratories, it is recommended that its use and adaptation be the objective of collaborative study.*

REFERENCES

(1) HARDIN, L. J., *This Journal*, **35**, 621 (1952).
(2) ———, *ibid.*, **36**, 237 (1953).

REPORT ON MOLYBDENUM IN SOILS

By W. O. ROBINSON (Research Branch, Plant Industry Station, U. S. Dept. of Agriculture, Beltsville, Md.), *Associate Referee*

There is a real need for a reliable method of analysis for molybdenum in soils and plants. In very small quantities (of the order of one part per million or less) it is now considered an essential element for plant growth. Smaller quantities are essential for the growth of nitrogen-fixing bacteria. However, 5–10 p.p.m. of molybdenum in pasturage, an amount only slightly larger than is required for plant growth, is decidedly toxic to ruminants. Serious molybdenum toxicity to cattle from pasturage has been reported in several states in this country, notably Florida and California.

It is possible for a skilled operator to obtain reliable analytical results for molybdenum in soils and plants by following the general thiocyanate colorimetric method of Hillebrand and Lundell (1) or that of Sandell (3). Analysts, however, apparently have trouble with the method. Analytical chemists outside the United States had reported as high as 69 p.p.m. molybdenum in one lot of arable soils, and up to 73 p.p.m. in another lot. Our results were 1 p.p.m. or less for the first lot and up to 18.6 p.p.m. for the second lot. The latter soils were especially difficult, for they contained relatively great concentrations of unusual trace elements, some of which might interfere with the molybdenum determination.

Early this year, four soil samples were sent to several collaborators for molybdenum determinations. So far, only one has reported results, which came in very late. It was learned indirectly that another collaborator obtained results too variable to report. Evidently the thiocyanate colorimetric method as outlined by the Associate Referee is not developed well enough for collaborative trials on soils.

Piper and Beckwith (2) describe a method for determining small amounts of molybdenum such as those which occur in plants and soils. This method has the advantage of determining copper at the same time.

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1954).

The reagent for this colorimetric method—dithiol-(4-methyl-1,2-dimercaptobenzene)—is now obtainable in gram lots in sealed glass tubes. It appears that the dithiol method is more sensitive than the thiocyanate method.

The spectrographic method should be considered. It is an excellent independent check on the chemical method. The Atomic Energy Commission has recently developed a new procedure for the determination of molybdenum, the neutron activation method, which is sensitive to one part in one hundred million.

It is known that rather extensive work on the determination of molybdenum in soils and plants is being done at several experiment stations and universities. It is hoped that their interest as collaborators can be enlisted. If so, it is recommended* that they employ the method now in use in their laboratories and describe it in detail. In this way it is hoped that one or perhaps two methods may be selected for further trials.

REFERENCES

(1) HILLEBRAND, W. F., and LUNDELL, G. E. F., *Applied Inorganic Analysis with Special Reference to the Analysis of Metals, Minerals, and Rocks*, John Wiley and Sons, Inc., New York, 1929.
(2) PIPER, C. S., and BECKWITH, R. S., *J. Soc. Chem. Ind.*, **67**, 374 (1948).
(3) SANDELL, E. B., *Colorimetric Determination of Traces of Metals*, Interscience Publishers, New York, 1944.

No reports were given on boron, exchangeable calcium and magnesium, exchangeable potassium, phosphorus, or zinc and copper.

REPORT ON SUGARS AND SUGAR PRODUCTS

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

At the 1953 meeting, reports by five Associate Referees have been presented. The Referee concurs in the recommendations contained therein.

In addition to the material covered by the reports of the Associate Referees, attention is called to matters of a general nature relating to the official methods of the Association.

Refractive Indices of Sucrose Solutions.—At the 1952 meeting of the Association, the Associate Referee on Densimetric and Refractometric Methods reported that measurements of the refractive indices of sucrose

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1954).

solutions in the range 60 to 70 per cent had been made at the New York Sugar Trade Laboratory; subsequently, a similar series was made at the National Bureau of Standards.

The results obtained at the two laboratories indicate possible errors in the official refractive index table, **41.7** (1), in this range, as well as the need for further measurements. The details of this work constitute the report of the subcommittee on Subject 7 of the U. S. National Committee on Sugar Analysis, March 1953, given by the Chairman, James Martin, and published in the proceedings (2) of that organization.

In the total of 28 concentrations measured, the observed indices were higher than the corresponding values in the existing table in 26 cases and lower in 2 cases; the mean was about 2 units higher in the fourth decimal place of the index.

Direct Polarization of Raw Sugars.—The official method for the determination of the direct polarization of raw sugars, **29.17** (3), directs that clarification be effected by the addition of dry basic lead acetate after the solution is made to volume. This method is in agreement with the provisions adopted by the International Commission for Uniform Methods of Sugar Analysis, 9th session, London, 1936. Since dry lead clarification was not then universally accepted the subject was included in the agenda of the 10th session of the Commission in 1949, and proved to be one of the most controversial subjects at that meeting. As a result, no action was taken.

In view of the importance of this test in trade transactions involving raw sugars, the subject will be included in the agenda for the 1954 meeting of the International Commission. Recently, Gaskin and Hands (4) reported the results of their investigation of wet and dry lead clarification of raw sugars; they recommend the mean of the polarization by wet and dry lead as the most satisfactory value.

These authors reported the effect of reagents of different basicities on the polarization of a Cuban raw sugar. The effect due to the basicity of the lead reagent is eliminated in the official method through the adoption of a basic lead acetate of specified composition, **29.18(c)** (5).

The amount of lead required for clarification is largely a matter of the judgment of the analyst. Experienced operators are skilled in estimating the minimum amount for satisfactory defecation. Although an excess is to be avoided, it is less serious with the lead solution than with dry lead. In the latter case, the volume increase caused by the excess may introduce significant errors.

It is the experience of the Referee that the amount of lead necessary for the clarification of present-day Cuban raws seldom exceeds 1 ml of the solution, or 0.33 g of dry lead.

The effect of different amounts of dry lead on the polarization is shown in the results obtained on twelve samples of Cuban raws, each defecated

with 0.3, 0.6, and 1.2 g of dry lead (Horne's). The average polarizations obtained were 96.52°*S*, 96.44°*S*, and 96.33°*S*, respectively. The decrease in polarization with increasing amounts of dry lead is roughly proportional to the increase in volume due to the solution of the dry reagent in the sugar solution; 1 gram of dry lead dissolved in 100 ml of the normal solution of Cuban raw sugar increases the volume by 0.204 ml (experimental average).

The effect of different amounts of lead solution on the polarization of raw sugars is shown by the following experiments. In each experiment a series of twelve or more Cuban raws were polarized in duplicate. In each case the duplicate solutions were identical except for the amount of the lead solution used in the defecation. The average polarizations of each series are given in Table 1.

TABLE 1.—*Effect of different amounts of lead solution on the polarization of raw sugars*

EXPERIMENT NO.	AMT. Pb SOLN USED	AV. POLARIZATION	DIFFERENCE
	ml	°*S*	
1	1	96.40	+.02
	2	96.42	
2	1	96.50	−.01
	3	96.49	
3	2	96.45	+.04
	4	96.49	
4	2	96.59	+.13
	6	96.72	

The observation of Gaskin and Hands, that a contraction of several hundredths of a milliliter occurred when 52 ml of the raw sugar solution was mixed with the water after making to volume, has been substantially confirmed. Repeating their experiment with seven Cuban raws, we found an average contraction corresponding to a rotation of 0.06°*S*. In these experiments, the average volume of sugar solution before making to volume with water was 68 ml.

It does not appear that conditions causing excessive contraction exist to an appreciable extent in routine polarization measurements, for the following reasons: After the raw sugar is dissolved, the neck of the flask is washed down with water and additional water is added until the level

is just below the lower end of the neck. The contents are mixed by shaking. The volume of the diluted solution is 90–95 ml and water is then added to complete to volume. Thus the final mixing, after completing the volume and adding the dry lead, is between 90–95 ml of sugar solution and 5–10 ml of water. The contraction under these conditions causes no significant error, according to these authors, and this statement is confirmed by present experiments.

The general question of wet and dry lead clarification was summarized in the report by F. W. Zerban on Subject 21 in the 1951 proceedings of the U. S. National Committee on Sugar Analysis.

Pending international agreement, no action on the method for polarization of raw sugars is recommended.

RECOMMENDATIONS

It is recommended*—

(1) That the study of methods for the determination of moisture in sugar products be continued.

(2) That the study on density of solutions of sugars at various temperatures be continued.

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

(4) That the method for carbohydrate analysis of honey, using carbon column pre-treatment, referred to in the report of the Associate Referee this year, be submitted to collaborative study.

(5) That the study of methods for the determination of reducing sugars, including those employing chromatographic separation, be continued.

(6) That the study of methods **29.132–29.154,** inclusive, be continued.

(7) That the methods described in this year's report for the determination of color in raw cane sugars be adopted, first action.

(8) That the collaborative study on refractive indices of sucrose solutions in the range 60 to 70 per cent be continued.

(9) That work on the Somogyi phosphate method be discontinued.

(10) That the anthrone method referred to in the Associate Referee's report for the determination of very small amounts of glucose be further studied.

(11) That collaborative work be carried out on determination of sucrose by determining reducing sugars by the Lane and Eynon method before and after hydrolysis.

(12) That collaborative work be conducted on methods for determining total sugars in hydrol.

(13) That work be continued on development of methods for the de-

* For report of Subcommittee D and action by the Association, see *This Journal*, 37, 79 (1954).

tection of adulteration of maple products and the identity of maple concentrates.

REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Wash. 4, D. C., 1950, p. 808.
(2) Proceedings of the Subcommittee on Subject 7, U. S. National Committee on Sugar Analysis, March 1953.
(3) *Official Methods of Analysis*, 7th Ed., 1950, p. 497.
(4) GASKIN, J. G. N., and HANDS, G. C., *The Analyst*, **78**, 334 (1953).
(5) *Official Methods of Analysis*, 7th Ed., 1950, p. 499.

REPORT ON DENSIMETRIC AND REFRACTOMETRIC METHODS

DETERMINATION OF TOTAL SOLIDS IN LIQUID SUGARS

By CARL F. SNYDER (*Associate Referee*) and M. RUTH DRYDEN (National Bureau of Standards, Washington 25, D. C.)

During the past few months, the Referee has received a number of inquiries regarding the applicability of the official methods of the A.O.A.C. to the determination of total solids in commercial liquid sugar products. Liquid sugars are marketed in bulk liquid form, and in 1952, production amounted to 691,026 tons of solids on the refined basis. (The marketing of these products and their commercial importance is discussed in detail in Report 52, "Marketing of Liquid Sugar," by F. J. Poats, Production and Marketing Administration, U. S. Department of Agriculture, June, 1953.)

The various methods employed for the determination of total solids in different types of liquid sugar products have been the subject of numerous investigations by this Association and others. Schneller (1) compared the total solids as sucrose by refractometer with the values obtained by the double dilution Brix method on a number of sirups which he prepared by the careful acid inversion of sucrose.

DeWhalley (2) studied the effect of the presence of invert sugar on total solids (estimated as sucrose by the refractometer) and proposed the addition of 0.022 per cent for each per cent invert to obtain true solids. Subsequently, Zerban (3) employed the data of Schneller and of de Whalley in the evaluation of the correction for invert sugar. It was shown that this correction factor is not constant, but steadily increases with the concentration; the average confirms the deWhalley value. On the basis of this work, the average value of the correction (0.022 for

each per cent of invert) was recommended and adopted for the refractometric method, **29.8.**

At the tenth session of the International Commission for Uniform Methods of Sugar Analysis in 1949, deWhalley (4) reported the observation of Hobbs that the correction should be obtained by means of the formula: $y=0.00031x$ (*1*), where y is the correction for each per cent invert and x is the per cent true solids. The report further stated that the Hobbs formula did not apply to commercially prepared samples, including so-called "Golden Sirups," where reversion products were undoubtedly present. For such commercial products, deWhalley suggested the formula: $y=0.00025x$ (*2*).

Another means of correcting "refractometer solids as sucrose" to true solids is by the application of the Vosberg rule as suggested by Zerban (3). This is accomplished by the use of the refractive index values of invert sugar solutions of Zerban and Martin (5), and those of sucrose, **41.7.**

In Table 2 is given a comparison of the results obtained by the application of the above corrections to the refractometer solids of the invert types of liquid sugars in Table 1.

COMPARISON OF METHODS

Since analytical data on commercial liquid sugars of current production are not readily available, a series of comparative tests were made by the existing official methods. Through the efforts of C. J. Broeg, Sugar Branch, Production and Marketing Administration, a number of manufacturers of liquid sugars kindly furnished the Referee with samples of their products.

The methods of analysis, results of which are given in Table 1, were as follows:

TOTAL SOLIDS

By refractometer **(29.8).**—The refractive indices were measured on a carefully calibrated Bausch and Lomb precision refractometer at 20°C. The corresponding values of total solids as sucrose were obtained from the five-place Bausch and Lomb table which is based on the official table **41.7** (col. 5). In the case of samples containing more than 0.2% invert sugar, the values for per cent solids were corrected by adding 0.022 for each per cent invert sugar present (col. 6).

Drying on quartz sand **(29.5).**—Preliminary mixing and drying was effected by placing the dishes on the top of a heated oven so that the temperature of the samples did not exceed 60°C. The final drying was accomplished in a vacuum oven at 60°C. and continued until the loss of weight during a 4–6 hour heating interval did not exceed a few tenths of a milligram (col. 2).

By pycnometer **(29.7** (b)**).**—The directions were followed except that the flask was filled nearly to the mark with sample so that only a few drops of water were required to complete to volume at 20°C. The corresponding per cent sugar solids (col. 7) as sucrose were obtained from Table **41.5.** For comparison, the values thus obtained were corrected by adding 0.022 for each per cent invert (col. 8).

TABLE 1.—*Comparison of determinations of total solids in liquid sugars*

SAMPLE NO.	BY VACUUM DRYING (60°C.)	INVERT SUGAR, (L. AND E.) *per cent*	ASH, *per cent*	BY REFRACTOMETER		BY PYCNOMETER		BY SPINDLE	(BRIX)
				TOTAL SOLIDS		TOTAL SOLIDS		DIRECT	DILD 1:1
				AS SUCROSE	CORRECTED	AS SUCROSE	CORRECTED		
Sucrose Type									
1	67.02	0.29	0.04	67.00	67.01	66.80	66.81	66.77	
2	67.11	0.18		67.27		67.18		67.22	
3	67.28	0.16		67.29		67.20		67.23	
4	67.23	0.09		67.38		67.27		67.29	
5	67.37	0.13		67.22		66.98		66.98	
6	67.64	0.28	0.002	67.51	67.52	67.39	67.40	67.55	
	67.275			67.278		67.137		67.173	
Medium Invert Type									
7	75.77	37.54	0.07	74.65	75.49	74.73	75.55		75.49
8	75.99	42.77	0.04	74.82	75.76	74.90	75.84		75.49
9	76.16	41.83	0.07	75.09	76.01	75.14	76.07		75.76
10	76.18	37.71	0.07	75.33	76.16	75.43	76.27		76.01
11	76.39	41.31	0.07	75.30	76.21	75.40	76.32		76.05
12	76.86	40.33	0.01	75.87	76.76	75.92	76.81		76.63
13	76.99	44.15	0.002	75.60	76.57	75.69	76.65		76.35
14	77.17	37.40	0.01	75.99	76.82	76.05	76.87		76.27
	76.439			75.331	76.223	75.408	76.298		76.006
Total Invert Type									
15	73.95	69.91	0.06	72.27	73.81	72.47	74.01		73.60
16	74.71	70.00	0.01	72.98	74.52	73.20	74.74		74.15
17	76.76	72.12	0.08	75.18	76.77	75.40	77.00		76.43
18	76.87	71.42	0.08	75.36	76.93	75.65	77.22		76.49
	75.573			73.948	75.508	74.180	75.743		75.168

TABLE 2.—*Corrected refractometer solids in invert liquid sugars*

SAMPLE NO.	BY METHOD 29.8[a]	BY VOSBERG'S RULE	BY HOBBS FORMULA (1)	BY DEWHALLEY FORMULA (2)
7	75.49	75.47	75.54	75.36
8	75.76	75.74	75.83	75.63
9	76.01	75.99	76.08	75.90
10	76.16	76.15	76.22	76.05
11	76.21	76.18	76.28	76.09
12	76.76	76.78	76.83	76.64
13	76.57	76.55	76.65	76.44
14	76.82	76.79	76.89	76.71
	76.223	76.206	76.290	76.103
15	73.81	73.72	73.87	73.56
16	74.52	74.47	74.60	74.28
17	76.77	76.71	76.90	76.56
18	76.93	76.89	77.07	76.73
	75.508	75.448	75.610	75.283

[a] See Table 1, col. 6.

By spindle **(29.6).**—The determinations were made at 20°C. with calibrated Brix spindles. The readings were made on the original samples of the sucrose type liquid sugars (col. 9). The invert types were diluted with approximately equal weights of water, the spindle reading was taken, and the Brix values of the original sample were calculated (col. 10).

INVERT SUGAR

Lane and Eynon volumetric method, **29.32,** was used (col. 3).

ASH

Official method, **29.9,** was used (col. 4).

CONCLUSIONS

No recommendations for revision of the methods are presented at this time. It is hoped that it will be possible to extend the experimental work on invert type sirups during the coming year.

The subject of refractive indices and densities of invert sugar solutions at different temperatures is being investigated at the National Bureau of Standards under a project supported by the Sugar Research Foundation, Incorporated.

REFERENCES

(1) SCHNELLER, M., *This Journal,* **9,** 156 (1926).
(2) DEWHALLEY, H. C. S., *Intern. Sugar J.,* **37,** 353 (1935).

(3) ZERBAN, F. W., *This Journal*, **26**, 143 (1943).
(4) DEWHALLEY, H. C. S., *Intern. Sugar J.*, **52**, 190 (1950).
(5) ZERBAN, F. W., and MARTIN, J., *This Journal*, **27**, 295 (1944).

REPORT ON MICRO METHODS OF SUGAR ANALYSIS

By BETTY K. GOSS (National Bureau of Standards, Washington 25, D. C.), *Associate Referee**

During the past year, many articles have appeared on the color reaction of anthrone with sugars. Among the materials analyzed by this method are glucose (1–3), polyglucose (4), low concentrations of sucrose (5), polysaccharides (6), pentoses (7), starch and cellulose (8), and methylcellulose (9). The method is reported to be extremely simple and sensitive, and since it appears to have almost unlimited application in the microanalysis of sugars, it was selected for study.

In 1946, Dreywood (10) published the first method based on the reaction. Morris (11) published further work on the method, and Sattler and Zerban (12, 13) postulated a connection between the anthrone reaction and carbohydrate structure. More recent work by Koehler (14) describes the differentiation of carbohydrates by the anthrone reaction rate and color intensity.

The method described in the latter paper forms the basis for the present work. Despite the many modifications (15–17) that depend on heat of reaction to develop the color, it was the experience of this laboratory that very careful standardization of heating and cooling was necessary to achieve quantitative results.

In the course of the investigation the question arose as to whether the purity of the solid anthrone was responsible for inconsistent results. Three anthrone solutions were made up; solutions A and B were made from recrystallized anthrone (3 parts benzene to 1 part petroleum ether in the cold), and solution C was made from the commercial product as received from the Eastman Kodak Company. At intervals over a period of eight days, duplicate 5 ml portions of these solutions were carried through the procedure with 2.5 ml of water (not glucose solution; see procedure, below) and read on the Evelyn colorimeter with the 635 mμ filter against a distilled water blank set at 100. Colorimeter readings and their deviations from the first day's reading are shown in Table 1.

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* Present address: 1403 North Vernon St., Arlington, Va.

TABLE 1.—*Colorimeter readings of anthrone "blanks"*

	1ST DAY	2ND DAY	3RD DAY	4TH DAY	5TH DAY	8TH DAY
Solution A						
Reading (av.)	96.6	97.7	96.8	96.2	94.5	91.0
Deviation from 1st day		+ 1.1	+ 0.2	− 0.4	− 2.1	− 5.6
Solution B						
Reading (av.)	95.4	96.7	96.1	95.3	93.8	88.5
Deviation from 1st day		+ 1.3	+ 0.7	− 0.1	− 1.6	− 6.9
Solution C						
Reading (av.)	96.2	97.2	96.6	95.6	93.5	91.6
Deviation from 1st day		+ 1.0	+ 0.4	− 0.6	− 2.7	− 4.6
	(*8th day*)	(*9th day*)	(*10th day*)	(*11th day*)	(*12th day*)	(*15th day*)
Solution D (made up 1 wk. earlier)						
Reading (av.)	91.8	92.8	91.4	91.0	89.2	83.8
Deviation from 8th day		+ 1.0	− 0.4	− 0.8	− 2.6	− 8.0

The direct readings on solution C and their successive deviations from those on the fresh solution are not appreciably different from those on solutions A and B. This indicates that inconsistent results are not attributable to the purity of the anthrone and that recrystallization is probably not necessary.

Readings on solution D, which was made up one week previously, further illustrate the break-down of anthrone solutions. All of these solutions were kept refrigerated when not in actual use.

The figures indicate that the anthrone solutions should give accurate results over a period of four days. Consequently, any laboratory set up to do routine anthrone analyses could make up the fresh reagent on Monday morning and use it from Tuesday through Friday. It is desirable in testing sugar products with anthrone, as with so many other reagents, to standardize the fresh solution against pure sugars and to refer the results obtained from the unknown sugars to the standard curve for the particular batch of reagent.

METHOD

APPARATUS

(a) *Matched test-tubes.*—20 mm×170 mm, absolutely clean and free from dust, lint, filter paper, etc.

(b) *Evelyn colorimeter, or similar instrument.*—Equipped with red filter (near 635 mμ. An Evelyn is preferable, as solns may then be read in the reaction vessels).

REAGENTS

Anthrone soln.—Commercial product recrystallized from benzene, 3 parts, and petr. ether, 1 part. Make 0.2% in 95% H_2SO_4 (950 ml concd H_2SO_4 added to 50 ml distd H_2O.)

DETERMINATION

To 2.5 ml glucose soln in a test tube, add, with shaking, 5 ml anthrone soln while the test tube is immersed in ice-H_2O. This addn should be made by pipet to avoid contamination by stopcock lubricants used in burets. Immerse in boiling H_2O bath for exactly 10 min. Remove and cool in ice-H_2O for 5 min. Let stand in air until ready to read. If a series is being run it may be necessary for some test tubes to stand a few min. Read all the tubes in consecutive order, allowing a sufficient interval between reading any two to allow every soln to stand in air a standard length of time before reading. Read in a colorimeter at 635 mμ with the instrument set at 100 against the blank.

In a collaborative study,* the method was investigated over the range 0–80 mmg glucose in 10 mmg increments, and between 30 and 40 mmg glucose in 5 mmg increments. The results of two analysts are shown in Table 2. Each collaborator used a single anthrone preparation. Analyst 1 read the colors on an Evelyn colorimeter at 635 mμ and Analyst 2 used a Lumetron colorimeter with a No. 66 red filter (660 mμ); thus readings are not comparable. However, the tabulated readings serve to delineate standard curves for the two instruments and how the curves may change from day to day. They also indicate the expected precision of the method with pure solutions of glucose.

Neither operator experienced difficulty in reading the solutions, as evidenced by the good agreement between the original reading and the check on each day's run.

In general, it should be possible to obtain results on very dilute dextrose solutions accurate to the nearest 5 mmg.

SOMOGYI'S PHOSPHATE REAGENT

The 1952 report on micro methods for sugar analysis (18) discussed the use of Somogyi's phosphate reagent when the reduced copper is determined iodometrically, and recommended (1) that further studies be made on Somogyi's phosphate method, and (2) that comparative studies be made between Somogyi's carbonate and phosphate methods. Somogyi's carbonate method appears as first action in *Official Methods of Analysis*, **29.61,** but there was evidence that the phosphate reagent is preferable to the carbonate reagent.

During the intervening year, however, it has come to the attention of the Associate Referee that use of the Somogyi phosphate reagent has

* Acknowledgment is made to Miss M. R. Dryden, of the National Bureau of Standards, for her cooperation in the collaborative project.

TABLE 2.—*Colorimeter readings for two ranges of glucose concentration*

GLUCOSE (*mmg*)	ANALYST 1 (EVELYN)		ANALYST 2 (LUMENTRON)	
	(5/6)	(5/7)	(6/30)	(7/1)
Series I (0–80 mmg)				
0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
10	82.2 82.9	81.8 82.0	89.9 90.3	89.7 90.0
20	65.9 66.1	64.4 64.5	82.4 82.6	82.0 82.5
30	52.2 52.4	49.1 49.2	74.9 75.1	74.5 74.7
40	42.0 42.0	40.9 40.9	67.8 67.8	66.4 67.0
50	33.8 33.9	32.3 32.3	61.8 62.1	59.9 59.5
60	26.8 26.9	26.3 26.4	54.8 55.2	52.9 53.4
70	21.7 21.8	20.9 20.9	48.3 48.6	48.0 48.4
80	17.2 17.2	17.1 17.1	44.4 44.6	43.8 44.2
Series II (30–40 mmg)				
	(5/20)	(5/21)	(7/2)	(7/2)
0	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
0	99.8 99.8	97.0 97.1	99.6 100.0	95.4 95.0
30	49.9 50.0		75.4 75.2	
30	51.1 51.0		74.7 74.9	
30	49.2 49.1		75.2 75.5	
35	43.5 43.8	43.2 43.5	69.1 69.5	70.5 71.0
35	43.9 43.8	44.9 45.0	69.3 69.6	71.3 71.4
40		39.8 39.8		66.2 66.5
40		40.0 40.0		67.5 67.8
40		40.0 39.9		65.9 66.0

been discontinued in many laboratories. Dr. Somogyi stated: "After several years of experience, we observed, however, that the sugar equivalents are subject to change. Such changes were not observable during the first year, but they came to our attention as we eventually prepared the reagent from varying batches of chemicals, and as we checked on some of our solutions that were several months old. Since we were unable to find a full explanation of the trouble, we have reverted to the use of carbonate instead of phosphate as the alkali" (19). With reference to the colorimetric modification of the method, he further states that he is convinced "that this reagent is not suitable for accurate colorimetric work, because it interferes with the stability of the colors developed with chromogenic reagents."

Dr. O. L. Baril at the College of the Holy Cross, Worcester, Massachusetts, is presently conducting extensive studies on the reagent in hopes of discovering the reason for the inconsistent results obtained. He is also canvassing various laboratories to learn of their experiences with the phosphate reagent.

Therefore, it seems inadvisable to continue work on the method, and it is recommended*—

(1) That the Somogyi phosphate studies be abandoned.

(2) That the anthrone method for the determination of very small amounts of glucose be further considered.

REFERENCES

(1) MOTEGI, K., *J. Japan. Biochem. Soc.*, **21**, 40 (1949).
(2) GREEN, P., and WADE, E., *Can. Med. Assoc. J.*, **66**, 175 (1952).
(3) ZIPF, R. E., and WALDO, A. L., *J. Lab. Clin. Med.*, **39**, 497 (1952).
(4) VAN SLYKE, D. D., SINEX, F. M., and MACMULLEN, J., *Proc. Soc. Exptl. Biol. Med.*, **79**, 163 (1952).
(5) MORSE, E. E., *Proc. Am. Soc. Sugar Beet Technol.*, **5**, 540 (1948).
(6) SHETLAR, M. R., *Anal. Chem.*, **24**, 1844 (1952).
(7) BRIDGES, R. R., *ibid.*, **24**, 2004 (1952).
(8) VILES, F. J., JR., and SILVERMAN, L., *ibid.*, **21**, 950 (1949).
(9) SAMSEL, E. P., and DELAP, R. A., *ibid.*, **23**, 1795 (1951).
(10) DREYWOOD, R., *Ind. Eng. Chem., Anal. Ed.*, **18**, 499 (1946).
(11) MORRIS, D. L., *Science*, **107**, 254 (1948).
(12) SATTLER, L., and ZERBAN, F. W., *ibid.*, **108**, 207 (1948).
(13) ———, *J. Am. Chem. Soc.*, **72**, 3814 (1950).
(14) KOEHLER, L. H., *Anal. Chem.*, **24**, 1576 (1952).
(15) LOEWUS, F. A., *ibid.*, **24**, 219 (1952).
(16) KIBRICK, A. C., ROGERS, H. E., and SKUPP, S., *J. Biol. Chem.*, **190**, 107 (1951).
(17) KOWALD, J. A., and MCCORMACK, R. B., *Anal. Chem.*, **21**, 1383 (1949).
(18) GOSS, B. K., *This Journal*, **36**, 245 (1953).
(19) SOMOGYI, M., *J. Biol. Chem.*, **195**, 19 (1952).

* For report of Subcommittee D and action of the Association, see *This Journal*, 37, 80 (1954).

REPORT ON TRANSMITTANCE OF SUGAR SOLUTIONS

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

The report given last year (1) showed that four of the seven collaborators on this subject obtained satisfactory agreement, with a standard deviation of 2.7 per cent at the important wavelength 560 mμ, but for all collaborators the standard deviation at this wavelength was over twice as high, i.e., 6 per cent. It was therefore decided to repeat the work this year, improving the method of preparing the samples and the directions for the analyses. V. R. Deitz called attention to the probable inhomogeneity of raw sugar samples prepared by hand mixing, and offered to have the bulk samples intended for distribution among the collaborators made up in his laboratory by the process of riffling, previously designed and adopted at the National Bureau of Standards for mixing bonechar. The changes in the analytical procedure, largely suggested by A. B. Cummins, are shown in the directions for this year's work (1).

Three samples were again distributed this year, a Cuban and a Puerto Rican raw sugar, kindly furnished by R. Winston Liggett of the American Sugar Refining Company, Philadelphia, and a Hawaiian raw sugar contributed by T. R. Gillett of the California and Hawaiian Sugar Refining Corporation, Crockett, California. Each sample was prepared by riffling in the laboratory at the National Bureau of Standards, and portions were sent to the collaborators, together with the required quantity of Celite from the thoroughly mixed contents of one bottle of this filter aid (analytical grade) to insure that all collaborators used material of the same quality. The Associate Referee is greatly indebted to all those who assisted in these preparations and took part in the analytical work. The following directions were sent to each collaborator:

DIRECTIONS TO COLLABORATORS

Three samples of raw sugar are being distributed: a Cuban, a Puerto Rican, and a Hawaiian; and a bottle of Celite analytical filter aid is also being sent to each collaborator. The transmittance determinations are to be made as nearly as possible between January 10 and 20, according to the following method, which differs from that of last year in several details:

COLOR IN RAW CANE SUGARS

REAGENTS

Filter aid.—Celite analytical filter aid (Johns-Manville Corp.).

APPARATUS

Fractionator.—Construct fractionator of 35 mm internal diam. heavy wall Pyrex tubing, 145 mm long from top to bottom shoulder where it is sealed to a

standard taper stopcock with a 3 mm bore and 9 mm outside diam. tubing. A 55 mm stem is left below the stopcock. Seal a 9 mm outside diam. tube 45 mm long

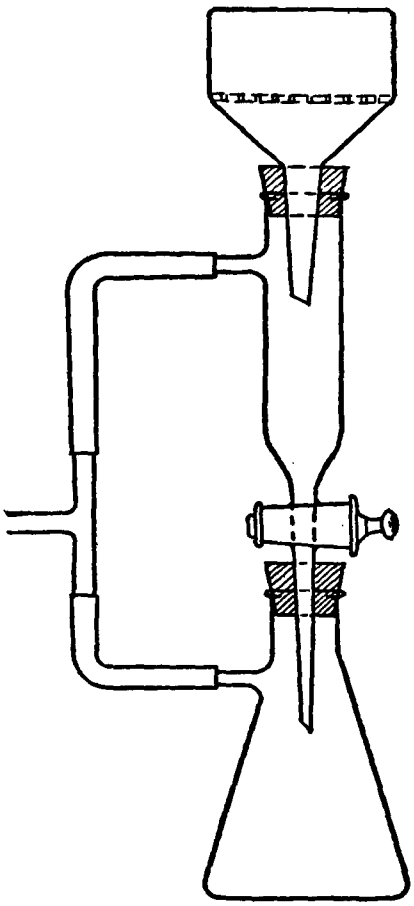

FIG. 1.—Fractionator.

to the body of the fractionator 45 mm below top. Connect to Büchner and source of vacuum thru a "T" tube as shown in Figure 1.

DETERMINATION

Place 60 g of the sugar in a flask, add 40 g of water that has been heated to boiling, and rotate flask until all sugar has been dissolved. Add 3 g of Celite and shake mixt. vigorously for 1 min. Assemble special filtration app. and, with stopcock open, place filter paper, Schleicher and Schuell No. 589 blue ribbon, 7 cm diam., in Büchner funnel, wet down with H_2O, and suck excess water by vacuum thru filtering tube into filtering flask. Close stopcock and pour the well shaken mixt. of sugar soln

and Celite evenly over filter paper. Carry out filtration at a constant vacuum of 24 inches. Collect ca 10 ml of the first filtrate, which is somewhat turbid, in the filtering tube and run into filtering flask by opening stopcock. Close stopcock, collect another 10 ml of filtrate in the filtering tube, and then run into filtering flask as before to wash inner walls of filtering tube free from any small particles of turbidity. Keep bed of Celite well covered with sugar soln during entire filtration. *Do not allow to run dry.* Collect final clear filtrate in filtering tube, transfer to a small bottle or to a small glass-stoppered Erlenmeyer flask, and mix thoroly. Det. refractometer Brix on a portion of the soln, and calc. concn, c, (g dry substance per ml soln) by multiplying Brix by corresponding true density and dividing by 100.

Det. % transmittance at 560 mμ on a spectrophotometer with wavelength and transmittance scales that have been checked, preferably with a standard glass filter supplied by the National Bureau of Standards. Use distd H_2O as the 100% transmittance standard. The cell thickness used should be such that readings are within the range 25–75% transmittance.

Calc. attenuation index, $a_c = -\log$ transmittance.

Supplementary directions were furnished as follows: Determine the pH of the solution, without dilution, electrometrically with the glass electrode and use the remainder of the solution for the transmittance determinations.

It is presupposed that the collaborators are well versed in spectrophotometry, and are familiar with the exacting cleanliness required. The wavelength and the transmission scales should be checked, preferably with a standard glass filter of the National Bureau of Standards. The cell thickness should preferably be selected so that the readings fall as much as possible within the range of 25 to 75 per cent transmittance, to avoid percentage errors in the corresponding negative logarithms.

The transmittances are to be determined at six wavelengths, 375, 420, 480, 560, 640, and 720 mμ, with distilled water as the 100 per cent transmittance standard.

Three separate complete tests of each of the three sugars are to be made, each of the nine tests starting with the solid raw sugar. The details of the procedure described should be strictly adhered to in order to obtain the closest possible agreement among the results for each of the three sugars.

The following data are to be reported for each of the nine series of determinations:

(1) The observed transmittance at each of the six wavelengths, and the cell thickness used for each of the transmittance measurements.

(2) The concentration of each of the nine final filtrates used for the transmittance measurements.

(3) The pH of the final filtrates used for the transmittance measurements.

COLLABORATIVE RESULTS

Reports have been received from all seven collaborators, and each carried out the work within the stipulated period of time. The following instruments, concentrations, and cell thicknesses were used by them:

(1) Louis Sattler, New York Sugar Trade Laboratory, New York, N. Y.: Beckman Model B; $c = .7717$ to $.8066$; cell thickness, 0.246 cm.

(2) Carl Erb, New York Sugar Trade Laboratory, New York, N. Y.: Beckman Model B; $c = .7691$ to $.7850$; cell thickness, 0.246 cm.

(3) V. R. Deitz, National Bureau of Standards, Washington, D. C.; Beckman Model DU; $c = .782$ to $.803$; cell thicknesses, 0.041, 0.2, and 1.0 cm.

(4) T. R. Gillett, California and Hawaiian Sugar Refining Corp., Crockett,

California, experimental work by W. D. Heath: Beckman Model DU; $c = .788$ to .805; cell thickness, 1.0 cm.

(5) R. Winston Liggett, assisted by J. A. Devlin and D. C. Nelson, American Sugar Refining Company Research Laboratory, Philadelphia, Pa.; (*a*) Beckman Model DU; $c = .8049$ to .8234; cell thicknesses, 0.1 and 1.0 cm.; (*b*) Cary Recording Spectrophotometer; $c = .8049$ to .8234 (same solutions as under (*a*)); cell thickness, 0.3 cm.

(6) R. T. Balch, U. S. Department of Agriculture, Houma, La.: Coleman Jr. Spectrophotometer; $c = .7776$ to .7842; cell thicknesses, 0.1 and 0.8 cm.

(7) C. A. Fort, Southern Regional Research Laboratory, New Orleans, La.; Beckman Model DU; $c = .7735$ to .8175; cell thicknesses, 0.5, 1.0 and 2.0 cm.

The results obtained by all the collaborators are shown separately for each sugar in Table 1. They are termed "attentuation indexes," $(a_c)^1$ in conformity with the latest definitions, since all the filtered solutions contained small quantities of light scattering particles.

The *p*H of each final solution, reported by six of the collaborators, is shown in Table 2.

Differences recorded in the *p*H of the individual solutions of each single sugar are evidently without significant effect on the transmittance. Solution No. 2 of the Cuban sugar of collaborator 4 had a much lower *p*H than solutions No. 1 and No. 3, and should have the lowest attenuation index, but at four of the wavelengths tested it gave the highest index, and at the remaining wavelength it gave a figure between the highest and lowest. In other words, the effect of variations in the *p*H of individual filtrates of the same sugar appears to be within the limit of error of transmittance determinations. T. R. Gillett has expressed the opinion that the discrepancies of the *p*H results of collaborator 4 may be due to experimental error. The Cary recording spectrophotometer, the results of which are given for each sugar in Column 5 (b) of Table 1, was a new instrument, received only recently, and the operators had gained little experience with it. Although the averages of the triplicate determinations with this instrument generally agreed satisfactorily with those of the others, there were in many cases large discrepancies between the individual results of the same triplicate sets. The results in column 5 (b) are discussed separately.

REPRODUCIBILITY

Reproducibility of the results of each single collaborator.—The difference between the maximum and minimum attenuation index, in per cent of the average of each set of triplicate results, is shown in Table 3 to permit comparison with the corresponding values obtained last year (1).

The over-all average for the three sugars, at all wavelengths, by all collaborators (with the exception of 5(b) with admittedly unsatisfactory

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[1] $a_c = -\log$ transmittance.

TABLE 1.—*Attenuation indexes*

WAVELENGTH	Collaborator 1	2	3	4	5(a)	6	7	5(b)
	Instrument: BECKMAN MODEL B	BECKMAN MODEL B	BECKMAN DU	BECKMAN DU	BECKMAN DU	COLEMAN JR.	BECKMAN DU	CARY
	Cuban Sugar							
375 mμ								
No. 1	7.597	7.658	7.537	——	7.592	——	——	7.776
No. 2	7.603	7.743	7.346	——	7.601	——	——	7.842
No. 3	7.650	7.686	7.494	——	7.560	——	——	7.699
Av.	7.620	7.696	7.457	——	7.584	——	——	7.772
420 mμ								
No. 1	2.842	2.879	2.631	1.616[a]	2.747	2.657	2.865	2.798
No 2	2.848	2.885	2.619	1.628[a]	2.766	2.653	2.863	2.873
No. 3	2.877	2.898	2.619	1.627[a]	2.774	2.656	2.883	2.810
Av.	2.856	2.887	2.623	1.622[a]	2.762	2.655	2.870	2.827
480 mμ								
No. 1	1.311	1.336	1.261	1.330	1.292	1.260	1.337	1.246
No. 2	1.304	1.356	1.238	1.346	1.291	1.288	1.336	1.342
No. 3	1.315	1.358	1.240	1.332	1.287	1.304	1.348	1.343
Av.	1.310	1.350	1.246	1.336	1.290	1.284	1.340	1.310
560 mμ								
No. 1	0.598	0.608	0.608	0.592	0.560	0.575	0.589	0.534
No. 2	0.594	0.601	0.603	0.598	0.561	0.576	0.590	0.596
No. 3	0.611	0.615	0.607	0.591	0.560	0.576	0.593	0.595
Av.	0.601	0.608	0.606	0.594	0.560	0.576	0.591	0.575
640 mμ								
No. 1	0.280	0.275	0.280	0.279	0.264	0.297	0.279	0.241
No. 2	0.276	0.268	0.276	0.283	0.266	0.297	0.279	0.307
No. 3	0.266	0.270	0.276	0.292	0.262	0.297	0.281	0.301
Av.	0.274	0.271	0.277	0.285	0.264	0.297	0.280	0.283
720 mμ								
No. 1	0.117	0.115	0.115	0.115	0.106	——	0.119	0.089
No. 2	0.114	0.113	0.113	0.119	0.106	——	0.118	0.140
No. 3	0.116	0.120	0.112	0.115	0.103	——	0.118	0.140
Av.	0.116	0.116	0.113	0.116	0.105	——	0.118	0.123

[a] Transmittance read about 5%.

TABLE 1—(*continued*)

WAVELENGTH	Collaborator							
	1	2	3	4	5(a)	6	7	5(b)
	Instrument							
	BECKMAN MODEL B	BECKMAN MODEL B	BECKMAN DU	BECKMAN DU	BECKMAN DU	COLEMAN JR.	BECKMAN DU	CARY
	Puerto Rican Sugar							
375 mμ								
No. 1	8.430	8.690	8.542	——	8.205	——	——	8.519
No. 2	8.473	8.624	8.047	——	8.453	——	——	8.781
No. 3	8.526	8.678	8.057	——	8.414	——	——	8.785
Av.	8.476	8.664	8.215	——	8.357	——	——	8.695
420 mμ								
No. 1	3.612	3.549	3.160	2.266[b]	3.287	3.198	3.460	3.378
No. 2	3.620	3.549	3.101	2.270[b]	3.417	3.179	3.487	3.531
No. 3	3.658	3.558	3.118	——	3.396	3.202	3.493	3.558
Av.	3.630	3.552	3.126	2.268[b]	3.361	3.193	3.480	3.489
480 mμ								
No. 1	1.690	1.718	1.605	1.697	1.603	1.624	1.690	1.603
No. 2	1.700	1.703	1.582	1.703	1.642	1.607	1.697	1.707
No. 3	1.710	1.717	1.573	——	1.639	1.618	1.698	1.734
Av.	1.700	1.713	1.587	1.700	1.628	1.616	1.695	1.681
560 mμ								
No. 1	0.676	0.722	0.722	0.700	0.651	0.692	0.686	0.616
No. 2	0.686	0.724	0.718	0.699	0.668	0.679	0.690	0.679
No. 3	0.702	0.723	0.740	——	0.671	0.688	0.691	0.715
Av.	0.688	0.723	0.727	0.700	0.663	0.686	0.689	0.670
640 mμ								
No. 1	0.336	0.304	0.331	0.329	0.299	0.356	0.319	0.253
No. 2	0.328	0.305	0.321	0.329	0.306	0.343	0.322	0.330
No. 3	0.332	0.311	0.321	——	0.304	0.348	0.324	0.344
Av.	0.332	0.307	0.324	0.329	0.303	0.349	0.322	0.309
720 mμ								
No. 1	0.139	0.137	0.146	0.146	0.130	——	0.146	0.068
No. 2	0.138	0.141	0.144	0.151	0.132	——	0.146	0.160
No. 3	0.159	0.139	0.143	——	0.130	——	0.148	0.172
Av.	0.145	0.139	0.144	0.149	0.131	——	0.147	0.133

[b] Transmittance read below 2%.

TABLE 1—(*continued*)

WAVELENGTH	Collaborator							
	1	2	3	4	5(a)	6	7	5(b)
	Instrument							
	BECKMAN MODEL B	BECKMAN MODEL B	BECKMAN DU	BECKMAN DU	BECKMAN DU	COLEMAN JR.	BECKMAN DU	CARY
	Hawaiian Sugar							
375 mμ								
No. 1	7.176	7.365	7.097	——	7.203	——	——	7.330
No. 2	7.265	7.233	6.893	——	7.132	——	——	7.398
No. 3	7.094	7.028	6.965	——	6.939	——	——	7.283
Av.	7.178	7.212	6.985	——	7.091	——	——	7.337
420 mμ								
No. 1	3.005	3.109	2.721	1.721[c]	2.919	2.804	2.954	2.831
No. 2	3.071	3.039	2.703	1.728[c]	2.897	2.792	3.057	2.994
No. 3	3.004	2.989	2.746	1.728[c]	2.755	2.730	2.990	2.981
Av.	3.027	3.046	2.723	1.726	2.857	2.782	3.000	2.935
480 mμ								
No. 1	1.416	1.492	1.358	1.408	1.395	1.396	1.411	1.373
No. 2	1.448	1.457	1.341	1.435	1.388	1.397	1.456	1.431
No. 3	1.414	1.423	1.363	1.450	1.368	1.400	1.420	1.444
Av.	1.426	1.457	1.354	1.431	1.384	1.398	1.429	1.416
560 mμ								
No. 1	0.645	0.691	0.659	0.661	0.641	0.655	0.647	0.581
No. 2	0.657	0.663	0.665	0.665	0.632	0.652	0.681	0.640
No. 3	0.640	0.654	0.662	0.667	0.627	0.628	0.655	0.659
Av.	0.647	0.669	0.662	0.664	0.633	0.645	0.661	0.627
640 mμ								
No. 1	0.334	0.352	0.349	0.341	0.323	0.362	0.333	0.255
No. 2	0.338	0.341	0.342	0.346	0.317	0.362	0.352	0.334
No. 3	0.316	0.333	0.342	0.347	0.309	0.346	0.339	0.350
Av.	0.329	0.342	0.344	0.345	0.316	0.357	0.341	0.313
720 mμ								
No. 1	0.145	0.162	0.158	0.153	0.141	——	0.154	0.081
No. 2	0.164	0.167	0.155	0.156	0.140	——	0.161	0.161
No. 3	0.154	0.164	0.153	0.158	0.134	——	0.155	0.173
Av.	0.154	0.164	0.155	0.156	0.138	——	0.157	0.138

[c] Transmittance read about 4%.

TABLE 2.—*pH values of final solutions*

COLLABORATORS	1	2	3	4	6	7
Cuban						
No. 1	5.92	6.10	6.00	6.10	5.91	5.95
No. 2	5.92	6.10	5.88	5.45	5.91	5.95
No. 3	5.92	6.10	5.90	5.91	5.91	5.95
Puerto Rican						
No. 1	5.35	5.35	5.29	5.60	5.30	5.40
No. 2	5.35	5.35	5.24	5.45	5.22	5.40
No. 3	5.35	5.35	5.24	——	5.22	5.40
Hawaiian						
No. 1	5.70	5.90	5.60	5.75	5.73	5.80
No. 2	5.70	5.90	5.57	5.75	5.73	5.80
No. 3	5.70	5.90	5.65	5.83	5.75	5.80

results because of inexperience with the instrument) is 2.4 per cent, only about one-half of the corresponding figure found last year, 4.7 per cent. This result is probably largely due to better mixing in preparing the bulk sugar samples. At a wavelength of 560 mμ, the over-all average value is 2.0 per cent, against 3.5 per cent last year.

Reproducibility of the results obtained by different collaborators.—This was again ascertained by calculating the standard deviation according to the formula $\sqrt{S/(n-1)}$, where S is the sum of the squares of the individual deviations from the mean, and n is the number of all single determinations (not averages of triplicates). Table 4 gives the results for all collaborators, leaving out those of collaborator 5 with the Cary Recording Spectrophotometer for reasons explained previously. Some of the results of collaborator 4, as indicated by footnotes in Table 1, have also been omitted as unreliable because the transmittance read was too near the zero point.

In Table 5, the mean standard deviations found for the three sugars this year are compared with those found last year by the four collaborators who showed close agreement, as well as by all seven collaborators.

Table 5 shows that the mean standard deviation found this year at wavelengths 480, 560, and 640 mμ was about the same as that obtained by four collaborators last year, with a value a little below 3 per cent for 560 mμ in both years. At wavelengths 420 and 720 mμ there was some improvement over last year. It is noteworthy that at 375 mμ the mean standard deviation was lower than at any other wavelength used. Although the three single determinations in each triplicate set agreed much more closely than last year, the over-all standard deviation at 560 mμ showed no improvement over last year's results of four collaborators. It may therefore be concluded that a standard deviation of 3 per cent at 560 mμ is about as low as may be expected.

TABLE 3.—*Difference between maximum and minimum results of each triplicate set, in per cent of average result of the same triplicate set*

WAVELENGTH	COLLABORATORS								
	1	2	3	4	5(a)	6	7	AV.	5(b)
	Cuban Sugar								
375	0.7	1.1	2.6	—	0.5	—	—	1.2	1.8
420	1.2	0.7	0.5	0.7	1.0	0.2	0.7	0.7	2.7
480	0.8	1.6	1.8	1.2	0.4	3.4	0.9	1.4	7.4
560	2.8	2.3	0.8	1.2	0.2	0.2	0.7	1.2	10.8
640	5.1	2.6	1.4	4.6	1.5	0.0	0.7	2.3	23.3
720	2.6	6.0	2.6	3.4	2.9	—	0.8	3.2	41.5
	Puerto Rican Sugar								
375	1.1	0.8	5.6	—	3.0	—	—	2.0	3.1
420	1.3	0.3	1.9	0.2	3.9	0.7	0.9	1.3	5.2
480	1.2	0.9	2.0	0.4	2.4	1.1	0.5	1.2	7.8
560	3.8	0.3	2.9	0.1	3.0	1.9	0.7	1.8	14.7
640	2.4	2.3	3.1	0.0	2.3	3.7	1.6	2.2	29.4
720	14.5	2.9	2.1	3.4	1.5	—	1.4	4.3	78.2
	Hawaiian Sugar								
375	2.4	4.8	2.9	—	3.7	—	—	3.5	1.6
420	2.2	3.9	1.6	0.4	5.6	2.7	3.4	2.8	5.6
480	2.4	4.7	1.6	2.9	2.0	0.3	3.1	2.4	5.0
560	2.6	5.5	0.9	0.9	2.2	4.1	5.1	3.0	12.4
640	6.7	5.6	2.0	1.7	4.4	4.5	5.5	4.3	30.4
720	12.3	3.0	3.2	3.2	5.1	—	4.5	4.5	66.7
	Mean for the Three Sugars								
375	1.4	2.2	3.7	—	2.4	—	—	2.4	2.2
420	1.6	1.6	1.3	0.4	3.2	1.2	1.7	1.6	4.5
480	1.5	2.4	1.8	1.5	1.6	1.6	1.5	1.7	6.7
560	3.1	2.7	1.2	0.7	1.7	2.1	2.2	2.0	12.6
640	4.7	3.5	2.2	2.1	2.7	2.7	2.6	2.9	27.7
720	9.8	4.0	2.6	3.3	3.2	—	2.2	4.2	62.1

The original title of this subject of the Association (1941 to 1948) was "Color and Turbidity in Sugar Products." Zerban and co-workers (2) have confirmed the conclusion of Peters and Phelps (3) that the specific absorptive index, now termed attenuation index, of the appropriately filtered solution of a sugar, measured at wavelength 560 mμ, is, within the limit of error of transmittance measurements, equivalent to the color as perceived by the eye. The recommendation offered by this Associate

TABLE 4.—*Mean standard deviation of individual determinations*

WAVELENGTH	MEAN a_c	STANDARD DEVIATION AS a_c	STANDARD DEVIATION (PER CENT)
	Cuban Sugar		
375	7.588	0.102	1.3
420	2.776	0.108	3.9
480	1.308	0.037	2.8
560	0.591	0.017	2.9
640	0.278	0.011	3.8
720	0.114	0.005	4.2
Av.			3.2
	Puerto Rican Sugar		
375	8.428	0.219	2.6
420	3.391	0.190	5.6
480	1.661	0.050	3.0
560	0.696	0.023	3.3
640	0.323	0.016	4.8
720	0.142	0.008	5.3
Av.			4.1
	Hawaiian Sugar		
375	7.117	0.144	2.0
420	2.905	0.136	4.7
480	1.413	0.038	2.7
560	0.655	0.016	2.4
640	0.339	0.014	4.1
720	0.154	0.009	5.8
Av.			3.6

TABLE 5.—*Mean standard deviation for all sugars*

WAVELENGTH	1952, ALL COLLABORATORS	1952, FOUR CLOSELY AGREEING COLLABORATORS	1953
	per cent	*per cent*	*per cent*
375	——	——	2.0
420	10.2	7.7	4.7
480	7.7	2.7	2.8
560	6.0	2.7	2.9
640	8.0	3.9	4.2
720	10.4	6.4	5.1
Av.	8.5	4.7	3.6

Referee therefore relates directly to the determination of the color of sugars in solution.

It is recommended* that the method described in this report for the determination of the color of raw cane sugars, by measuring at wavelength 560 mμ with a spectrophotometer of adequate accuracy and precision the transmittance of the solution, prepared and filtered as specified, and converting it into the corresponding attenuation index, be adopted, first action.

REFERENCES

(1) ZERBAN, F. W., *This Journal*, **36**, 250 (1953).[1]
(2) ———, *et al.*, *Anal. Chem.*, **23**, 303 (1951); **24**, 168 (1952).
(3) PETERS, H. H., and PHELPS, F. P., *Nat. Bur. Standards Technol. Paper*, **No. 338** (1927).

REPORT ON REDUCING SUGAR METHODS

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

In accord with last year's recommendations, work has been done on the method of Lane and Eynon. In Fort's modification and also in the procedure recently proposed by the U. S. Customs Laboratory, a given amount of an unknown sugar solution is added to the copper reagent and the final titration is made with a standard invert sugar solution. The procedure has been used in this laboratory, and it has been found to possess considerable advantage when a series of different samples is being analyzed.

A collaborative project is in progress for determining the agreement among results that can be expected when determining sucrose by Clerget's method and by reducing sugar before and after hydrolysis. Preliminary experiments performed with a soft sugar, a specially prepared reference sugar, and a washed raw sugar indicate that a considerable number of determinations must be made and the results must be treated statistically before definite conclusions can be drawn. It is proposed to make such a study of Cuban raw sugar samples.

It is recommended† that the studies on sucrose determination by the method of Lane and Eynon be continued.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 80 (1954).
[1] Correction to be applied to Reference 1: The heading of Column 2 on Table 1, pp. 253, 254, and 255 should read "Beckman DU" rather than "Cary."
† For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 80 (1954).

REPORT ON METHODS FOR MAPLE PRODUCTS

By C. O. WILLITS (Eastern Utilization Research Branch,* Philadelphia, Pa.), *Associate Referee*

The program of work on methods for the analysis of maple products (sirup and sugar) by the Association has been held in abeyance for the past several years. Because of the all-time high in the prices of maple products, with the corresponding large differential in price between cane sugar and maple sugar, attention has again been focused on the need for a satisfactory method of analysis which would serve for detection of adulteration.

The methods for the analysis of maple products given in *Official Methods of Analysis*, 7th Ed., procedures **29.107–29.129,** are completely adequate for obtaining the different chemical and physical measurements of these products.

Unfortunately, these methods do not give a measure of adulteration; at best they can give only presumptive evidence. This was well demonstrated by a collaborative study conducted recently in Canada in which it was shown that relatively large amounts of adulterants could be added without positive identification.

This situation exists because the detection of adulteration based upon the use of these methods presupposes that the addition of an adulterant will sufficiently alter the analytical values from those for pure maple to disclose adulteration. However, the chemical and physical measurement values which now serve as criteria of purity are not constants, but vary between rather wide limits. This range is so large that, even though the maple product being examined has been adulterated, the resultant analytical value will usually be within this range.

The method which can be used to disclose adulteration must be one that will either (*a*) detect something that is peculiar to the adulterant (which is usually cane or brown sugar); or (*b*) measure some chemical or physical property of pure maple that is nearly constant.

It is recommended† that work be continued on development of methods for the detection of adulteration of maple products with emphasis on (*b*), above.

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* One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

† For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 80 (1954).

REPORT ON ANALYSIS OF HONEY

By JONATHAN W. WHITE, JR. (Eastern Utilization Research Branch,* Philadelphia 18, Pa.), *Associate Referee*

No collaborative work on honey has been carried out within the past year. However, the following work of the Associate Referee has application to honey analysis and is therefore briefly mentioned.

A recent paper from this laboratory (1) described a comparison of five methods for the determination of levulose and dextrose in honey, including two described in *Official Methods of Analysis* of the A.O.A.C. These were applied to fourteen floral types of honey. It was concluded that "variance due to methods was as great as that due to differences in dextrose and levulose content of the samples from fourteen different floral sources." Among the possible reasons for this situation is that an oversimplified view of the carbohydrate composition of honey is generally taken.

A method, which will be described later, has been developed for carbohydrate analysis of honey which gives a somewhat more realistic idea of its composition. With the use of carbon column adsorption (2, 3) as a pretreatment, three solutions are obtained for analysis, containing monosaccharides, disaccharides, and higher sugars, respectively. These solutions are analyzed by modifications of existing methods. Interference by disaccharides and other sugars in the determination of dextrose and levulose has been eliminated, with resulting increase in accuracy. Analysis of the disaccharide fraction shows that reducing disaccharides are a general component of honey, as suggested by Hurd, *et al.* (4). The procedure has been applied to 22 floral types of honey, some of which have also been analyzed by other methods.

It is recommended† that the analytical method referred to above, using carbon column pretreatment, be tested collaboratively.

REFERENCES

(1) WHITE, J. W., JR., RICCIUTI, C., and MAHER, J., *This Journal*, **35**, 859 (1952).
(2) WHISTLER, R. L., and DURSO, D. F., *J. Am. Chem. Soc.*, **72**, 677 (1950).
(3) MCDONALD, E. J., and PERRY, R. E., JR., *J. Research Nat. Bur. Standards*, **47**, 363 (1951) (R. P. 2263).
(4) HURD, C. D., ENGLIS, D. T., BONNER, W. A., and ROGERS, M. A., *J. Am. Chem. Soc.*, **66**, 2015 (1944).

* One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.
† For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 80 (1954).

No reports were given on corn sirup and corn sugar, drying methods, or starch conversion products.

REPORT ON FRUITS AND FRUIT PRODUCTS

By R. A. OSBORN (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.), *Referee*

RECOMMENDATIONS

It is recommended*—

(1) That the method for total malic acid, laevo and inactive, as given in the Associate Referee's report, be adopted, first action.

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

(3) That the study of methods for the examination of frozen fruits for fruit, sugar, and water content be continued.

(4) That the study of methods for the determination of fill of container for frozen fruits be continued.

REPORT ON FRUIT ACIDS

DETERMINATION OF LAEVO-MALIC AND INACTIVE MALIC ACID

By L. W. FERRIS (Food and Drug Administration, Department of Health, Education, and Welfare, Buffalo 3, N. Y.), *Associate Referee*

A method for the determination of laevo-malic acid (1) in the absence of isocitric acid was presented at the 1952 meeting of this Association. The laevo-malic acid was estimated by the difference in polarization before and after saturating the filtrate from the tartaric acid determination with uranium acetate. It was found that, by inserting a chromatographic step in the above method, both laevo-malic acid and inactive malic acid could be separated from interfering isocitric acid and tartaric acid. Details of such a procedure were sent to collaborators during the past year and their results are presented in this report.

The chromatographic procedure used follows that of Isherwood (2) with two exceptions: first, the silicic acid column is packed in the tube with a piston as suggested by Neish (3), and secondly, in preparing the sample for chromatography, the isolated acid solution is not neutralized,

* For report of Subcommittee D and action of the Association, see *This Journal*, 37, 79 (1954).

and a large excess of H_2SO_4 is added to the sample before transfer to the column. By these variations, a large amount of malic acid can be concentrated in a small portion of the eluate. This is especially desirable so that an accurate polariscopic reading can be made on the laevo-malic acid.

Samples for the collaborative study of the method were prepared by adding pure laevo- and inactive malic acid to a commercial blackberry juice. The Associate Referee determined the amount of isocitric acid in the blackberry juice by the enzymatic method of Hargreaves, *et al.*, (4) using the A.O.A.C. method (5) for citric acid before and after the enzyme treatment (instead of the halide titration procedure of Hargreaves). The blackberry juice used was found to contain 0.75 per cent isocitric acid. The collaborative samples were made as shown in Table 1, and results of five collaborators are shown in Table 2.

TABLE 1.—*Composition of collaborative samples (grams per 100 ml)*

SAMPLE NO.	1	2	3
Blackberry juice	49.5	17.5	17.5
Isocitric acid	0.37	0.13	0.13
Natural *l*-malic acid[a]		0.04	0.04
Added *l*-malic acid		0.36	0.15
Total *l*-malic acid		0.40	0.19
Natural inactive malic acid[a]		0.02	0.02
Added inactive malic acid		0.16	0.36
Total inactive malic acid		0.18	0.38

[a] Calculated from average results of collaborators.

Details of the procedure for the determination of both laevo- and inactive malic acids sent with the samples to the collaborators follow:

DETERMINATION OF LAEVO- AND INACTIVE MALIC ACID

(Either or both isocitric acid and tartaric acid may be present.)

REAGENTS

(a) *Thymol blue indicator.*—**34.79(k).**

(b) *Phenolphthalein.*—1% in alcohol.

(c) *Solvent.*—Either 30% tertiary amyl alcohol, or 30% *n*-butyl alcohol in chloroform. (Eastman's Practical tert-amyl alcohol and U.S.P. $CHCl_3$ without further treatment have been found satisfactory.)

(d) *Silicic acid suitable for chromatography.*—Analytical reagent 100 mesh (No. 2847 Mallinckrodt Chemical Co. or equivalent).

TABLE 2.—*Collaborative results for malic acid (grams per 100 ml.)*

SAMPLE	1			2			3		
THEORETICAL	TOTAL	LAEVO	INACTIVE	TOTAL	LAEVO	INACTIVE	TOTAL	LAEVO	INACTIVE
(*Analyst*)	(See Table 1)			.58	.40	.18	.57	.19	.38
LWF	.16	.15	.01	.55	.39	.16	.54	.18	.36
	.16	.15	.01	.56	.38	.18	.54	.20	.34
HPE	.16	.14	.02	.51	.36	.15	.51	.17	.34
	.16	.14	.02	.51	.35	.15	.51	.18	.33
FEY	.16	.12	.04	.53	.37	.16	.55	.12	.43
	.16	.13	.03	.54	.37	.17	.54	.12	.42
TES	.13	.04	.09	.52	.27	.25	.53	.10	.43
	.15	.09	.06	.53	.28	.25	.52	.10	.41
	.13	.06	.07				.52	.16	.35
							.52	.16	.36
TJK	.17	.10	.07	.52	.34	.18	.55	.18	.37
	.17	.11	.06	.55	.37	.18	.54	.19	.35

APPARATUS

(a) *Chromatographic tubes.*—Ca 13 mm internal diam. and 400 mm long; may have a perforated disc or coarse sintered disc at beginning of constriction. Glass piston to fit tube.

(b) *Source of pressure.*—**18.11(c).**

STANDARDIZATION OF SILICIC ACID COLUMN

Mix in mortar to uniform powder 6 g of silicic acid and the amount of 0.5 *N* H_2SO_4 that will allow solvent to be eluted at rate of 1–1.5 ml per min. with a pressure of less than 1 atmosphere. The amount of 0.5 *N* H_2SO_4 required may vary with different batches of silicic acid; however, the silicic acid and 0.5 *N* H_2SO_4 must be measured accurately and the column for the detn made up exactly as under standardization. (3 ml of 0.5 *N* H_2SO_4 has been found satisfactory for one batch of silicic acid.)[1] Slurry with enough $CHCl_3$ to fill chromatograph tube. Place a small amount of cotton at bottom of tube and pour slurry into tube so that no air bubbles are occluded. Cut disc of filter paper with a cork borer to fit tightly inside tube and pack the silicic acid with piston until no more $CHCl_3$ is forced out. A column packed in this manner permits the sample to be stirred with solvent without disturbing column and also gives a sharper sepn of acids. Remove piston, pour the remaining $CHCl_3$ out top of the tube, and place 10 ml cylinder under the tube. Dissolve ca 10 mg malic acid in 1 ml 5 *N* H_2SO_4 in small beaker. Stir with 2 g of silicic acid—or enough to make a free flowing powder that does not adhere to beaker. Transfer thru funnel to column, rinse beaker with ca 5 ml of solvent (c), and pour thru funnel into tube. With long, thin rod stir powder and solvent in tube until all air bubbles are removed. Remove rod and stand it in sample beaker. With pressure, pack sample until solvent just disappears into jel. Rinse the long rod, beaker, and funnel with

[1] (See p. 553.—Ed.)

ca 2 ml solvent and sink into jel. Repeat washing with another 2 ml of solvent. Place plug of cotton in top of tube, wet it with solvent, and with rod push it down to top of sample. Fill tube with solvent and apply pressure so that eluate is forced out at rate of 1–1.5 ml per minute. Titrate eluate in 10 ml portions, rinsing cylinder with 10 ml CO_2-free H_2O and using thymol blue indicator. If the mixt. being titrated is swirled gently so that no emulsion is formed the end point is sharp and easily seen. When excess acid is present the indicator goes into the lower layer and as neutrality is reached, the indicator turns yellow and enters the aq. phase. Swirl and add alkali until lower layer is colorless and aq. layer is blue. Note amount of solvent (c) required to bring the malic acid to bottom of jel and the amount required to elute all the malic acid. In the same manner, det. the threshold vol. of citric acid. The vol. of solvent necessary to bring citric acid to bottom of column should be at least 20 ml more than that required to elute all the malic acid. During elution, the column will become semi-transparent at the top and progressively downward, but when malic acid is all eluted, 1 cm or more of the column should be unchanged in appearance. When the semi-transparency reaches the bottom of the column, H_2SO_4 may be carried into the eluate. (It has been found, using the above procedure, that the first 70 ml of eluate contained no malic acid and that the next 70 ml contained all the malic acid and no citric acid. However, the vol. required should be detd for the app. and the particular batch of silicic acid used.)

DETERMINATION

Proceed as in tartaric acid method, *This Journal*, **36**, 79 (1953), to line 12 under "Determination." (The 30% KOH and device for filtering at 0° are not used.) Conc. 200 ml of filtrate—do *not* neutralize—to ca 15 ml. Transfer with a small amount of H_2O to a tared beaker with bottom ca 3 cm diam. and evap. on steam bath to 1 g (±.05 g). A jet of air over surface of liquid may be used to hasten the evapn but no portion of the bottom of the beaker should be allowed to dry, as darkening of soln may occur with loss of malic acid. Cool, add 0.25 ml H_2SO_4 (1+1), 2 g silicic acid or slightly more if necessary to make a free flowing powder, and transfer to a prepd column as under standardization. Discard the vol. of eluate equal to the found threshold vol. of malic acid and collect in a 150 ml beaker the eluate that will contain all the malic acid and none of the citric. Evap. solvent on steam bath. (A jet of air over liquid hastens evapn and prevents loss by bumping.) Dissolve residue in ca 10 ml CO_2-free H_2O and titrate with 0.02 *N* NaOH, using phenolphthalein indicator. Correct titration for a blank on the eluate put thru a column as under detn. 1 ml 0.02 *N* NaOH = 1.34 mg malic acid; mg malic acid ÷ 0.64 = total malic acid in sample.

Acidify soln contg the neutralized malic acid with a drop of *N* acetic acid, evap. to ca 15 ml, transfer to a 25 ml volumetric flask, and proceed as under "Laevo-malic Acid" beginning at line 5, *This Journal*, **36**, 80 (1953). Total malic acid in sample minus laevo-malic acid = inactive malic acid.

Four of the five collaborators obtained quantities of total, laevo, and inactive malic acid approaching the theoretical, and although the fifth analyst reported some difficulty in obtaining check results, his recoveries are considered by the Associate Referee to be satisfactory for this type of determination.

ACKNOWLEDGMENT

The Associate Referee wishes to express appreciation to the following members of the Food and Drug Administration for suggestions and for

collaborative work: Dr. R. A. Osborn, Referee on Fruit Products, and other members of the Division of Food, Washington, D. C.; F. E Yarnall, Kansas City District; T. E. Strange, Portland Sub-District; T. J. Klayder, Denver District; and H. P. Eiduson, Buffalo District.

RECOMMENDATIONS

It is recommended*—

(1) That the method presented in this report for total malic acid, laevo, and inactive, be adopted, first action.

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

REFERENCES

(1) FERRIS, L. W., *This Journal*, **36**, 268 (1953).
(2) ISHERWOOD, F. A., *Biochem. J.*, **40**, 688 (1946).
(3) NEISH, A. C., *Can. J. Research*, **B27**, 6 (1949).
(4) HARGREAVES, C. A., ABRAHAMS, M. D., and VICKERY, H. B., *Anal. Chem.*, **23**, 467 (1951).
(5) *This Journal*, **34**, 74 (1951).

REPORT ON FILL OF CONTAINER, AND FRUIT, SUGAR, AND WATER IN FROZEN FRUITS

By R. A. OSBORN, *Referee* and C. G. HATMAKER (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

A. FILL OF CONTAINER STUDIES

The volume of a given weight of frozen fruit and sugar depends to some extent on the ratio of the ingredients, since the specific gravity of fruit is less than that of sugar. Slack filling of containers with fruit and sugar to be frozen has the effect of increasing the volume occupied by a given weight of the mixture. These conclusions can be drawn from consideration of the data in Table 1.

Marshall strawberries (sliced) from the Stanwood, Washington area (INV 78387K) were packed in containers ($3\frac{1}{2} \times 1\frac{3}{4} \times 5''$; water capacity, 486 ml) capable of holding 1 lb. net of 4+1 fruit and sugar. Three ratios of fruit to sugar: 4+1, 3+1, and 2+1, were packed with net weights of exactly 16, 15, and 14 oz. The table gives volumes of the frozen samples, the volumes calculated to net weights of 1 lb, and the headspace (before freezing) measured in sixteenths of an inch from top of container to top of contents. The procedure used for measuring the volumes of the frozen samples by displacement was that of Wallace and Osborn (1).

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 79 (1954).

TABLE 1.—*Volume of sliced strawberries plus sugar*

RATIO OF FRUIT TO SUGAR	NET WT.	VOLUME	FILL OF CONTAINER[a]	VOLUME CALC. FOR 16 OZ.	HEAD SPACE IN 1/16" BEFORE FREEZING
	(oz.)	(ml)	(per cent)	(ml)	
4+1	16	442	91	442	14
3+1	16	435	90	435	14
2+1	16	415	85	415	16
4+1	15	420	86	451	16
3+1	15	411	85	440	17
2+1	15	383	79	410	19
4+1	14	399	82	458	21
3+1	14	394	81	450	21
2+1	14	358	74	412	23

[a] Canco (tin ends, waxed paper sides) 3⅛×1⅞×5"; 486 ml.

Red Sour (Tart) Pitted Cherries, Fill of Tin Cans.—Commercial and experimental packs of frozen red sour pitted cherries, with sugar and with 65 per cent sugar syrup, were prepared at Geneva, New York (July 16, 1952; INV 8660L), and at Traverse City, Michigan (July 24, 1952; INV 54932L). Lithographed tin containers were used: *No. 1 Picnic*, 211×400: capacity, 310 ml=18.90 cu. in. (for commercial, 10.5 oz. net wt. 5+1 cherries); *No. 303*, 303×406: capacity, 479 ml=29.20 cu. in (for commercial 1 lb. net 5+1 cherries); and *No. 2 cans*, 307×409: capacity, 583 ml =35.55 cu. in. (for commercial, 1 lb. 4 oz. declared net weight cherries with 65.4 per cent sugar syrup—actually 71:29 ratio cherries to syrup; net 22.0 oz.). Table 2 contains a summary of the data obtained on examination of the commercial and experimental packs. The data represent averages from the examination of 6 cans of each subdivision.

It will be observed that commercial fills of 94 per cent, 89 per cent, and 99 per cent were obtained with the three can sizes; that one pound of 5+1 RSP cherries and sugar occupy approximately 425 ml when frozen (and a little larger volume when the containers are slack filled); that one pound of cherries with syrup, packed 3+1 fruit to syrup, occupies approximately 438 ml when frozen; and that a lower ratio of fruit to syrup results in smaller volumes for one pound.

In carrying out the laboratory work with frozen cherries it was found that care must be exercised to keep the sample intact as a frozen unit. Should the cherries break apart, the measurement of volume is affected.

Frozen Sliced Strawberries with Sugar and with Sugar Syrup.—The use of a flexible paper outer box in combination with an inner bag as a container for frozen fruits was observed in the Dayton, Tennessee area (32881L). The outer box measured 1¾×5¼×4" with a capacity of 467 ml. Sliced Blakemore strawberries and sugar were commercially packed with a declaration of 11 oz. net. Average fill on six commercial packages was 69 per cent of the capacity of the outer box. The net weight averaged 10.6 oz. An experimental pack of 4+1 sliced strawberries and sugar, accurately weighed, with put in weights of 12.5 oz. net, gave a fill (for six packages)

TABLE 2.—*Fill data for frozen sour pitted cherries*

LAB. NO.	CONTAINER	SIZE	CAPACITY	NET WEIGHT DECLARED	FOUND	TYPE OF PACK	VOL. CALCD. FOR 1 LB. FROZEN	FILL OF CONTAINER
			ml	*oz.*	*oz.*			*per cent*
8660 L	No. 1 picnic	211×310	310	10.5	11.0	5+1 sugar (commercial)	416[a] 421[b]	92[a] 96[b]
8660 L	No. 303	303×406	479	16	16.2	5+1 sugar (commercial)	416[a] 431[b]	87[a] 91[b]
8660 L	No. 303	303×406	479	—	16.0	5+1 sugar (exact)	423[a] 429[b]	88[a] 90[b]
8660 L	No. 303	303×406	479	—	16.0	12 oz. RSP cherries; 4 oz. 65% syrup (exact 75:25)	434[a] 441[b]	90[a] 92[b]
8660 L	No. 303	303×406	479	—	12.0	5+1 sugar (exact) (slack filled)	458[a] 450[b]	72[a] 70[b]
54932 L	No. 2	307×409	583	20.0	22.0	71:29 with 65% syrup (commercial)	420[a]	99[a]
54932 L	No. 2	307×409	583	—	20.0	15 oz. RSP cherries 5 oz. 65% syrup (exact 75:25)	438[a]	94[a]
54932 L	No. 2	307×409	583	—	22.0	16 oz. RSP cherries 6 oz. 65% syrup (exact 73:27 syrup)	424[a]	100[a]
54392 L	No. 2	307×409	583	—	18.0	5+1 sugar (exact) (slack filled)	436[a]	84[a]

[a] Food and Drug Adm., Food Division, Washington, D. C.
[b] Food and Drug Adm., Buffalo Laboratory.

of 75 per cent. These packages did not appear to be well filled. With exactly 9 oz. of sliced strawberries and 3 oz. of 61 per cent sugar syrup, an average fill of 74 per cent was obtained with six packages. They too were not well filled. It was calculated that one lb. of the 4+1 sliced strawberries and sugar requires a 445 ml volume when frozen, and that 1 lb. of the syrup pack would require a 461 ml volume.

Commercial and experimental packs of sliced Blakemore strawberries with sugar, from the Knoxville, Tennessee area, were prepared on May 12, 1952 (32880L). The subdivisions were packed and frozen in *No. 1 Picnic* tin cans, 211×400, 312 ml capacity. Table 3 summarizes the data on fill.

Since the mixing of the sliced strawberries with sugar in the commercial operation was continuous, it was not possible to state the ratio of the ingredients. The commercial cans had a fill of 96 per cent. Fill measurements were not made with a third strawberry pack (32882 L) since the shipment was partially thawed on arrival.

TABLE 3.—*Fill data for frozen sliced strawberries and sugar*

CONTAINER	TYPE OF PACK	AVERAGE VOLUME OF SAMPLE	FILL	CALCULATED VOLUME FOR 1 LB.
		(*ml*)	(*per cent*)	(*ml*)
No. 1 Picnic 211×400. (Capacity—312 ml for all)	Exact 4+1 ratio fruit to sugar. Experimental 10.5 oz. net	291	93	443
No. 1 Picnic 211×400. (Capacity—312 ml for all)	Exact 3+1 ratio fruit to sugar. Experimental 10.5 oz. net	279	89	425
No. 1 Picnic 211×400. (Capacity—312 ml for all)	Commercial Mixture 10.5 oz. declared 10.6 oz. found	300	96	453

B. THE COMPOSITION OF FROZEN FRUITS

Procedures for the examination of the frozen fruits for estimation of fruit, sugar, and water content have been described by Fallscheer and Osborn (2). This report gives additional data from other areas of production, including three strawberry packs from Tennessee, a red raspberry and a cherry pack from Michigan, and a cherry pack from New York State. The preparation and examination of the additional packs were along lines described in the earlier report.

Table 4 contains data on the chemical composition of the straight fruits used in the experimental fruit packs. The analyses are normal and in agreement with those previously reported by Sale (3) and by Fallscheer and Osborn (2).

The addition of sugar or sugar syrup to fruit contributes soluble solids

TABLE 4.—*Chemical composition of authentic fruits*[a]

	STRAWBERRIES (TENNESSEE)			LATHAM RED RASPBERRIES (MICHIGAN)	RED SOUR PITTED MONTMORENCY CHERRIES (MICHIGAN)	RED SOUR PITTED MONTMORENCY CHERRIES (NEW YORK STATE)
Sample No.	32880 L[b]	32881 L[b]	32882 L[b]	54931 L[c]	54932 L[c]	8660 L[d]
Soluble solids, %	7.2	7.3	8.9	8.7	13.9	18.2
*p*H	3.31	3.23	3.15	3.28	3.50	3.39
Water Insoluble solids, %	2.25	2.09	2.34	4.26	1.35	1.48
Acidity as anhyd. citric, %	0.95	0.95	1.26	1.32	1.39	1.46
Ash, %	0.36	0.35	0.44	0.36	0.39	0.36
K_2O, mg/100 g	180	174	210	159	224	189
K_2O, % of ash	50.0	49.7	47.7	44.2	57.4	52.5
P_2O_5, mg/100 g	33.3	28.2	32.3	32.2	32.5	40.2
P_2O_5, % of ash	9.3	8.1	7.3	8.9	8.3	11.2
Total sugars as invert, %	4.1	3.9	5.2	6.2	8.4	10.7

[a] *Official Methods of Analysis*, A.O.A.C., 7th Ed. (1950).
[b] Analysis by Frank H. Collins and Arthur C. Thompson, Food and Drug Adm., Cincinnati Laboratory.
[c] Analysis by John H. Bornmann, Food and Drug Adm., Chicago Laboratory.
[d] Analysis by J. Thomas Welch, Food and Drug Adm., Buffalo Laboratory.

to the mixture. It reduces the percentages of water-insoluble solids, acidity, ash, K_2O, and P_2O_5 in direct proportion to the weight of sugar and water added. The conventional procedure for determining the amount of fruit in mixtures is based on chemical analyses for the fruit constituents and comparison of the values obtained with those obtained from analyses of authentic samples of fruits.

Fallscheer and Osborn (2) suggest rapid alternate procedures for the estimation of fruit, sugar, and added water. The formula for per cent of fruit, X, in a mixture of fruit and sugar only, is:

$$X = \frac{(100 - M)100}{100 - F}$$

where M is the soluble solids of the fruit and sugar mixture, and F the soluble solids of the fruit. Soluble solids are readily obtained by use of the refractometer on a portion of the well mixed sample. Table 5 summarizes the data obtained for fruit content from the examination of the fruit and sugar packs. Calculations are based on: (a) the soluble solids of the samples of fruit going into the packs; and (b) on the average soluble solids of many authentic samples of strawberries (8.0 per cent), sour cherries (13.9 per cent), and red raspberries (10.5 per cent). (Food and Drug Administration personnel collected these samples over a period of years from the areas of production in the United States.)

It will be observed that the use of the average soluble solids for authentic fruits results in a calculated fruit content in good agreement with

TABLE 5.—*Fruit content of fruit and sugar packs calculated from soluble solids (SS)*

SAMPLE NO.	FRUIT	SUB. NO.	AV. SS OF SAMPLE *per cent*	FRUIT:SUGAR RATIO	CALCULATED FRUIT CONTENT	
					USING SS OF PUT-IN FRUIT	USING AV. SS OF AUTHENTICS[b]
32880 L	Strawberry	A	7.1 7.2[a]	Fruit only Fruit only		
32880 L	Strawberry	D	30.7 31.4[a]	75:25	75 74	75 75
32880 L	Strawberry	G	25.8 26.6[a]	80:20	80 79	81 80
32880 L	Strawberry	C	20.4 19.8[a]	Commercial Mixture	85.7 86.4	86 87
32881 L	Strawberry	A	7.0 7.3[a]	Fruit only		
32881 L	Strawberry	D	24.8 26.2[a]	80:20	81 80	82 80
32881 L	Strawberry	C	23.4 24.4[a]	Commercial Mixture	82 82	83 82
32882 L	Strawberry	A	9.7 8.9[a]	Fruit only		
32882 L	Strawberry	D	32.2 30.2[a]	79:21	75 77	74 76
32882 L	Strawberry	E	26.2 26.2[a]	5+1 83:17	82 81	80 80
32882 L	Strawberry	C	34.2 32.9[a]	Commercial Mixture	73 74	72 73
8660 L	RSP Cherries	A	18.2[a]	Fruit only		
8660 L	RSP Cherries	D	32.7	5+1 83:17	82	78
8660 L	RSP Cherries	F	29.9	5+1 83:17	86	81
8660 L	RSP Cherries	C	31.1	Commercial (5+1) No. 303 can	84	80
8660 L	RSP Cherries	V	31.2	Commercial (5+1) 83:17 (5+1) No. 1 Picnic	84	80
54932 L	RSP Cherries	A	14.5 13.9[a]	Fruit only		
54932 L	RSP Cherries	F	28.0 28.5[a]	5+1 83:17	84 83	84 83

[a] Collaborative analyses by F. H. Collins and A. C. Thompson (32880-2L), H. Thomas Welch (8660 L) and John Bornmann (54931-2L). See Table 4.
[b] Strawberry 8.0%, cherry 13.9%, Red Raspberry 10.5%.

TABLE 6.—*Drained weights (20°C.) of frozen fruits, and soluble solids (refractometer) of whole samples*

SAMPLE NO.	SUB. NO.	PACKING MEDIUM	RATIO OF FRUIT TO PACKING MEDIUM	SOLUBLE SOLIDS (WHOLE SAMPLE)	DRAINED WEIGHT ×100 DIVIDED BY PUT-IN WEIGHT OF FRUIT	
RSP Cherries	A	none	fruit only	14.5		
(54932 L	C	65.4%	71:29	31.4	89	
Michigan)		syrup	(average)[d]	29.6[a]	89[a]	84[b]
	D	"	75:25	29.2	91	
			(exact)	29.3[a]	93[a]	
	E	"	73:27	27.9	85	81[b]
			(exact)	28.3[a]	87[a]	
	F	sugar	5+1	28.0	81	
			(exact)	28.5[a]	85[a]	
RSP Cherries	A	none	fruit only	18.1		
(8660 L	D	sugar	5+1	32.7	92	
New York			(exact)			
State)	E	60.3%	75:25	29.2	93	83[b]
		syrup	(exact)	28.8		
	F	"	75:25	29.9	91	80[b]
			(exact)	29.5		
Strawberries,	A	none	fruit only	7.1	58	
sliced.				7.2[c]	59[c]	
(32880 L	D	sugar	3+1	30.7	70	
Tenn.)			(exact)	31.4[c]	67[c]	
				30.2		64[b]
	G	sugar	4+1	25.8	71	
			(exact)	26.6[c]	65[c]	
				25.3		66[b]
Strawberries,	A	none	fruit only	7.0	62	
sliced.				7.3[c]	64[c]	
(32881 L	D	sugar	4+1	30.7	70	
Tenn.)			(exact)	31.4[c]	67[c]	
				30.2		64[b]
	E	61%	75:25	21.0	64	
		syrup	(exact)	21.1[c]	68[c]	
				20.7		61[b]
Strawberries,	A	none	fruit only	9.7	55	
sliced.				8.9[c]	54[c]	
(32882 L	D	sugar	79:21	32.2	69	
Tenn.)			(exact)	30.2[c]	63[c]	
				30.8		65[b]

[a] Analyses by Chicago Laboratory, Food and Drug Administration.
[b] Not thawed in original container.
[c] Analyses by Cincinnati Laboratory, Food and Drug Administration.
[d] Commercial pack; some variation in ratio of fruit to syrup from can to can.

TABLE 6—(*continued*)

SAMPLE NO.	SUB. NO.	PACKING MEDIUM	RATIO OF FRUIT TO PACKING MEDIUM	SOLUBLE SOLIDS (WHOLE SAMPLE)	DRAINED WEIGHT ×100 DIVIDED BY PUT-IN WEIGHT OF FRUIT	
	E	sugar	5+1	26.2	64	
			(exact)	26.2[c]	60[c]	
				25.7		61[b]
	G	60%	75:25	24.2	68	
		syrup	(exact)	24.0[c]	69[c]	
				24.1		63[b]
Red	A	none	fruit only	9.7		
Raspberries				8.7[a]		
54931 L Mich.)	D	56%	67:33	26.3	74	
		syrup	(exact)	25.7		66[b]
				25.5[a]	78[a]	
	C	"	59:41	29.3	73	
			(average)[a]	29.2	74	
				29.2		71[b]
				28.8[a]	79[a]	
				28.5[a]	84[a]	

[a] Commercial pack with variation of syrup put-in from 3.9 to 4.9 oz., av. 4.6 oz. Weight of fruit in cans estimated by subtracting 4.6 oz. from net weight of mixture.

that actually put in. In the examination of commercial lots of frozen fruits for fruit content, it is not to be expected that the analyst will have available a sample of the actual fruit going into the pack.

Commercial packers can use the refractometer to check their ratio of fruit to packing medium during the packing operation. They will thus be able to determine whether they are obtaining reasonable uniformity in their mixtures of fruit and sugar, or fruit and syrup.

Drained Weight of Frozen Fruit Packs.—The procedure employed in obtaining drained weight of subdivisions of the frozen fruit packs described in this report is given by Fallscheer and Osborn (2). In the present investigation it was found preferable to allow the packages to thaw in their original containers, without disturbing the contents before draining. For comparison, a number of subdivisions were first examined for fill of container; the packages were then thawed, and drained weights were determined. In those instances where the frozen samples were transferred to a plastic bag (in order to measure fill) and then thawed (by immersing in water), the drained weights were lower than corresponding samples which thawed undisturbed.

Collaborative results for drained weight were obtained. The data reported in Table 6 represent averages from the examination of 6 to 12 packages of each subdivision.

The drained weight values obtained for cherries (89 per cent), sliced strawberries (66 per cent), and red raspberries (77 per cent), are in agreement with values previously reported (2). The use of drained weight determinations under controlled conditions provides a rapid alternate procedure for estimating the proportion of fruit in frozen fruit packs.

ACKNOWLEDGMENT

Assistance and helpful suggestions in the preparation of the fruit packs were obtained from Food and Drug Administration inspectors John S. Shanly (Buffalo District), Theodore R. Steiskal (Chicago District), and John Fletcher (Cincinnati District). Collaborative analyses and constructive comments were made by Food and Drug Administration chemists J. Thomas Welch and O. C. Kenworthy (Buffalo District); John H. Bornmann and Iman Schurman (Chicago District); and Frank H. Collins, Arthur C. Thompson, and C. B. Stone (Cincinnati District).

The following frozen fruit packers provided facilities and assistance in the preparation of the investigational packs, and their cooperation is acknowledged:

William E. McIntosh, Inc., Geneva, New York
Producers Service Corporation, Benton Harbor, Michigan
Cherry Growers, Inc., Traverse City, Michigan
Winter Garden Co., Inc., Knoxville, Tennessee
Southern Freezing and Preserving Co., Dayton, Tennessee
Southland Frozen Foods, Inc., Portland, Tennessee

REFERENCES

(1) WALLACE, W. W., and OSBORN, R. A., *This Journal*, **36**, 860 (1953).
(2) FALLSCHEER, H. O., and OSBORN, R. A., *ibid.*, **36**, 270 (1953).
(3) SALE, J. W., *ibid.*, **21**, 502 (1938).

REPORT ON FERTILIZERS

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

Reports were received from Associate Referees on ammoniacal solutions and liquid fertilizers; boron; magnesium and manganese; nitrogen; phosphoric acid; and potash. Their recommendations are approved.

It is recommended*—

* For report of Subcommittee A and action of the Association, see *This Journal*, 37, 61 (1954).

(1) That investigation of methods for the direct determination of available P_2O_5 in fertilizers be continued, and that the use of perchloric acid in the preparation of phosphate fertilizer solutions be studied.

(2) That the interference of minor elements in methods for potash and the use of the flame photometer for that determination be studied further.

(3) That collaborative study of methods for boron be continued and that a procedure for active water-insoluble boron be devised.

(4) That methods for collection, storage, and transfer of aliquots of samples of ammoniacal liquids be studied further.

(5) That the method for water-soluble magnesium in coarse particles presented in 1952 by the Associate Referee be changed to prescribe a sieve with openings of 420 microns and be adopted, first action.

(6) That methods for sampling and sample preparation be studied further.

(7) That all the Referees continue their work during the coming year.

REPORT ON SAMPLING AND PREPARATION OF FERTILIZER SAMPLE*

By STACY B. RANDLE (New Jersey Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

The Associate Referee's report on this topic last year was based upon a preliminary study conducted during 1952. In accordance with the recommendations of Subcommittee A, the investigation has been continued and expanded.

The purpose of this investigation is to study procedures for fertilizer sampling and preparation of the sample for analysis. A survey last year revealed that some regulatory agencies reduced the size of the sample by quartering in the field while others forwarded the entire sample to the laboratory for preparation for analysis. In order to formulate a basis for comparison of these two procedures, the present study was undertaken. The Associate Referee realizes the procedures for this study are arbitrary and the comparisons may be limited; yet this represents an attempt to simulate current sampling practices.

The procedure for sampling and hand quartering of the fertilizer sample was continued essentially as directed last year, but for the sake of clarity, minor changes in the directions were made. The project was enlarged by the inclusion of directions for riffle quartering for those collaborators who were able to undertake such a study. The instructions for these studies were as follows:

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* Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, State University of New Jersey, Department of Chemistry.

HAND QUARTERING STUDY

Sample shipment of high analysis fertilizer according to A.O.A.C. sampling procedure 2.1 (*Official Methods of Analysis*, 7th Ed., p. 6). Quarter sample in field as follows: Thoroughly mix portions taken on clean oilcloth, paper, or rubber sheet. After thoroughly mixing, roll sample so as to produce a cone or pyramid. Divide sample into quarters with spatula or metal sheet. Take two opposite quarters and repeat mixing and quartering until sample of approximately 8 ounces is obtained. Transfer this sample to an air-tight, screw top bottle. Save *all* the remaining portions removed by the coning and quartering procedure and transfer them to an air-tight, screw top container. Transmit both samples to the laboratory.

Grind each sample for analysis. In order to have a workable laboratory sample it may be necessary to reduce the larger sample by coning and quartering after grinding.

Thoroughly mix each sample and make triplicate determinations for nitrogen, total phosphoric acid, and potash. The insoluble phosphoric acid may be determined, if time permits. Report each analysis on the attached sheet.

The results are to reach the Associate Referee by August 1, if possible. It is suggested that each collaborator secure three or four samples for this study.

FOR RIFFLE QUARTERING STUDY

Sample shipment of high analysis fertilizer according to A.O.A.C. sampling procedure 2.7 (*Official Methods of Analysis*, 7th Ed.). Thoroughly mix portions taken on clean oilcloth, paper, or rubber sheet. Reduce sample by passing through a riffle sampler. Remove portion from one side of riffle and save. Then reduce the other portion by passing it through riffle again. Continue this reduction until a sample of approximately 8 ounces is obtained. Place it in an air-tight, screw top container. Then combine all other portions from the riffle separations into one sample and place it in an air-tight, screw top container. Transmit both samples to the laboratory.

Grind each sample for analysis. In order to have a workable laboratory sample it may be necessary to reduce the larger sample by riffling again after the sample is ground.

Thoroughly mix each sample and make triplicate determinations for nitrogen, total phosphoric acid, and potash. The insoluble phosphoric acid may be determined, if time permits. Report each analysis on the attached sheet.

The results are to reach the Associate Referee by August 1, if possible. It is suggested that each collaborator secure three or four samples for this study.

DISCUSSION

Table 1 presents the chemical analysis of the one-quarter and three-quarter portions of 37 fertilizer samples, representing 29 grades, studied by 10 collaborators who used the hand quartering procedure. Table 2 presents the chemical analysis of the one-quarter and three-quarter portions of 22 fertilizer samples, representing 16 grades, studied by 7 collaborators who used the riffle quartering procedure. It should be noted that some collaborators used both the hand and the riffle quartering procedures. In several instances the collaborator subjected the same grade to both types of quartering. There were a total of 12 collaborators participating in the study.

TABLE 1

HAND QUARTERED

Analyses of one-quarter and three-quarter fertilizer samples[a]

COLLABORATOR	GRADE	ONE-QUARTER SAMPLE ¼				THREE-QUARTER SAMPLE				DIFFERENCE			
		N	TPA	APA	K_2O	N	TPA	APA	K_2O	N	TPA	APA	K_2O
1	10–10–10	9.74	10.45		10.60	9.79	10.65		10.81				
		9.83	10.39		10.57	9.83	10.54		10.75				
							10.76						
	Av.	9.78	10.42		10.58	9.81	10.65		10.78	+.03	+.23		+.20
	8–16–16	7.79	16.92		16.53	7.70	17.07		16.50				
		7.83	16.83		16.53	7.75	16.98		16.52				
	Av.	7.81	16.88		16.53	7.72	17.02		16.51	−.09	+.14		−.02
	5–15–15	5.02	15.92		15.51	5.00	15.87		15.65				
		5.04	15.73		15.49	5.02	15.88		15.56				
			15.83										
	Av.	5.03	15.83		15.50	5.01	15.88		15.60	−.02	+.05		+.10
	0–20–20		20.05		21.33		20.70		20.20				
			19.93		21.27		20.48		20.24				
			20.20				20.68						
	Av.		20.06		21.30		20.62		20.22		+.56		−.08
2	14– 0–14	13.80			15.60	13.80			15.30				
		13.80			15.70	13.90			15.40				
		13.90			15.65	14.00			15.50				
	Av.	13.83			15.65	13.90			15.40	+.07			−.25
	8– 0–24	7.70			24.50	7.70			24.35				
		7.80			24.70	7.60			24.35				
		7.70			24.50	7.60			24.25				
	Av.	7.73			24.57	7.63			24.32	−.10			−.25
	4–16–16	3.68	18.90	18.10	14.85	4.10	18.32	17.42	15.20				
		3.70	18.95	18.15	14.95	4.15	18.30	17.40	15.20				
		3.70	18.95	18.15	14.85	4.13	18.35	17.45	15.35				
	Av.	3.69	18.93	18.13	14.88	4.13	18.32	17.42	15.25	+.44	−.61	−.71	+.37

[a] N = Nitrogen; TPA = Total Phosphoric Acid; APA = Available Phosphoric Acid; K_2O = Potash.

	0–10–30		10.55	10.25	30.05		10.55	10.25	30.90			
			10.70	10.40	30.15		10.55	10.25	30.90			
			10.60	10.30	30.25		10.55	10.25	30.55			
	Av.		10.62	10.32	30.15		10.55	10.25	30.78		−.07	−.07	+.63
	10–10–10	9.50	10.45	10.15	9.60	9.35	10.55	10.25	9.70			
		9.50	10.55	10.25	9.70	9.25	10.55	10.25	9.80			
		9.50	10.55	10.25	10.00	9.45	10.55	10.25	9.90			
	Av.	9.50	10.52	10.32	9.77	9.35	10.55	10.25	9.80	−.15	+.03	−.07	+.03
3	5–10–13	4.00	12.50		11.30	4.00	12.90		11.00			
		4.10	12.60		11.30	4.00	12.90		10.80			
	Av.	4.05	12.55		11.30	4.00	12.90		10.90	−.05	+.35		−.40
	4–24–12	3.90	17.70		8.70	3.90	17.50		8.90			
		3.90	17.50		8.60	3.90	17.40		9.10			
	Av.	3.90	17.60		8.65	3.90	17.45		9.00	.00	−.15		+.35
	10–10–10	9.30	11.50		9.50	9.30	11.50		9.60			
		9.30	11.60		9.80	9.30	11.40		9.90			
	Av.	9.30	11.55		9.65	9.30	11.45		9.75	.00	−.10		+.10
6	15– 8– 4	14.88	8.80	8.60	4.73	14.88	8.45	8.30	4.68			
		14.92	8.85	8.65	4.68	14.86	8.35	8.20	4.70			
		14.92	8.75	8.55	4.73	14.96	8.30	8.15	4.68			
	Av.	14.91	8.80	8.60	4.71	14.90	8.37	8.22	4.69	−.01	−.43	−.38	−.02
	15– 8– 4	15.10	8.40	8.10	4.16	15.16	7.95	7.70	4.29			
		15.00	8.35	8.05	4.13	15.28	8.05	7.80	4.27			
		15.02	8.30	8.00	4.18	15.26	7.90	7.65	4.23			
	Av.	15.04	8.35	8.05	4.16	15.23	7.97	7.72	4.26	+.19	−.38	−.33	+.10
	10–16– 8	10.03	16.15	15.25	9.28	10.17	16.20	15.30	8.94			
		10.07	16.15	15.25	9.24	10.13	16.30	15.40	8.95			
		9.99	16.15	15.25	9.27	10.10	16.40	15.50	9.00			
	Av.	10.03	16.15	15.25	9.26	10.13	16.30	15.40	8.96	+.10	+.15	+.15	−.30
	14–14–7	12.96	15.25	15.15	6.73	12.80	15.90	15.80	6.74			
		13.00	15.35	15.25	6.76	12.80	15.85	15.75	6.69			
		12.94	15.45	15.35	6.73	12.84	15.70	15.60	6.67			
	Av.	12.97	15.35	15.25	6.74	12.81	15.82	15.72	6.70	−.16	+.47	+.47	−.04

TABLE 1—(*continued*)

COLLABORATOR	GRADE	ONE-QUARTER SAMPLE				THREE-QUARTER SAMPLE				DIFFERENCE			
		N	TPA	APA	K_2O	N	TPA	APA	K_2O	N	TPA	APA	K_2O
7	6–12–12	6.12	13.20		12.05	6.14	12.56		12.29				
		6.17	13.26		12.05	6.18	12.64		12.17				
		5.97	12.94		12.05	6.20	12.45		12.29				
	Av.	6.09	13.13		12.05[b]	6.17	12.55		12.25[b]	+.08	+.42		+.20
	8–16–16	7.89	18.10		14.46	7.86	17.95		14.46				
		7.94	18.10		14.46	7.89	18.10		14.46				
		7.99	18.20		14.46	7.87	17.92		14.46				
	Av.	7.94	18.13		14.46[b]	7.87	17.99		14.46[b]	−.07	−.14		.00
	0–20–20		21.22		19.88		21.49		19.88				
			21.18		19.88		21.39		19.88				
			21.20		19.88		21.30		19.88				
	Av.		21.20		19.88[b]		21.40		19.88[b]		+.20		.00
8	3–12–12	3.04	12.65	12.05	12.00	3.01	12.65	12.06	12.20				
		3.05	12.23	11.75	12.21	2.93	12.42	11.80	12.04				
		3.00	12.17	11.57	12.28	3.01	12.65	12.00	12.33				
	Av.	3.03	12.35	11.79	12.16	2.98	12.57	11.95	12.19	−.05	+.22	+.16	+.03
	4–12– 4	3.93	13.19	12.52	4.16	4.00	13.02	12.30	4.14				
		3.91	12.53	11.79	4.23	3.90	12.56	11.79	4.25				
		4.01	13.15	12.37	4.16	3.81	12.67	11.95	4.16				
	Av.	3.95	12.99	12.23	4.18	3.90	12.75	12.01	4.17	−.05	−.24	−.22	−.01
	3– 9–27	2.96	10.00	9.22	26.39	2.96	10.14	9.44	26.88				
		2.93	10.20	9.55	26.44	2.92	9.95	9.22	26.61				
		2.94	10.30	9.60	26.54	3.01	10.15	9.42	26.55				
	Av.	2.94	10.20	9.46	26.46	2.96	10.08	9.36	26.68	+.02	−.12	−.10	+.22
9	7– 7– 7	7.02	7.54	7.28	7.14	7.05	7.60	7.33	7.26				
		6.96	7.54	7.31	7.14	7.05	7.57	7.35	7.26				
		7.07	7.60	7.34	7.18	7.01	7.68	7.46	7.30				
	Av.	7.02	7.56	7.31	7.15	7.04	7.62	7.38	7.27	+.02	+.06	+.07	+.12

[b] Flame photometer.

9	6– 9–12	6.08	9.36	9.26	11.82	6.15	9.40	9.29	12.06			
		6.15	9.40	9.31	11.76	6.19	9.44	9.33	12.14			
		6.08	9.34	9.23	11.78	6.11	9.39	9.24	12.06			
	Av.	6.10	9.37	9.27	11.79	6.15	9.41	9.29	12.09	+.05	+.04	+.02	+.30
	9–14–18	8.53	14.97	14.24	20.36	8.92	15.73	15.00	19.42			
		8.44	14.98	14.39	20.36	8.94	15.62	14.96	19.66			
		8.51	15.22	14.56	20.48	9.00	15.24	14.47	19.58			
	Av.	8.49	15.06	14.40	20.40	8.95	15.53	14.81	19.55	+.46	+.47	+.41	−.85
	10–12–20	9.05	12.58	12.38	21.60	9.23	12.05	11.74	21.80			
		9.02	12.54	12.39	21.68	9.26	12.35	12.22	21.72			
		9.08	12.53	12.39	21.60	9.23	12.19	12.00	21.72			
	Av.	9.05	12.55	12.39	21.63	9.24	12.20	11.99	21.75	+.19	−.35	−.40	+.12
	6–12– 6	6.06	11.91	11.83	6.60	6.04	11.81	11.70	6.52			
		6.02	11.89	11.75	6.52	6.08	11.91	11.82	6.56			
		6.06	12.00	11.89	6.56	6.06	11.86	11.75	6.60			
	Av.	6.05	11.93	11.82	6.56	6.06	11.86	11.76	6.56	+.01	−.07	−.06	.00
10	7–14–14	7.11	13.30	13.00	14.40	6.95	13.25	12.95	14.62			
		7.03	13.22	12.92	14.28	6.95	13.29	12.99	14.62			
		7.07	13.20	12.90	14.35	6.86	13.07	12.77	14.62			
	Av.	7.07	13.24	12.94	14.34	6.92	13.20	12.90	14.62	−.15	−.04	−.04	+.28
	14–28–14	14.81	27.70	27.70	12.55	14.27	27.68	27.68	13.44			
		14.73	27.80	27.80	12.67	14.19	27.96	27.96	12.96			
		14.68	27.74	27.74	12.72	14.23	28.34	28.34	13.39			
	Av.	14.74	27.75	27.75	12.65	14.23	28.00	28.00	13.26	−.51	+.25	+.25	+.61
	10–20–20	10.32	19.70	19.30	17.33	10.23	19.66	19.26	17.35			
		10.28	19.60	19.20	17.30	10.28	19.64	19.24	17.40			
		10.19	19.64	19.24	17.24	10.19	19.90	19.50	17.62			
	Av.	10.26	19.65	19.25	17.33	10.23	19.73	19.33	17.46	−.03	+.08	+.08	+.13
	10–10–10	9.90	10.10	9.90	9.00	9.90	10.12	9.92	9.12			
		9.98	10.05	9.85	9.00	9.82	10.12	9.92	9.18			
		9.98	10.14	9.94	9.00	9.86	10.11	9.91	9.16			
	Av.	9.95	10.10	9.90	9.00	9.86	10.12	9.92	9.15	−.09	+.02	+.02	+.15

TABLE 1—(*continued*)

COLLABORATOR	GRADE	ONE-QUARTER SAMPLE				THREE-QUARTER SAMPLE				DIFFERENCE			
		N	TPA	APA	K_2O	N	TPA	APA	K_2O	N	TPA	APA	K_2O
	8–16–16	7.24	17.81	16.71	15.24	7.24	17.02	16.52	16.03				
		7.28	17.65	16.95	15.26	7.20	16.85	16.35	15.96				
		7.20	17.64	16.94	15.12	7.28	16.98	16.48	15.86				
	Av.	7.24	17.70	16.88	15.21	7.24	16.95	16.45	15.95	.00	−.75	−.43	+.74
12	13–13–13	12.84	14.30	13.98	13.09	12.72	14.85	14.60	13.38				
		12.88	14.00	13.72	13.09	12.64	15.15	14.90	13.26				
		12.80	14.00	13.75	12.99	12.72	14.85	14.64	13.48				
	Av.	12.84	14.10	13.82	13.06	12.69	14.95	14.71	13.37	−.15	+.85	+.89	+.31
	12–24–12	11.80	26.30	25.92	12.20	11.84	26.30	25.87	12.02				
		11.84	26.30	25.90	11.89	11.80	26.30	25.87	12.02				
		11.82	25.90	25.58	11.89	11.80	25.90	25.52	11.96				
	Av.	11.82	26.17	25.80	11.99	11.81	26.17	25.75	12.00	−.01	.00	−.05	+.01
	16–20– 0	15.64	21.75	21.12		15.68	21.75	21.09					
		15.72	21.75	20.99		15.72	21.75	21.02					
		15.68	21.75	20.99		15.76	21.75	21.06					
	Av.	15.68	21.75	21.03		15.72	21.75	21.06		+.04	.00	+.03	
	10–30–10	10.28	32.80	31.48	10.79	10.22	32.80	31.31	10.92				
		10.31	32.80	31.42	10.79	10.22	32.10	30.67	10.92				
		10.26	32.80	31.42	10.79	10.26	32.80	31.42	10.92				
	Av.	10.28	32.80	31.44	10.79	10.23	32.57	31.13	10.92	−.05	−.23	−.31	+.13
	10–10–10	8.60	11.57	10.71	8.90	8.90	11.57	10.77	8.94				
		8.64	11.57	10.74	8.75	8.86	11.83	11.00	8.94				
		8.62	11.83	10.94	8.86	8.86	11.83	11.00	9.02				
	Av.	8.62	11.66	10.80	8.84	8.87	11.74	10.92	8.97	+.25	+.08	+.12	+.13

TABLE 2

RIFFLE QUARTERED

Analyses of one-quarter and three-quarter fertilizer samples

COLLABORATOR	GRADE	ONE-QUARTER SAMPLE				THREE-QUARTER SAMPLE				DIFFERENCE			
		N	TPA	APA	K_2O	N	TPA	APA	K_2O	N	TPA	APA	K_2O
1	10–10–10	9.65	10.63		10.64	9.67	10.68		10.52				
		9.73	10.66		10.62	9.62	10.66		10.62				
	Av.	9.69	10.64		10.63	9.64	10.67		10.57	−.05	+.03		−.06
	8–16–16	7.94	16.47		16.87	7.80	16.57		16.66				
		7.86	16.38		16.87	7.86	16.48		16.74				
	Av.	7.90	16.42		16.87	7.83	16.52		16.70	−.07	+.10		−.17
	5–15–15	5.02	15.92		15.54	5.02	16.17		15.56				
		5.02	15.83		15.55	5.02	16.23		15.54				
	Av.	5.02	15.88		15.54	5.02	16.20		15.55	.00	+.32		+.01
	0–20–20		21.02		20.11		20.43		20.26				
			20.95		20.14		20.70		20.32				
							20.55						
	Av.		20.98		20.12		20.56		20.29		−.42		+.17
2	3– 9–27	3.10	10.45	9.90	25.70	3.20	10.55	10.05	25.50				
		3.20	10.45	9.90	25.70	3.20	10.55	10.05	25.60				
		3.10	10.45	9.90	25.95	3.20	10.55	10.05	25.60				
	Av.	3.13	10.45	9.90	25.78	3.20	10.55	10.05	25.57	+.07	+.10	+.15	−.21
3	5–10–13	4.00	12.90		11.10	4.00	12.80		11.10				
		4.00	12.90		11.20	4.00	12.80		11.10				
	Av.	4.00	12.90		11.15	4.00	12.80		11.10	.00	−.10		−.05
	4–24–12	3.80	17.70		8.80	3.80	17.40		8.40				
		3.90	17.60		9.00	3.80	17.70		8.60				
	Av.	3.85	17.65		8.90	3.80	17.55		8.50	−.05	−.10		−.40
	10–10–10	9.30	11.40		9.80	9.10	11.30		10.10				
		9.30	11.40		9.80	9.10	11.50		10.10				
	Av.	9.30	11.40		9.80	9.10	11.40		10.10	−.20	.00		+.30

TABLE 2—(*continued*)

RIFFLE QUARTERED

Analyses of one-quarter and three-quarter fertilizer samples

COLLABORATOR	GRADE	ONE-QUARTER SAMPLE				THREE-QUARTER SAMPLE				DIFFERENCE			
		N	TPA	APA	K_2O	N	TPA	APA	K_2O	N	TPA	APA	K_2O
4	10–20–10	9.47	21.05	20.01	12.13	9.55	21.05	20.05	12.09				
		9.47	21.05	20.01	12.07	9.47	21.10	20.12	12.10				
		9.48	21.15	20.13	11.96	9.51	21.15	20.15	12.12				
	Av.	9.47	21.08	20.05	12.05	9.51	21.10	20.11	12.10	+.04	+.02	+.06	+.05
	3– 9–27	3.32	8.50	7.87	15.78	3.23	8.18	7.54	15.81				
		3.34	8.48	7.85	15.85	3.20	8.10	7.49	15.89				
		3.31	8.48	7.82	15.89	3.17	8.20	7.57	15.74				
	Av.	3.32	8.49	7.85	15.84	3.20	8.16	7.53	15.81	−.12	−.33	−.32	−.03
	4–16–16	5.04	15.55	14.87	18.05	5.00	15.35	14.65	17.95				
		5.04	15.65	14.93	17.83	5.02	15.33	14.67	17.96				
		5.05	15.45	14.71	17.88	5.02	15.33	14.75	18.12				
	Av.	5.04	15.55	14.87	17.92	5.01	15.34	14.69	18.01	−.03	−.21	−.18	+.09
	12–24–12	11.70	24.25	23.97	13.62	11.70	24.20	23.93	13.62				
		11.69	24.20	23.94	13.64	11.77	24.30	24.03	13.65				
		11.73	24.20	23.96	13.69	11.70	24.25	23.97	13.51				
	Av.	11.71	24.22	23.96	13.65	11.72	24.25	23.98	13.59	−.01	+.03	+.02	−.06
	12–12–12	12.04	12.00	11.36	12.48	11.90	12.40	11.90	12.28				
		12.09	11.95	11.35	12.45	11.90	12.33	11.75	12.21				
		12.09	11.90	11.45	12.46	11.89	12.33	11.73	12.16				
	Av.	12.07	11.95	11.39	12.46	11.90	12.35	11.79	12.22	−.17	+.40	+.40	−.24
5	3–18– 9	3.28	20.30		9.54	3.26	20.10		9.44				
		3.27	20.35		9.54	3.27	20.15		9.52				
		3.10	20.00		9.60	3.11	20.25		9.52				
	Av.	3.22	20.22		9.56	3.21	20.16		9.49	−.01	−.06		−.07

	3–12–12	2.99	13.00		13.56	2.97	12.80		13.38			
		3.04	13.10		13.40	3.10	13.10		13.56			
		3.04	13.00		13.40	3.07	13.00		12.90			
	Av.	3.02	13.03		13.45	3.05	12.97		13.28	+.03	−.06	−.17
	8– 8– 8	6.97	9.50		9.60	7.06	9.70		9.54			
		7.07	9.45		9.62	7.02	9.45		9.60			
		6.73	9.50		9.60	6.55	9.60		9.64			
	Av.	6.92	9.48		9.61	6.88	9.58		9.59	−.04	+.10	−.02
8	3–12–12	3.00	12.65	12.07	11.88	3.03	12.70	12.14	12.18			
		2.90	12.64	12.08	11.96	3.01	12.46	11.93	12.04			
		3.10	12.55	12.00	12.10	2.98	12.53	12.03	12.11			
	Av.	3.00	12.61	12.05	11.98	3.01	12.56	12.03	12.11	+.01	−.05	−.02	+.13
	4–12– 4	3.98	13.30	12.60	4.15	3.95	13.35	12.63	4.21			
		4.00	13.45	12.67	4.39	3.91	12.91	12.24	4.26			
		4.07	13.20	12.37	4.25	4.00	13.15	12.38	4.22			
	Av.	4.02	13.32	12.55	4.26	3.95	13.14	12.42	4.23	−.07	−.18	−.13	−.03
	3– 9–27	2.89	9.71	8.99	27.37	3.00	9.85	9.17	27.13			
		2.98	9.85	9.12	27.37	2.94	10.10	9.45	26.98			
		3.01	9.75	9.12	27.19	2.89	10.40	9.77	26.88			
	Av.	2.96	9.77	9.08	27.31	2.94	10.12	9.46	26.99	−.02	+.35	+.38	−.32
11	0–20–20		21.10	20.23	20.38		20.80	20.00	20.00			
			21.12	20.27	20.58		20.82	20.02	19.94			
			21.15	20.32	20.34		20.79	20.02	20.42			
	Av.		21.12	20.27	20.43		20.80	20.01	20.12		−.32	−.26	−.31
	0–14–14		15.10	14.60	15.32		14.80	14.40	15.70			
			15.00	14.40	15.46		14.84	14.38	15.56			
			15.05	14.45	15.54		14.82	14.37	15.42			
	Av.		15.05	14.48	15.44		14.82	14.38	15.56		−.23	−.10	+.12
	8– 8– 8	7.27	9.65	9.35	7.78	7.36	9.65	9.45	7.72			
		7.27	9.65	9.35	7.78	7.36	9.65	9.45	7.70			
		7.27	9.65	9.35	7.76	7.36	9.65	9.45	7.76			
	Av.	7.27	9.65	9.35	7.77	7.36	9.65	9.45	7.73	+.09	.00	+.10	−.04

A statistical analysis of the results will be presented later.

Collaborators are listed alphabetically and not in the same order as the collaborator numbers in the tables. The results of one collaborator were not included because of insufficient data.

COLLABORATORS

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Lineweaver, A. N., F. S. Royster Guano Company, Norfolk, Va.
Marshall, C. V., Canada Dept. of Agriculture, Ottawa, Canada
Miller, P. H., Arkansas State Plant Board, Little Rock, Ark.
Tobey, E. R., Maine Agricultural Experiment Station, Orono, Me.
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It is recommended* that there be further study of sampling and sample preparation.

REPORT ON NITROGEN SOLUTIONS

PROCEDURES FOR SAMPLING AND ANALYSIS

By J. F. FUDGE (Texas Agricultural Experiment Station, College Station, Texas), *Associate Referee*

The use of solutions containing important quantities of free ammonia as a source of fertilizer nitrogen is a comparatively new development, but one which promises to be of increasing importance. This type of product poses new questions which must be answered by fertilizer control officials. In view of this situation, an Associate Referee to investigate problems and methods of collecting and handling samples of these materials was appointed after the 1952 meeting of the A.O.A.C. The present report covers the work done this year; the work was confined to a compilation and collation of reports from a number of fertilizer control officials.

Requests for information as to how these samples were handled locally were sent to fertilizer control officials of all states where this type of material is being sold. Replies were received from fifteen States. While all of the replies indicated a definite interest in the problem and a realiza-

* For report of Subcommittee A and action of the Association, see *This Journal*, 37, 61 (1954).

tion of its probable importance in the future, only five of the States were actively engaged in the collection and analysis of samples of this type of material. Among this small group, certain points in the procedure seem to be fairly uniform, but there is a conspicuous lack of uniformity on other points.

Sample container.—Some use pressure bottles, such as Sargent No. S-9015, while others simply use Mason jars. In the interest of safety, it appears advisable to guard against excessive pressures in some way.

Collection of sample.—Wherever possible, samples are drawn from a valve outlet by flushing the line, attaching a rubber tube which leads to the bottom of the sample container, and allowing the solution to flow until the sample container has been satisfactorily flushed out. Where a valve is not available, a glass sampling tube is inserted to the bottom of the container, removed as quickly as possible, and the sample transferred to the sample container. The container is immediately capped, carefully packed, and shipped to the laboratory in the usual way.

Storage.—The sample should be kept as cool as possible prior to analysis. Storage in a refrigerator is preferable and the storage period should be short as practicable.

Sampling for analysis.—The sample must be cooled before removing a portion for analysis. Removal of a portion for analysis should be made as rapidly as possible. The special glass sampling pipet developed by the Nitrogen Division of Allied Chemical and Dye Corporation has been used successfully by a number of laboratories. This consists of a glass bulb of about 100 ml capacity, to which two stopcocks have been attached. The bulb is evacuated, a small amount of the sample is drawn up into the bulb, an excess of acid is added in the same way, and nitrogen is determined on the acidified sample. The weight of the sample taken is determined by weighing the bulb before and after the introduction of the sample, using another bulb as an approximate counterpoise. The primary sample should be open to the air for the shortest possible time.

A sampling procedure suitable for all nitrogen solutions and liquid fertilizers in which free ammonia is present has been developed in considerable detail by the Barrett Division for analytical control of their Nitrogen Solution II. Fertilizer control officials may obtain this procedure by writing to: Technical Service Bureau, Nitrogen Division, Allied Chemical and Dye Corporation, 40 Rector Street, New York 6, New York.

RECOMMENDATIONS

It is recommended*—

That the study of these products be continued with emphasis upon two phases:

* For report of Subcommittee A and action of the Association, see *This Journal*, 37, 61 (1954).

(a) Methods of collection and storage of primary sample.

(b) Methods for transfer of portions of the primary sample to the Kjeldahl flask, with particular emphasis on the Barrett procedure.

REPORT ON METHODS FOR THE DETERMINATION OF BORON IN FERTILIZERS

By RODNEY C. BERRY (Virginia Dept. of Agriculture, State Office Building, Richmond, Va.), *Associate Referee*

Two methods for the determination of borax in mixed fertilizers have been developed and accepted, first action, by the Association. In most cases, both of these methods give reasonably accurate results in the hands of the experienced chemist; however, some difficulties have been encountered with both of them.

In the "modified" method,[1] only boron in a water-soluble form is extracted, while in the identical *p*H method,[2] some boron which is not in a water-soluble form is extracted.

In the work reported last year,[1] only one analyst out of seventeen obtained satisfactory results when using the identical *p*H method on sample 3, whereas satisfactory results were generally obtained with the modified method. Sample 3 contained 145 pounds per ton of process tankage, and higher percentages of sulfates and carbonates than samples 1 and 2.

The collaborative study this year (1953) was not as extensive as in 1952 and only six laboratories reported (five of these did collaborative work in 1952). The same samples used in the 1952 study were sent out this year in order to observe the shelf-life of these mixtures, the improvement of laboratory techniques, and the adaptability of the methods.

On samples 1 and 2, the reported results showed a greater deviation by the same analyst than those reported last year. One analyst encountered difficulty in filtering, with and without the use of filter-cel. Two analysts preferred the "modified" method.

The work to date indicates that borax in most mixed fertilizers can be determined with equal accuracy by either method. All analysts have reported low results with the identical *p*H method on mixed fertilizers containing process tankage and a high percentage of sulfates and carbonates, whereas good results were obtained with the modified method. This discrepancy should be further investigated. One analyst experienced difficulty this year with sample 3; his results last year on this sample, however, were very satisfactory.

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[1] *This Journal*, 36, 623 (1953).
[2] *Official Methods of Analysis, A.O.A.C.*, 2.45.

RECOMMENDATIONS

It is recommended*—

That the identical *p*H method, and the "modified" method, remain in their present status and that collaborative study be continued.

That a method be provided for the determination of boron not readily soluble in water but known to be available to plants when it is applied to the soil.

REPORT ON MAGNESIUM AND MANGANESE IN FERTILIZERS†

By JOHN B. SMITH (Agricultural Experiment Station, Kingston, R. I.), *Associate Referee*

The objectives were a further study of the method proposed for magnesium in coarse particles of magnesic limestone in mixed fertilizers, and of the applicability of official methods to newly proposed carriers of magnesium, manganese, and other elements, namely, a glass or enamel frit, and the complex salts of ethylenediamine tetraacetic acid.

WATER-INSOLUBLE MAGNESIUM IN COARSE PARTICLES

This procedure was proposed in 1951 and studied collaboratively last year.[1] Fifteen-gram portions of unground fertilizer are boiled with water, the aggregates are disintegrated in a porcelain dish with a rubber pestle, and the coarse material is separated on a 40 mesh (420 microns) sieve by running water. The residues are analyzed for magnesium.

The three samples of commercial brands of fertilizer reported last year were submitted to a small group of analysts. The results listed in Table 1 are similar to those reported last year and indicate that satisfactory agreement may be attained. Results are averages of three runs and it is inadvisable to rely on a single determination. The collaborators agree that the chief limitation of the procedure is variance among the weighed samples, but the differences among analysts are quite similar to those for acid-soluble magnesium in ground samples. The method published last year should be changed to specify a sieve with openings of 420 microns rather than merely as 40 mesh.

The magnesium segregated in this fraction should not be labeled "unavailable to plants." Its activity depends on many factors: particle size distribution within the fraction, soil acidity and texture, climate, and crop.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 61 (1954).
† Contribution No. 830 of this Station.
[1] *This Journal*, **36**, 628 (1953).

TABLE 1.—*Collaborators' results for Mg, per cent*

COLLABORATOR NUMBER	ACID-SOLUBLE Mg			WATER-INSOLUBLE Mg IN COARSE PARTICLES[a]		
	Sample 1	*Sample 2*	*Sample 3*	*Sample 1*	*Sample 2*	*Sample 3*
1	2.24	2.71	1.59	0.17	1.56	1.14
2	2.47	2.72	1.55	0.15	1.35	0.91
3	2.37	2.53	1.48	0.14	1.33	0.98
Average	2.36	2.65	1.54	0.15	1.41	1.01
Average, 1952[b]	2.28	2.63	1.54	0.18	1.36	1.07

[a] On sieve with openings of 420 microns.
[b] Averages of results reported by 13 collaborators for the same samples in 1952. All results are averages of 3 determinations.

It is based on the particle size most often chosen as the dividing line between coarse and fine limestone, and the procedure is recommended for trial to satisfy a need not supplied at present in any other way.

MAGNESIUM AND MANGANESE IN ENAMEL FRIT AND IN COMPLEX SALTS OF ETHYLENEDIAMINE TETRAACETIC ACID

Two new types of materials have recently been suggested as carriers of secondary and minor fertilizer elements. Frit is a glassy or enamel silicate which contains many essential fertilizer elements and it is proposed as a slowly available source of secondary and minor elements. Its low solubility is demonstrated by the analyses shown in Table 2. The present official methods will include only very small quantities of magnesium or manganese in this material, and more agronomic data are needed to show the correlation between those quantities and the degree of availability of frit in various soils under various cropping conditions.

There is no official method for water-soluble manganese. It might be assumed that manganese from manganese sulfate, the principal carrier now in use, remains soluble in fertilizer mixtures, but change to insoluble forms has been shown,[2] and this is confirmed by work in this laboratory. Three dry-mixed fertilizers containing 2.04, 5.30, and 8.50 per cent of manganese yielded 1.38, 4.30, and 8.05 per cent, respectively, after boiling 1 gram samples for 1 hour with 350 ml of water. Therefore, although the manganese from frit used as a fertilizer ingredient will not be included in a water-soluble fraction, neither will all of the manganese from manganese sulfate. The addition of superphosphate to the frit before water extraction had no significant effect (Table 2).

Chelated complexes of magnesium and manganese with sodium salts of ethylenediamine tetraacetic acid have been proposed for direct use on plants or in the soil. The analyses of a sample of the magnesium complex,

[2] *Fertilizer Review*, 25, No. 2, 12–14 (1950).

TABLE 2.—*Applicability of Official Methods to frit and complex magnesium and manganese salts of ethylenediamine tetraacetic acid*

METHOD	FRIT	SALTS OF ETHYLENEDIAMINE TETRAACETIC ACID	
	Magnesium per cent		
Total Mg, sample decomposed with H_2SO_2-HF, Method **3.16**	0.91	—	
Total Mg. sample ashed, Method **3.16**	—	4.40	
Acid-soluble Mg, HNO_3-HCl, Method **2.56**	0.21	4.37	
	Manganese, per cent	*(Sample 1)*	*(Sample 2)*
Total Mn, sample decomposed with H_2SO_4-HF, Method **3.17**	2.25	—	—
Total Mn, sample ashed, Method **3.17**	—	8.6	11.7
Acid-soluble Mn, H_2SO_2-HNO_3, colorimetric Method **2.58**	0.21	8.7	11.8
Acid-soluble Mn, H_2SO_2-HNO_2, bismuthate Method **2.60**	0.05	8.5	11.9
Water-soluble Mn, 1 g boiled 1 hr with 350 ml H_2O **2.57(b), 2.60.**	0.03	8.6	11.7
Water-soluble Mn, as above with 0.5 g superphosphate added	0.04	8.4	11.7

and two containing manganese, shown in Table 2, indicate that these materials present no analytical difficulties by current methods. The addition of superphosphate to the sample before water extraction did not prevent complete recovery of manganese.

It is recommended* that the method for water-soluble magnesium in coarse particles published last year be changed to specify a sieve with openings of 420 microns and adopted, first action, and that the study be continued.

ACKNOWLEDGMENT

Sincere appreciation is expressed to Bernie E. Plumner, Jr., Agricultural Experiment Station, Orono, Me., and E. D. Schall, Agricultural Experiment Station, Lafayette, Ind., for collaboration with the analyses for magnesium in coarse particles.

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 48 (1954).

REPORT ON PHOSPHORIC ACID IN FERTILIZERS

DIRECT DETERMINATION OF AVAILABLE PHOSPHORIC ACID

By K. D. JACOB, *Associate Referee*, and W. M. HOFFMAN (Division of Fertilizer and Agricultural Lime, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.)

The report on phosphoric acid presented at the 1952 meeting of this Association (7) supported the findings of other workers (1, 2, 3, 11) as to the possibilities in the direct determination of available phosphorus in fertilizers by volumetric and photometric methods. Further investigation of such methods was made in a collaborative study of six samples, the results of which are reported herein.

The collaborators' results for total, citrate-insoluble, and available P_2O_5 in the samples, as determined by the official methods, are summarized in Table 1. Samples 1 to 4 were ground to pass the 35-mesh Tyler sieve, and Samples 5 and 6 were ground to pass the 10- and 60-mesh sieves, respectively.

TABLE 1.—*Total, citrate-insoluble, and available P_2O_5 in samples, as determined by official methods*

SAMPLE	TOTAL P_2O_5		INSOLUBLE P_2O_5		AVAILABLE P_2O_5	
	RANGE	AVERAGE[a]	RANGE	AVERAGE[a]	RANGE	AVERAGE[a]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1[b]	20.20–21.55	20.77	0.07–1.50	0.22	19.12–21.44	20.55
2[c]	49.71–51.52	50.57	1.36–4.06	2.21	46.69–49.43	48.36
3[d]	20.43–21.92	21.05	0.57–1.35	0.83	19.76–21.07	20.22
4[d]	19.87–22.68	20.59	1.94–5.01	3.84	15.42–18.35	16.75
5[e]	2.08– 2.43	2.35	0.04–0.21	0.16	1.95– 2.35	2.19
6[f]	63.23–65.17	64.05	0.64–3.73	1.21	59.90–64.46	62.83

[a] Averages of triplicate determinations by each collaborator.
[b] Normal superphosphate; 34 collaborators.
[c] Triple superphosphate; 28 collaborators.
[d] Ammoniated superphosphate; 29 collaborators.
[e] Cottonseed meal; 11 collaborators.
[f] Calcium metaphosphate; 7 collaborators.

Samples 1 and 2 (normal and triple superphosphates, respectively) were commercial, well-cured materials made from Florida land-pebble phosphate.

Samples 3 and 4 (ammoniated normal superphosphate) were laboratory prepared, thoroughly aged materials made from commercially produced Florida land-pebble superphosphate. They contained approximately 2.2 and 4.0 per cent of ammonia, respectively.

Sample 5 was commercial feed-grade cottonseed meal.

Sample 6 was calcium metaphosphate supplied by the Division of Chemical Development, Tennessee Valley Authority, Wilson Dam, Ala. The composition and characteristics of the material as received were stated to be typical of the product planned for distribution by the Authority in 1950.

INSTRUCTIONS TO COLLABORATORS

Without further grinding, mix each sample thoroughly and analyze by the following methods. Make all determinations in triplicate, each on a separate portion of the sample, and report the individual results on the form enclosed with these instructions. If for any reason it is necessary or desirable to repeat a determination the repetition should be made in triplicate and the three results reported should be those obtained in simultaneous replications. Your comments and observations concerning this investigation are requested.

TOTAL P_2O_5

Samples 1 to 4.—Prep. the soln as directed in *Official Methods of Analysis*, 7th Ed., 1950, p. 8, sect. **2.9(a)**. Det. P_2O_5 as directed on pp. 9–10, sect. **2.13(a)**.

Samples 5 and 6.—Prep. the soln as directed on p. 353, sect. **22.49(a)**, using a 2 g sample and finally adjusting the vol. of the soln to 200 ml in a volumetric flask. Det. P_2O_5 as directed on pp. 9–10, sect. **2.13(a)**.

AVAILABLE P_2O_5

Preparation of Solution.—Place 1 g of sample on a 9 cm paper (Whatman No. 2 or equivalent) and wash by gravity filtration with twelve 10 ml portions of H_2O into a 1 liter filter flask contg 10 ml of HNO_3 (1+1). Allow each portion of wash H_2O to pass thru the filter before adding the next portion. After the final washing, allow the filter to drain thoroly, remove from the funnel, and rinse the funnel with 10 ml of H_2O with drainage into the filter flask. Within 1 hr, digest the filter and H_2O-insol. residue with neutral NH_4 citrate soln as directed on p. 11, sect. **2.17(a)**, either with shaking at 5 min. intervals or preferably with continuous agitation during the citrate digestion. Filter the citrate ext. into the flask contg the H_2O ext., and wash the residue with H_2O at 65°C. to a total vol. of 450 ml in the filter flask. Reserve the citrate-insol. residue. Transfer the contents of the filter flask to a 500 ml volumetric flask, cool to room temp., adjust the vol. to 500 ml, and mix thoroly (Solution A).

VOLUMETRIC METHOD

Procedure 1.—With Samples 1 to 4, use aliquots of Soln A corresponding approximately to 0.10 and 0.05 g of sample for materials contg less than 25, and 25 to 50%, of available P_2O_5, resp., and proceed as follows:

Transfer the appropriate aliquot to a 250 ml Erlenmeyer flask or other suitable vessel, add 15 ml of NH_4NO_3 soln contg 10 g of P-free NH_4NO_3, adjust the vol. to 75–100 ml with H_2O, and heat to 50°C. Then add slowly, with agitation, 60 ml of hot (50°C.) molybdate soln. omitting the supplemental HNO_3 (5 ml/100 ml of molybdate) customarily added in prepg the volumetric molybdate soln. Without further application of heat, shake or stir continuously for 30 min., and complete the detn as directed on pp. 9–10, sect. **2.13(a)**.

With Sample 5, digest 200 ml of Soln A with HNO_3 and $HClO_4$ as directed on p. 353, sect. **22.49(a)** and finally adjust the vol. to 200 ml in a volumetric flask (Soln B). Det. P_2O_5 in a 50 ml aliquot of Soln B as directed in the preceding paragraph.

With Sample 6, digest 100 ml of Soln A with HNO_3 and $HClO_4$ as directed in the preceding paragraph, and finally adjust the vol. to 200 ml in a volumetric flask (Soln B). Det. P_2O_5 in a 25 ml aliquot of Soln B as directed in the preceding paragraph.

Procedure 2.—With Samples 1 to 4, using another aliquot (same vol.) of Soln A, proceed as directed under Procedure 1 thru the point where the molybdate soln is added, and then shake or stir continuously for 30 min. while maintaining the temp. at 50°C. Complete the detn as before.

With Samples 5 and 6, using another aliquot of Soln B (50 ml for Sample 5, 25 ml for Sample 6), proceed as directed in the preceding paragraph.

Omit the determinations by Procedure 2 if apparatus for continuous agitation at 50°C. is not available or cannot be assembled readily.

PHOTOMETRIC METHOD

Vanadomolybdate reagent.—Dissolve 40 g of NH_4 molybdate (analytical reagent quality) in 400 ml of hot H_2O. Dissolve 1 g of NH_4 metavanadate (C.P.) in 300 ml of H_2O and add 200 ml of HNO_3. Allow the solns to cool, and mix by pouring the molybdate soln into the vanadate soln. Dil. to 1 liter.

Standard phosphate soln.—Dissolve 0.3826 g of KH_2PO_4 in 1 liter of H_2O. This soln contains 0.20 mg of P_2O_5/ml. The KH_2PO_4 that is specially prepd and standardized for use in buffer solns is recommended.

Procedure.—Transfer an aliquot (0.2 to 0.8 mg of P_2O_5) of the soln of the sample (Soln A of Samples 1 to 4, Soln B of Samples 5 and 6) to a 100 ml volumetric flask, add 25 ml of H_2O and 25 ml of the vanadomolybdate reagent, bring the vol. to 100 ml, mix thoroly, and let stand 15 min. for development of color. Det. the absorbance of the soln by means of a photometer, with prior adjustment of the instrument against an appropriate blank soln contg the vanadomolybdate and other reagents in the same concn as with the soln (Soln A or Soln B) being analyzed. Estimate the P_2O_5 by interpolation of the photometric reading on a curve prepd from analyses of aliquots of the standard KH_2PO_4 soln under similar conditions of temp. and reagent concn.

(A wavelength of about 400 mμ appears to be satisfactory for this detn when using the Beckman DU, Coleman Universal, or Evelyn instruments. For other instruments the optimum photometric conditions, if not known, should be detd by the collaborator. It is believed that the indicated quantities of P_2O_5 (0.2–0.8 mg) can be detd satisfactorily with most if not all photometric instruments. Higher concns can be readily detd with some instruments.)

CITRATE-INSOLUBLE P_2O_5

Samples 1 to 4.—Digest the citrate-insol. residue as directed on p. 8, sect. **2.9(a)**, and det. P_2O_5 as directed on pp. 9–10, sect. **2.13(a)**.

Samples 5 and 6.—Digest the citrate-insol. residue as directed on p. 353, sect. **22.49(a)**, and det. P_2O_5 as directed in the preceding paragraph.

NOTES ON THE PROCEDURES

In the preparation of the citrate extracts of the samples, 18 of the 34 collaborators used continuous agitation devices of one kind or another, whereas the other collaborators employed manual agitation at 5 minute intervals during the citrate digestion (Table 2). Both methods of agitation are officially permitted, but calcium metaphosphate is the only material for which continuous agitation is specifically designated (4–7). All the collaborators who determined available P_2O_5 in the calcium metaphosphate (Sample 6) used continuous agitation. Previous investiga-

tions (5, 6, 8–10) have shown that the degree of agitation (continuous or at 5 minute intervals) during the citrate digestion has very little or no effect on the values for citrate-insoluble P_2O_5 in superphosphates, but that somewhat higher values may be obtained with continuous agitation of ammoniated superphosphates and especially the coarser grinds of certain furnace-made phosphates. In the present investigation the values for citrate-insoluble P_2O_5 (Table 1) obtained with the aid of continuous agitation were not consistently different from the comparable values with intermittent agitation.

TABLE 2.—*Types of apparatus used in determining available P_2O_5*

APPARATUS	COLLABORATOR NO.
Continuous Agitation During Citrate Digestion[a]	
End-over-end[b]	4, 9, 18, 25, 26, 27
Wrist action[c]	3, 5, 8, 20
Miscellaneous types	1, 12, 16, 21, 24, 28, 30, 33
Photometric Apparatus	
Beckman, Model B	1[d], 29
Beckman, Model DU	3[e], 4, 6[f], 10, 12, 17, 18, 22, 24, 28, 34
Coleman Universal	19[g], 20, 21[h], 30[h], 31
Evelyn	5[i], 8[i], 27, 33
Fisher	9, 15
Klett-Summerson	11[j], 25

[a] Collaborators 2, 6, 7, 10, 11, 13, 14, 15, 17, 19, 22, 23, 29, 31, 32, and 34 used manual agitation at 5-minute intervals during the citrate digestion.
[b] Precision Scientific Co.
[c] Burrell Technical Supply Co.
[d] Wavelength, 430 mμ; constant voltage by battery and charger.
[e] Slit width, 0.12 mm; constant temperature, 25°C.
[f] Slit width, 0.03 mm.
[g] Model 11.
[h] Model 14.
[i] Wavelength, 420 mμ.
[j] No. 42 blue filter.

Among the 26 collaborators who determined P_2O_5 by the photometric method, 11 used the Beckman Model DU spectrophotometer, 5 used various models of the Coleman Universal instrument, 4 the Evelyn instrument, and 2 each the Beckman Model B, Fisher, and Klett-Summerson instruments, respectively (Table 2).

COLLABORATIVE RESULTS FOR AVAILABLE P_2O_5

For convenience the several methods used for determination of available P_2O_5 in this investigation are designated as follows:

Method I.—The available P_2O_5 is the difference between the total and the citrate-insoluble P_2O_5 as determined by the methods indicated in the Instructions to Collaborators.

Method II.—The combined water-citrate extract is analyzed by the volumetric method, Procedure 1, as outlined in the Instructions.

Method III.—The combined water-citrate extract is analyzed by the volumetric method, Procedure 2, as outlined in the Instructions.

Method IV.—The combined water-citrate extract is analyzed for P_2O_5 by the photometric method as outlined in the instructions.

The averages of the individual collaborator's replicated results for available P_2O_5 in each of the samples by the several methods are shown in Table 3, which also indicates the individual differences between the results by the official method (I) and those by the direct methods. The data

TABLE 3.—*Percentages of available P_2O_5 as determined by several methods*

COLLABORATOR	METHOD[a]				DIFFERENCE IN RESULTS BY METHOD I AND METHOD:		
	I[b]	II[c]	III[d]	IV[e]	II	III[f]	IV[f]
	Sample 1. Normal Superphosphate						
1	20.06	20.40	20.63	19.93	0.34	0.57	−0.13
2	20.80	20.68	—	—	−0.12	—	—
3	20.48	20.29	20.31	20.27	−0.19	−0.17	−0.21
4	20.87	20.67	20.75	20.07	−0.20	−0.12	−0.80
5	20.58	20.55	20.50	20.50	−0.03	−0.08	−0.08
6	20.51	20.33	—	20.00	−0.18	—	−0.51
7	20.05	20.18	20.23	—	0.13	0.18	—
8	20.54	20.61	20.51	20.27	0.07	−0.03	−0.27
9	20.51	20.33	20.56	20.21	−0.18	0.05	−0.30
10	20.87	21.01	—	20.93	0.14	—	0.06
11	21.09	21.08	—	21.40	−0.01	—	0.31
12	20.87	20.57	20.77	21.42	−0.30	−0.10	0.55
13	20.57	20.53	—	—	−0.04	—	—
14	20.53	20.33	—	—	−0.20	—	—
15	20.43	20.93	—	21.37	0.50	—	0.94
16	20.51	20.09	20.17	—	−0.42	−0.34	—
17	20.74	21.30	—	20.07	0.56	—	−0.67
18	20.44	20.32	20.30	20.38	−0.12	−0.14	−0.06
19	20.64	20.44	—	19.27	−0.20	—	−1.37
20	20.69	20.72	—	20.67	0.03	—	−0.02
21	20.08	20.60	20.55	20.22	0.52	0.47	0.14
22	20.55	20.45	—	20.13	−0.10	—	−0.42
23	20.52	20.67	—	—	0.15	—	—
24	20.87	21.17	21.20	19.52	0.30	0.33	−1.35
25	20.51	20.53	—	20.11	0.02	—	−0.40
26	20.93	20.95	20.92	—	0.02	−0.01	—
27	21.44	20.17	—	20.00	−1.27	—	−1.44
28	20.20	20.73	20.78	20.62	0.53	0.58	0.42
29	20.55	—	—	20.60	—	—	0.05
30	20.89	20.62	20.85	20.27	−0.27	−0.04	−0.62
31	20.30	20.27	—	20.38	−0.03	—	0.08
32	20.47	20.35	—	—	−0.12	—	—
33	20.34	20.32	20.38	20.33	−0.02	0.04	−0.01
34	19.12	19.58	—	19.29	0.46	—	0.17

TABLE 3—(*continued*)

COLLABORATOR	METHOD[a]				DIFFERENCE IN RESULTS BY METHOD I AND METHOD:		
	I[b]	II[c]	III[d]	IV[e]	II[f]	III[f]	IV[f]
Sample 2. Triple Superphosphate							
1	47.72	47.32	47.35	47.52	−0.40	−0.37	−0.20
2	49.04	49.00	—	—	−0.04	—	—
3	48.45	48.15	48.39	48.57	−0.30	−0.06	0.12
4	49.32	48.73	49.13	50.20	−0.59	−0.19	0.88
5	49.02	48.79	48.90	48.70	−0.23	−0.12	−0.32
6	47.97	47.93	—	48.33	−0.04	—	0.36
7	47.72	47.73	47.87	—	0.01	0.15	—
8	48.79	48.94	48.83	48.09	0.15	0.04	−0.70
10	48.41	48.13	—	48.03	−0.28	—	−0.38
11	48.75	47.98	—	48.80	−0.77	—	0.05
12	49.43	49.87	49.93	51.31	0.44	0.50	1.88
13	48.18	48.17	—	—	−0.01	—	—
14	47.97	48.00	—	—	0.03	—	—
15	49.17	49.07	—	49.43	−0.10	—	0.26
17	48.42	50.07	—	47.73	1.65	—	−0.69
18	47.85	47.87	47.57	47.90	0.02	−0.28	0.05
19	48.29	47.89	—	42.33	−0.40	—	−5.89
20	49.25	49.07	—	49.49	−0.18	—	0.24
22	47.87	48.43	—	47.23	0.56	—	−0.64
23	48.17	48.50	—	—	0.33	—	—
24	48.18	48.27	48.40	47.72	0.09	0.22	−0.46
25	47.73	47.80	—	43.81	0.07	—	−3.92
26	48.81	48.28	48.38	—	−0.53	−0.43	—
28	46.69	48.77	48.83	48.12	2.08	2.14	1.43
29	48.54	—	—	48.27	—	—	−0.27
31	47.75	47.73	—	48.63	−0.02	—	0.88
32	48.60	48.21	—	—	−0.39	—	—
33	47.85	47.93	47.41	45.53	0.08	−0.44	−2.32
Sample 3. Ammoniated Superphosphate							
1	19.66	20.15	20.10	19.95	0.49	0.44	0.29
2	20.24	17.67	—	—	−2.57	—	—
3	20.07	20.05	19.77	19.77	−0.02	−0.30	−0.30
4	20.55	20.32	20.38	19.73	−0.23	−0.17	−0.82
5	20.21	20.16	20.25	20.20	−0.05	0.04	−0.01
6	19.90	19.83	—	20.03	−0.07	—	0.13
7	19.81	19.80	19.91	—	−0.01	0.10	—
8	20.01	20.20	20.26	19.82	0.19	0.25	−0.19
10	20.37	20.58	—	20.10	0.21	—	−0.27
11	20.68	20.83	—	20.42	0.15	—	−0.26
12	20.93	20.57	20.67	21.32	−0.36	−0.26	0.39
13	20.22	20.23	—	—	0.01	—	—

TABLE 3—(*continued*)

COLLABORATOR	METHOD[a]				DIFFERENCE IN RESULTS BY METHOD I AND METHOD:		
	I[b]	II[c]	III[d]	IV[e]	II[f]	III[f]	IV[f]
14	20.17	20.03	—	—	−0.14	—	—
15	20.29	20.67	—	20.45	0.38	—	0.16
17	20.31	21.05	—	19.70	0.74	—	−0.61
18	20.01	20.05	19.95	19.98	0.04	−0.06	−0.03
19	20.20	19.97	—	17.27	−0.23	—	−2.93
20	20.37	20.45	—	20.53	0.08	—	0.16
22	20.22	20.17	—	19.38	−0.05	—	−0.84
23	20.17	20.17	—	—	0.00	—	—
24	21.07	21.05	21.10	18.07	−0.02	0.03	−3.00
25	20.28	20.03	—	18.66	−0.25	—	−1.62
26	20.50	20.67	20.63	—	0.17	0.13	—
28	19.93	20.45	20.47	20.32	0.52	0.54	0.39
29	20.27	—	—	20.23	—	—	−0.04
31	19.81	19.72	—	19.56	−0.09	—	−0.25
32	20.10	19.77	—	—	−0.33	—	—
33	20.03	20.17	19.95	20.22	0.14	−0.08	0.19
34	19.87	19.88	—	19.32	0.01	—	−0.55
Sample 4. Ammoniated Superphosphate							
1	16.14	16.35	16.36	15.97	0.21	0.22	−0.17
2	16.97	17.03	—	—	0.06	—	—
3	18.11	18.13	18.11	18.03	0.02	0.00	−0.08
4	17.18	17.02	17.03	16.50	−0.16	−0.15	−0.68
5	16.52	16.47	16.54	16.37	−0.05	0.02	−0.15
7	17.32	17.14	17.25	—	−0.18	−0.07	—
8	16.62	16.76	16.84	16.57	0.14	0.22	−0.05
9	16.60	16.41	16.65	16.34	−0.19	0.05	−0.26
10	16.46	16.56	—	16.37	0.10	—	−0.09
13	16.32	16.23	—	—	−0.09	—	—
14	16.83	16.63	—	—	−0.20	—	—
16	15.42	15.12	15.30	—	−0.30	−0.12	—
17	16.13	16.93	—	15.80	0.80	—	−0.33
19	16.84	16.61	—	13.14	−0.23	—	−3.70
20	16.93	16.59	—	16.60	−0.34	—	−0.33
21	15.78	16.23	16.20	15.98	0.45	0.42	0.20
22	16.90	16.48	—	15.02	−0.42	—	−1.88
23	16.70	16.90	—	—	0.20	—	—
24	16.65	14.68	14.77	14.50	−1.97	−1.88	−2.15
25	16.19	16.07	—	14.38	−0.12	—	−1.81
26	16.72	16.78	16.77	—	0.06	0.05	—
27	17.26	16.37	—	16.00	−0.89	—	−1.26
28	18.35	16.61	16.79	15.79	−1.74	−1.56	−2.56
29	16.85	—	—	17.00	—	—	0.15
30	16.34	16.23	16.38	15.27	−0.11	0.04	−1.07
31	15.87	15.90	—	15.89	0.03	—	0.02

TABLE 3—(*continued*)

COLLABORATOR	METHOD[a]				DIFFERENCES IN RESULTS BY METHOD I AND METHOD:		
	I[b]	II[c]	III[d]	IV[e]	II[f]	III[f]	IV[f]
32	17.34	16.39	—	—	−0.95	—	—
33	16.16	16.03	16.08	15.37	−0.13	−0.08	−0.79
34	18.29	17.02	—	16.93	−1.27	—	−1.36
	Sample 5. Cottonseed Meal						
5	2.22	2.23	—	2.14	0.01	—	−0.08
6	1.95	0.67	—	0.45	−1.28	—	−1.50
8	2.11	2.22	—	2.29	0.11	—	0.18
9	2.19	2.22	2.45	2.21	0.03	0.26	0.02
11	2.24	2.43	—	1.98	0.19	—	−0.26
15	2.33	2.67	—	2.60	0.34	—	0.27
16	2.17	1.33	1.22	—	−0.84	−0.95	—
18	2.23	1.06	1.07	0.90	−1.17	−1.16	−1.33
21	2.18	0.83	0.80	0.71	−1.35	−1.38	−1.47
27	2.23	0.60	—	0.93	−1.63	—	−1.30
30	2.21	0.73	0.67	—	−1.48	−1.54	—
	Sample 6. Calcium Metaphosphate						
5	63.36	63.09	—	63.13	−0.27	—	−0.23
8	64.46	64.34	—	64.34	−0.12	—	−0.12
9	63.05	63.40	63.68	61.56	0.35	0.63	−1.49
16	59.90	60.32	60.91	—	0.42	1.01	—
21	62.58	64.20	63.73	64.07	1.62	1.15	1.49
27	63.72	61.08	—	58.67	−2.64	—	−5.05
30	62.75	62.97	62.53	61.60	0.22	−0.22	−1.15

[a] Average results of 3 or more replications.
[b] Official method.
[c] Direct volumetric method, Procedure 1.
[d] Direct volumetric method, Procedure 2.
[e] Direct photometric method.
[f] The minus sign denotes that the result by this method was lower than that by Method I.

are summarized in Table 4, which shows the average results obtained on each sample in simultaneous comparisons of the official method with the other methods. Discussion of the results on Samples 5 and 6 (cottonseed meal and calcium metaphosphate) is reserved mostly for later sections of this report, because of certain complicating factors involving the chemical nature of these materials and the methods used for their analysis.

The average results on the individual samples (Table 4) are not strictly comparable because of the variability in the number of individual comparisons. It should be noted, however, that the net differences between the average values for available P_2O_5 in Samples 1, 2, and 3 (normal, triple, and lightly ammoniated superphosphates) by the official method and those

TABLE 4.—*Summary of average results for available P_2O_5*

SAMPLE[a]	METHOD I[b] VS. METHOD II[c]				METHOD I[b] VS. METHOD III[d]				METHOD I[b] VS. METHOD IV[e]			
	COMPARISONS	AVAILABLE P_2O_5[f]			COMPARISONS	AVAILABLE P_2O_5[f]			COMPARISONS	AVAILABLE P_2O_5[f]		
		METHOD I	METHOD II	DIFFERENCE[g]		METHOD I	METHOD III	DIFFERENCE[g]		METHOD I	METHOD IV	DIFFERENCE[g]
	number	*per cent*	*per cent*	*per cent*	*number*	*per cent*	*per cent*	*per cent*	*number*	*per cent*	*per cent*	*per cent*
1	33	20.55	20.54	−0.01	16	20.51	20.59	0.08	26	20.55	20.32	−0.23
2	27	48.35	48.39	0.04	12	48.32	48.42	0.10	21	48.35	47.89	−0.46
3	28	20.21	20.17	−0.04	12	20.23	20.29	0.06	22	20.23	19.77	−0.46
4	28	16.75	16.49	−0.26	14	16.71	16.51	−0.20	21	16.77	15.90	−0.87
5	11	2.19	1.54	−0.65	5	2.20	1.24	−0.96	9	2.19	1.58	−0.61
6	7	62.83	62.77	−0.06	4	62.07	62.71	0.64	6	63.32	62.23	−1.09
1 to 4	116	26.02	25.95	−0.07	54	25.64	25.64	0.00	90	26.07	25.58	−0.49

[a] Sample 1, normal superphosphate; Sample 2, triple superphosphate; Samples 3 and 4, ammoniated superphosphate; Sample 5, cottonseed meal; Sample 6, calcium metaphosphate.
[b] Official method.
[c] Direct volumetric method, Procedure 1.
[d] Direct volumetric method, Procedure 2.
[e] Direct photometric method.
[f] Average of comparative results by all collaborators.
[g] The minus sign denotes that Method I gave the higher result.

by the direct volumetric methods (II and III) do not exceed 0.10 per cent. The differences are larger (0.20 to 0.26 per cent) with Sample 4 (heavily ammoniated superphosphate). The average values for available P_2O_5 in each of these four samples by Method II are lower than those by Method III, and with one exception (Sample 4) they agree more closely with those by the official method.

With Samples 1 to 4 the average values for available P_2O_5 (Table 4) by the direct photometric method (IV) are considerably lower than those by the official method (0.23 to 0.87 per cent) and by Methods II and III.

Table 5 summarizes for Samples 1 to 4 the numerical values of the differences in the average results of the individual determinations of available P_2O_5 by the official method in comparison with the other methods. The distribution of the differences according to their magnitude and the number of determinations showing plus and minus values, respectively, is shown in Table 6. These data afford an indication of the precision of the results among the collaborators.

For all comparisons on Samples 1 to 4 the differences between individual collaborators' average results for available P_2O_5 by the official method and Method II range from -2.57 to 2.08 per cent (average, -0.07 per cent) (Table 5). With Method III the range is -1.88 to 2.14 per cent (average, 0.00 per cent), and Method IV, -5.89 to 1.88 per cent (average, -0.49 per cent). The minus sign denotes that the official method gave the higher result. Excluding the comparisons in which the values differ by ± 1 per cent or more, the respective average differences are -0.02, 0.03, and -0.11 per cent. Differences of ± 1 per cent or more of P_2O_5 are shown in 6 per cent of all the comparisons of the official method with Method II and with Method III and 18 per cent with Method IV (Tables 5 and 6). For differences of less than 0.25 per cent of P_2O_5 the comparable figures are 64, 65, and 36 per cent with Methods II, III, and IV, respectively (Table 6). In 57 per cent of all the comparisons (Samples 1 to 4) the values by the official method are higher than those by Method II (Table 6). The values by the official method are higher in 50 per cent of the comparisons with Method III and in 68 per cent of those with Method IV.

The differences between the high and low results in replicate determinations of available P_2O_5 by the several methods are summarized in Table 7. For Samples 1 to 4 the range is 0.01 to 0.70 per cent of P_2O_5 (average, 0.14 per cent) with Method I, 0.00 to 0.93 per cent (average, 0.21 per cent) with Method II, 0.00 to 0.80 per cent (average, 0.20 per cent) with Method III, and 0.00 to 3.00 per cent (average, 0.46 per cent) with Method IV. Excluding analyses in which the replicate values differ by more than 0.50 per cent of P_2O_5, the average differences are 0.13, 0.18, 0.17, and 0.27 per cent with Methods I, II, III, and IV, respectively.

In only 3 per cent of all the analyses of Samples 1 to 4 by Method I did the difference between the high and low results in replicate determi-

TABLE 5.—*Summary of differences in average results for available P_2O_5 in samples 1 to 4*

METHOD[a]	COMPARISONS		DIFFERENCE IN AVAILABLE P_2O_5[c]	
	TOTAL	SELECTED[b]	RANGE	AVERAGE
	number	*number*	*per cent*	*per cent*
		Sample 1. Normal Superphosphate		
I vs. II	33	—	−1.27, 0.56	−0.01
	—	32	−0.42, 0.56	0.03
I vs. III	16	—	−0.34, 0.58	0.08
	—	16	−0.34, 0.58	0.08
I vs. IV	26	—	−1.44, 0.94	−0.23
	—	23	−0.80, 0.94	−0.08
		Sample 2. Triple Superphosphate		
I vs. II	27	—	−0.77, 2.08	0.04
	—	25	−0.77, 0.56	−0.10
I vs. III	12	—	−0.44, 2.14	0.10
	—	11	−0.44, 0.50	−0.09
I vs. IV	21	—	−5.89, 1.88	−0.46
	—	16	−0.70, 0.88	−0.05
		Sample 3. Ammoniated Superphosphate		
I vs. II	28	—	−2.57, 0.74	−0.04
	—	27	−0.36, 0.74	0.05
I vs. III	12	—	−0.30, 0.54	0.06
	—	12	−0.30, 0.54	0.06
I vs. IV	22	—	−3.00, 0.39	−0.46
	—	19	−0.84, 0.39	−0.13
		Sample 4. Ammoniated Superphosphate		
I vs. II	28	—	−1.97, 0.80	−0.26
	—	25	−0.95, 0.80	−0.09
I vs. III	14	—	−1.88, 0.42	−0.20
	—	12	−0.15, 0.42	0.05
I vs. IV	21	—	−3.70, 0.20	−0.87
	—	13	−0.79, 0.20	−0.20
		Samples 1 to 4		
I vs. II	116	—	−2.57, 2.08	−0.07
	—	109	−0.95, 0.80	−0.02
I vs. III	54	—	−1.88, 2.14	0.00
	—	51	−0.44, 0.58	0.03
I vs. IV	90	—	−5.89, 1.88	−0.49
	—	71	−0.84, 0.94	−0.11

[a] Method I, official; Method II, direct volumetric, Procedure 1; Method III, direct volumetric, Procedure 2; Method IV, direct photometric.

[b] In which the average result of a replication differed from that by Method I by less than 1%.

[c] *Range* of the difference in the average replicated results of the individual comparisons; weighted *average* of the difference in all the comparisons. The minus sign denotes that Method I gave the higher result.

TABLE 6.—*Distribution of differences in the average results of the individual determinations of available P_2O_5 in Samples 1 to 4 by the official method in comparison with the other methods*

DIFFERENCE	COMPARISONS OF METHOD I[a] WITH:								
	METHOD II[b]			METHOD III[c]			METHOD IV[d]		
	PLUS[e]	MINUS[f]	TOTAL	PLUS[e]	MINUS[f]	TOTAL	PLUS[e]	MINUS[f]	TOTAL
per cent	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*
Sample 1. Normal Superphosphate									
<0.10	4	5	9	2	4	6	3	4	7
0.10–0.24	3	10	13	1	4	5	2	2	4
0.25–0.49	3	3	6	2	1	3	2	4	6
0.50–1.00	4	0	4	2	0	2	2	4	6
>1.00	0	1	1	0	0	0	0	3	3
—	14	19	33	7	9	16	9	17	26
Sample 2. Triple Superphosphate									
<0.10	6	4	10	1	1	2	2	0	2
0.10–0.24	1	3	4	2	2	4	2	1	3
0.25–0.49	2	5	7	0	4	4	2	4	6
0.50–1.00	1	3	4	1	0	1	2	3	5
>1.00	2	0	2	1	0	1	2	3	5
—	12	15	27	5	7	12	10	11	21
Sample 3. Ammoniated Superphosphate									
<0.10	4	7	12[g]	2	2	4	0	3	3
0.10–0.24	5	3	8	2	1	3	4	1	5
0.25–0.49	2	3	5	2	2	4	3	4	7
0.50–1.00	2	0	2	1	0	1	0	4	4
>1.00	0	1	1	0	0	0	0	3	3
—	13	14	28[g]	7	5	12	7	15	22
Sample 4. Ammoniated Superphosphate									
<0.10	4	2	6	4	2	7[g]	1	3	4
0.10–0.24	4	8	12	2	2	4	2	2	4
0.25–0.49	1	3	4	1	0	1	0	3	3
0.50–1.00	1	2	3	0	0	0	0	2	2
>1.00	0	3	3	0	2	2	0	8	8
—	10	18	28	7	6	14[g]	3	18	21
Samples 1 to 4									
<0.10	18	18	37[g]	9	9	19[g]	6	10	16
0.10–0.24	13	24	37	7	9	16	10	6	16
0.25–0.49	8	14	22	5	7	12	7	15	22
0.50–1.00	8	5	13	4	0	4	4	13	17
>1.00	2	5	7	1	2	3	2	17	19
—	49	66	116[g]	26	27	54[g]	29	61	90

[a] Official method.
[b] Direct volumetric method, Procedure 1.
[c] Direct volumetric method, Procedure 2.
[d] Direct photometric method.
[e] Method I gave the lower result.
[f] Method I gave the higher result.
[g] Including one comparison in which the methods gave the same result.

TABLE 7.—*Summary of differences in the high and low results of replicated determinations of available P_2O_5*

SAMPLE[a]	METHOD I[b]			METHOD II[c]			METHOD III[d]			METHOD IV[e]		
	ANALYSES	DIFFERENCE[f]		ANALYSES	DIFFERENCE[f]		ANALYSES	DIFFERENCE[f]		ANALYSES	DIFFERENCE[f]	
		RANGE	AVERAGE		RANGE	AVERAGE		RANGE	AVERAGE		RANGE	AVERAGE
	number	*per cent*	*per cent*	*number*	*per cent*	*per cent*	*number*	*per cent*	*per cent*	*number*	*per cent*	*per cent*
1	34	0.01–0.35	0.08	33	0.00–0.60	0.16	16	0.05–0.30	0.13	26	0.00–2.00	0.35
2	28	0.05–0.50	0.18	27	0.00–0.50	0.24	12	0.00–0.80	0.30	21	0.10–3.00	0.77
3	29	0.01–0.36	0.12	28	0.00–0.93	0.21	12	0.00–0.20	0.13	22	0.10–1.00	0.39
4	29	0.04–0.70	0.19	28	0.05–0.76	0.23	14	0.05–0.76	0.26	21	0.00–1.15	0.36
5	11	0.01–0.21	0.07	11	0.02–0.60	0.14	5	0.00–0.55	0.17	9	0.01–0.43	0.14
6	7	0.10–0.71	0.25	7	0.25–7.00	1.74	4	0.13–0.77	0.40	6	0.00–8.00	1.70
1 to 4[g]	120	0.01–0.70	0.14[h]	116	0.00–0.93	0.21[h]	54	0.00–0.80	0.20[h]	90	0.00–3.00	0.46[h]
1 to 4[i]	118	0.01–0.50	0.13[h]	109	0.00–0.50	0.18[h]	51	0.00–0.45	0.17[h]	69	0.00–0.40	0.27[h]

[a] Sample 1, normal superphosphate; Sample 2, triple superphosphate; Samples 3 and 4, ammoniated superphosphate; Sample 5, cottonseed meal; Sample 6, calcium metaphosphate.
[b] Official method.
[c] Direct volumetric method, Procedure 1.
[d] Direct volumetric method, Procedure 2.
[e] Direct photometric method.
[f] Difference between the high and low results of replicated determinations.
[g] All analyses.
[h] Weighted average.
[i] Excluding analyses in which the difference was more than 0.50%.

nations exceed 0.49 per cent, as compared with 9 per cent by Method II, 6 per cent by Method III, and 31 per cent by Method IV (Table 8). For differences of less than 0.25 per cent of P_2O_5, the figures are 84, 71, 74, and 39 per cent for Methods I, II, III, and IV, respectively.

It appears that the precision of the results among the replicates of an analysis is highest with the official method, somewhat lower with the direct volumetric methods, and considerably lower with the direct photometric method.

SELECTED COMMENTS OF COLLABORATORS

Collaborator 1.—With a wavelength of 430 mμ in the photometric method (Beckman Model B photometer), which was used instead of 400 mμ, the calibration factor was more nearly constant and the calibration curve was more nearly linear. The direct volumetric method took more time and seemed to have more sources of error.

Collaborator 2.—The direct volumetric method required a longer time for washing the sample to remove water-soluble P_2O_5 than did the official method. The official method seems to be more suitable for multiple routine determinations.

Collaborator 3.—Under definitely controlled conditions, such as a constant temperature for making the solutions to volume and pipetting aliquots, the photometric method has merit for accurate determination of phosphorus over a wide range of values. For control work involving many analyses, it is questioned whether a saving of time and expense would result from its use, when the costs of the photometer and of the additional calibrated glassware are taken into consideration.

Collaborators 5 and 8.—In the nitric-perchloric acid treatment of the combined water and citrate extracts for direct determination of available P_2O_5 in the cottonseed meal and the calcium metaphosphate (Samples 5 and 6) the digestion was halted at the appearance of dense white fumes, the solution was allowed to cool for several minutes, hydrogen peroxide (30 per cent) was added cautiously in small increments until the solution became water white, and the solution was then boiled to remove excess hydrogen peroxide. Hydrogen peroxide facilitated the digestion and apparently the conversion of the dissolved phosphorus to the orthophosphate form. For the volumetric analyses, aliquots of the digested solutions were treated in the usual way with ammonia and ammonium nitrate, and the phosphorus was precipitated by appropriate additions of the official volumetric molybdate solution with continuous stirring at room temperature. The photometric analyses were made on unneutralized portions of the digested solutions.

Collaborator 6.[1]—It is suggested that a study be made of the conditions affecting the stability of the color developed in the photometric method.

Collaborator 9.—In the determination of total P_2O_5 in Sample 5 the quantity of nitric acid specified seemed inadequate to destroy the easily oxidizable organic matter. This sample was digested with an additional 30 ml of nitric acid before adding the perchloric acid. Sample 5 was also decomposed as directed in *Official Methods of Analysis*, 7th Ed., 1950, p. 8, sect. **2.9(c)**, with values of 2.25, 2.26, and 2.28 per cent for total P_2O_5. With decomposition of Sample 6 as directed in sect. **2.9(b)**, values of 64.06, 64.06, and 64.26 per cent were obtained for total P_2O_5.

We do not like to decompose large quantities of organic matter with perchloric

[1] This collaborator, Rhodella Wilkins, and Chester Gordon, California Department of Agriculture, compared the Klett-Summerson and the Beckman DU instruments in the photometric determination of P_2O_5, with the results given in Table 10. Also, an extensive study was made of the direct determination of available P_2O_5 in cottonseed meal, as mentioned in a subsequent section (available P_2O_5 in calcium metaphosphate and cottonseed meal) of this report.

TABLE 8.—*Distribution of differences in the high and low results of replicated determinations of available P_2O_5 in Samples 1 to 4*

DIFFERENCE	METHOD I[a]		METHOD II[b]		METHOD III[c]		METHOD IV[d]	
	ANALYSES	PROPORTION	ANALYSES	PROPORTION	ANALYSES	PROPORTION	ANALYSES	PROPORTION
per cent	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
Sample 1. Normal Superphosphate								
<0.10	23	68	8	24	5	31	1	4
0.10–0.24	10	29	18	55	9	56	11	42
0.25–0.49	1	3	6	18	2	13	8	31
>0.49	0	0	1	3	0	0	6	23
—	34	100	33	100	16	100	26	100
Sample 2. Triple Superphosphate								
<0.10	5	18	3	11	2	17	0	0
0.10–0.24	16	57	13	48	4	33	5	24
0.25–0.49	6	21	9	33	4	33	5	24
>0.49	1	4	2	8	2	17	11	52
—	28	100	27	100	12	100	21	100
Sample 3. Ammoniated Superphosphate								
<0.10	16	55	5	18	4	33	0	0
0.10–0.24	9	31	18	64	8	67	10	46
0.25–0.49	4	14	2	7	0	0	6	27
>0.49	0	0	3	11	0	0	6	27
—	29	100	28	100	12	100	22	100
Sample 4. Ammoniated Superphosphate								
<0.10	7	24	5	18	2	14	3	14
0.10–0.24	16	55	13	46	6	43	5	24
0.25–0.49	4	14	6	22	5	36	8	38
>0.49	2	7	4	14	1	7	5	24
—	29	100	28	100	14	100	21	100
Samples 1 to 4								
<0.10	51	42	21	18	13	24	4	5
0.10–0.24	51	42	62	53	27	50	31	34
0.25–0.49	15	13	23	20	11	20	27	30
>0.49	3	3	10	9	3	6	28	31
—	120	100	116	100	54	100	90	100

[a] Official method.
[b] Direct volumetric method, Procedure 1.
[c] Direct volumetric method, Procedure 2.
[d] Direct photometric method.

acid, as in the case of the direct determination of available P_2O_5 in the combined water and citrate extracts of Samples 5 and 6. The addition of as much as 250 ml of nitric acid in 50 ml increments failed to prevent charring when the solution was evaporated to fumes of perchloric acid.

It appears to be necessary to heat the solution when the phosphomolybdate is precipitated in the presence of citrate, but this causes high results when citrate is not present. The recommended aliquots are too small to permit an adequate titer in the determination of available P_2O_5 by direct volumetric methods.

We are unable to explain the low results for available P_2O_5 in Sample 6 as determined by the direct photometric method. The intensity of the color was the same after the solution stood for 24 hours.

The direct methods should be useful for evaluating materials having approximately uniform composition, such as superphosphate. Their limited applicability is against their use as official procedures.

Collaborator 11.—In previous comparisons on other samples it was found that values for available P_2O_5 were usually lower by the direct volumetric method than by either the official method or the direct photometric method. The limited data indicate, however, that the volumetric method should be sufficiently accurate for routine work. It would save considerable time and chemicals.

Perchloric acid seems to have a bleaching effect on the color of the phosphovanadomolybdate solution. To overcome this effect it was necessary to take a very small aliquot for the photometric determination of available P_2O_5 in Sample 5.

Collaborator 16.—Digestion of Samples 5 and 6 for determination of total P_2O_5 required much time. We would be concerned about the danger of using perchloric acid in routine work. It was very difficult to destroy the organic matter in the combined water and citrate extracts of Samples 5 and 6 for determination of available P_2O_5 by the direct methods. It was necessary to evaporate the extract several times with nitric acid in addition to the perchloric acid. A heavy precipitate formed when the water and citrate extracts of Sample 5 were combined.

Collaborator 18.—With determination of total P_2O_5 in Sample 5 by decomposition of the material as directed in *Official Methods of Analysis*, 7th Ed., 1950, p. 8, sect. **2.9(c)**, and determination of citrate-insoluble P_2O_5 by ashing the citrate-insoluble residue and dissolving the ash in hydrochloric acid, together with precipitation of the phosphomolybdate at room temperature as directed in sect. **2.13(a)**, the results agreed closely with those obtained in the corresponding, much more tedious determinations involving nitric-perchloric acid digestions.

Collaborator 19.—Our Coleman spectrophotometer (Model 11) does not appear to be sufficiently sensitive for use in the direct photometric method. Successive readings on the same solution, without removing it from the photometer, varied as much as 0.020 mg of P_2O_5. The reported results are averages of 3 to 5 successive readings on the same solution.

Collaborator 20.[2]—From our experience it appears that the direct photometric method is applicable to routine analysis, provided four standard solutions containing different concentrations of P_2O_5 are included in each set of analyses. This is necessary because the standard curve tends to shift slightly from time to time, although the slope remains constant.

Collaborator 21.[3]—It appears that the phosphorus in the combined water and citrate extracts of Sample 5 is incompletely converted to the orthophosphate form under the conditions prescribed for the nitric-perchloric acid digestion. At any rate, such digestion of water-citrate extracts is highly impractical on a production basis.

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[2] Additional results obtained by this collaborator, Leo J. Faneuf, New Jersey Agricultural Experiment Station, with the direct photometric method are given in Tables 10, 11, and 12.

[3] Additional results reported by this collaborator, J. S. Pittard, R. T. Teague, Jr., and J. G. Jernigan, North Carolina Department of Agriculture, are given in Table 13.

Collaborator 22.—Difficulty was experienced in obtaining consistent results by the photometric method.

Collaborator 23.—We find no saving of time with the direct volumetric method. Furthermore, we are interested in knowing both the citrate insoluble and the available P_2O_5.

Collaborator 25.—The small size of the aliquot necessary with the photometric method makes it very difficult to obtain consistent results. Many replications were made, and the reported results are from those in which the best agreement was obtained.

Collaborator 27.—For determination of total P_2O_5 in calcium metaphosphate, it has been found much simpler and more dependable to dissolve the sample by boiling with nitric acid (1+1) and then to digest an appropriate aliquot with 5 ml of nitric acid for 15 minutes to complete the conversion of the phosphate to the ortho form.

The preparation of Solution B, as prescribed in the directions for available P_2O_5 in Samples 5 and 6 by the direct methods, is too complex and time-consuming for practical application. The operations introduce potential inaccuracies, but regardless of accuracy the available P_2O_5 in such materials can be determined in less time by the official method.

At present we cannot subscribe to the direct determination of available P_2O_5, although it may be feasible where only orthophosphate is present and acid digestion of the water-citrate extract is not required. Even so, when the P_2O_5 content is low and a large aliquot of the extract is required, our experience has been that the presence of considerable citrate, in relation to the P_2O_5, interferes with the molybdate precipitation.

Collaborator 28.—In work with a photometric method similar to the one used in this collaborative study we found it difficult to obtain accurate results unless the operator is thoroughly familiar with the procedural technics. There appears to be considerable possibility for error in the preparation and interpolation of the standard curve. We believe the direct methods for available P_2O_5, particularly the photometric method, have good possibilities for use in control work, but much more work is needed to determine whether better agreement with the official method can be obtained.

Collaborator 30.—Subsequent determinations (made in triplicate) of available P_2O_5 in Samples 1 and 4 by the photometric method gave higher results than those previously reported from this laboratory—Sample 1, 20.6–20.6 per cent, average 20.6 per cent; Sample 4, 15.6–15.9 per cent, average 15.7 per cent. Additional determinations showed no higher results for available P_2O_5 in Sample 5 by the direct volumetric methods.

Collaborator 32.—An appreciable saving of labor and time does not appear to be obtained with the direct volumetric method.

Collaborator 33.—It appears that the direct volumetric method could well serve as a screening method in control laboratories.

Collaborator 34.—With the photometric procedure we had much difficulty in setting up a standard curve in the concentration range 0.2–0.8 mg. A sharp break occurred in the 0.5–0.6 mg region.

PERFORMANCE OF PHOTOMETRIC METHOD WITH DIFFERENT INSTRUMENTS

As previously pointed out, several makes of photometers were used in the collaborative analyses. The differences in the results for available P_2O_5 in the normal superphosphate (Sample 1), for example, as determined

by the official method and with the aid of the several makes of instruments in the photometric method are summarized in Table 9. All of these comparisons are by different collaborators, none of whom used more than one make of photometer. Regardless of the make of the instrument, the ranges and averages of the differences between the high and low results in replicate determinations are higher with the photometric method than with the official method. According to these limited data, the agreement among the individual replications appears to be better with the Coleman, Fisher, and Klett-Summerson instruments than with the Beckman and Evelyn instruments. With each make of instrument, the average values

TABLE 9.—*Summary of differences in results for available P_2O_5 in normal superphosphate (Sample 1), as determined by official method (I) and photometric method (IV)*

PHOTOMETER	COMPARISONS	DIFFERENCE IN AVAILABLE P_2O_5 BETWEEN:					
		HIGH AND LOW RESULTS OF INDIVIDUAL REPLICATIONS				AVERAGE RESULTS OF METHODS[a]	
		RANGE		AVERAGE			
		METHOD I	METHOD IV	METHOD I	METHOD IV	RANGE	AVERAGE
	number	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Beckman	13	0.01, 0.35	0.10, 0.85	0.10	0.33	−1.35, 0.55	−0.22
Coleman[b]	4	0.02, 0.10	0.00, 0.40	0.06	0.18	−1.37, 0.14	−0.11
Evelyn	4	0.03, 0.23	0.15, 2.00	0.10	0.71	−1.44, −0.01	−0.45
Fisher	2	0.04, 0.06	0.12, 0.20	0.05	0.16	−0.30, 0.94	−0.32
Klett-Summerson	2	0.02, 0.15	0.20, 0.30	0.09	0.25	−0.40, 0.31	−0.05
	25	0.01, 0.35	0.00, 2.00	0.09[c]	0.35[c]	−1.44, 0.94	−0.23[c]

[a] The minus sign denotes that Method I gave the higher result.
[b] Omitting Collaborator 19; see "Selected Comments of Collaborators."
[c] Weighted average.

for available P_2O_5 by the photometric method are lower than those by the official method (Table 9, column 8); the differences are smaller with the Coleman and Klett-Summerson photometers than with the other instruments. It should be emphasized, however, that the indicated differences in the results obtained with the different instruments have little if any significance because of the few analyses and the disparity in the number of comparisons.

Table 10 gives the results of comparisons of the Beckman DU photometer with the Coleman Universal instrument and with the Klett-Summerson instrument on several of the collaborative samples, submitted as supplemental data by Leo. J. Faneuf, New Jersey Agricultural Experiment Station, and Rhodella Wilkins and Chester Gordon, Bureau of Chemistry, California Department of Agriculture. In general, the results with the Coleman instrument (Series I) agree more closely with those by

the official method than do the results with the Beckman instrument. In the other comparisons (Series II), however, the average results with the Beckman instrument are in closer agreement with the values by the official method than are those with the Klett-Summerson instrument. As compared with the other instruments, poorer agreement among replications is indicated for the Beckman instrument in both series.

TABLE 10.—*Comparative results for available P_2O_5 in collaborative samples, as determined by the official method and by the use of three types of instruments in the direct photometric method*

SAMPLE[a]	OFFICIAL METHOD[b]		PHOTOMETRIC METHOD[b]							
			COLEMAN UNIVERSAL				BECKMAN DU[c,d]		KLETT-SUMMERSON[c,e]	
			WATER BLANK		REAGENT BLANK					
	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
					Series I[f]					
1	20.69	0.08	20.85	0.24	20.59	0.02	20.27	0.40	—	—
2	49.25	0.34	48.92	1.25	47.67	2.50	47.33	2.50	—	—
3	20.37	0.08	20.59	0.04	20.59	0.02	20.13	0.60	—	—
4	16.93	0.20	17.41	0.04	17.30	0.18	17.07	0.40	—	—
Average	26.81	0.18	26.94	0.39	26.54	0.68	26.20	0.98	—	—
					Series II[g]					
1	20.51	0.12	—	—	—	—	20.00	0.40	21.97	0.10
2	47.97	0.05	—	—	—	—	48.33	3.00	48.58	1.25
3	19.90	0.15	—	—	—	—	20.03	0.60	22.33	0.95
Average	29.46	0.11	—	—	—	—	29.45	1.33	30.96	0.77

[a] Sample 1, normal superphosphate; Sample 2, triple superphosphate; Samples 3 and 4, ammoniated superphosphate.
[b] *Average* of triplicate determinations; *difference* between the high and low results of the replication.
[c] Reagent blank.
[d] In Series II the wave length was 400 mμ with slit width of 0.03 mm.
[e] Model 900-3, blue filter (420 mμ).
[f] Analyses by Leo J. Faneuf, New Jersey Agricultural Experiment Station. Continuous agitation during the citrate digestion.
[g] Analyses by Rhodella Wilkins and Chester Gordon, Bureau of Chemistry, California Department of Agriculture. Intermittent agitation during the citrate digestion.

The Coleman Universal and Beckman DU photometers were compared by Leo. J. Faneuf in unreplicated determinations of available P_2O_5 in 48 samples of commercial mixed fertilizers by the direct photometric method, with the results summarized in Tables 11 and 12. From the standpoint of the relation of the results by the photometric to those by the official method, no considerable advantage of the one instrument over the other is indicated. It will be noted, however, that the values with the Beckman instrument show less divergence from and are predominantly higher than those by the official method, whereas the Coleman values, with both the

water blank and the reagent blank, are predominantly lower than the official values.

TABLE 11.—*Differences in results for available P_2O_5 in commercial mixed fertilizers as determined by the official method and by the use of two types of instruments in the direct photometric method*[a]

OFFICIAL METHOD VS.:	DIFFERENCE IN AVAILABLE P_2O_5[b]				
	MINUS VALUES	PLUS VALUES	ZERO VALUES	ALL VALUES	
				RANGE	AVERAGE
	number	*number*	*number*	*per cent*	*per cent*
Photometric method:					
A1[c]	32[d]	12[e]	4	−1.06, 0.64	−0.16
A2[f]	29[g]	18[h]	1	−1.03, 0.80	−0.18
B[i]	20[j]	27[k]	1	−0.66, 0.90	0.11
Guarantee[l]	33	14	1	−0.96, 2.61	0.05

[a] 48 samples; analyses by Leo J. Faneuf, New Jersey Agricultural Experiment Station; continuous agitation during citrate digestion.
[b] The minus sign denotes that the official method gave the higher result.
[c] Coleman Universal spectrophotometer, water blank.
[d] Range, −1.06, −0.01%; average, −0.33%.
[e] Range, 0.01, 0.64%; average, 0.24%.
[f] Coleman Universal spectrophotometer, reagent blank.
[g] Range, −1.03, −0.02%; average, −0.45%.
[h] Range, 0.03, 0.80%; average, 0.25%.
[i] Beckman DU spectrophotometer, reagent blank.
[j] Range, −0.66, −0.03%; average, −0.24%.
[k] Range, 0.01, 0.90%; average, 0.37%.
[l] Range, 7–12% available P_2O_5.

TABLE 12.—*Distribution of differences in results for available P_2O_5 in commercial mixed fertilizers, as determined by the official method and by the use of two types of instruments in the direct photometric method*[a]

DIFFERENCE	OFFICIAL METHOD VS. PHOTOMETRIC METHOD:					
	A1[b]		A2[c]		B[d]	
	COMPARISONS	PROPORTION	COMPARISONS	PROPORTION	COMPARISONS	PROPORTION
per cent	*number*	*per cent*	*number*	*per cent*	*number*	*per cent*
<0.10	12	25	10	21	9	19
0.10–0.24	14	29	8	17	13	27
0.25–0.49	12	25	15	31	15	31
>0.49	10	21	15	31	11	33
—	48	100	48	100	48	100

[a] 48 samples; analyses by Leo J. Faneuf, New Jersey Agricultural Experiment Station.
[b] Coleman Universal spectrophotometer, water blank.
[c] Coleman Universal spectrophotometer, reagent blank.
[d] Beckman DU spectrophotometer, reagent blank.

The foregoing data do not afford a sound basis for recommendations as to the type or types of instruments for use in the photometric method. Obviously, much more work needs to be done, not only on the method it-

self but also on the characteristics and behavior of the various photometers in relation to the conditions encountered in practical operation of the method.

AVAILABLE P_2O_5 IN CALCIUM METAPHOSPHATE AND COTTONSEED MEAL

Analysis of metaphosphates and organic phosphorus materials for available P_2O_5 by the direct methods involves the additional step of converting the phosphorus dissolved by the combined water and citrate extractions into the orthophosphate form. With metaphosphates the conversion can be accomplished rather readily by low-temperature digestion of the solution with nitric acid (7), but much more drastic treatment is necessary with the organic phosphorus materials (seed meals, pomaces, Milorganite, etc.). Conversion of the organic phosphorus in the solution to the orthophosphate form requires the oxidation not only of the dissolved organic matter itself but, for the photometric method at least, also the large quantity of citrate present therein. Thus, it is possible to effect satisfactory oxidation of the organic material by digestion, for example, with nitric-perchloric acids as outlined in the Instructions to Collaborators or with sulfuric acid and sodium nitrate as proposed by Teague (11), but only with the expenditure of much time and attention.

For the determination of available P_2O_5 in both calcium metaphosphate (Sample 6) and cottonseed meal (Sample 5) by the direct methods, the collaborators in the present study were instructed to digest the combined water-citrate extract with nitric-perchloric acids. In the case of the metaphosphate the results by the direct volumetric methods are mostly in fairly good agreement with those by the official method (Tables 3 and 4). On the other hand, about half of the collaborators reported very low values for available P_2O_5 in the cottonseed meal by all of the direct methods.

The low values reported by many of the collaborators for available P_2O_5 in the cottonseed meal by the direct methods appear to be due, at least in part, to incomplete conversion of the dissolved organic phosphorus to the orthophosphate form. The results of an extensive study made by Rhodella Wilkins and Chester Gordon, Bureau of Chemistry, California Department of Agriculture, indicate rather plainly that the Instructions to Collaborators relative to nitric-perchloric acid digestion of the combined water-citrate extract lacked sufficient clarity and detail to insure complete conversion of the organic phosphorus. As pointed out by these collaborators, another adverse factor may have been the failure of the Instructions to indicate specifically that the highly acid solution from the nitric-perchloric digestion should be neutralized before proceeding with the determination. They report that in some instances low results were obtained on unneutralized solutions, especially by the volumetric method.

J. S. Pittard, R. T. Teague, Jr., and J. G. Jernigan compared nitric-perchloric acid digestion of the combined water-citrate extract, as outlined in the Instructions to Collaborators, with sulfuric acid-sodium nitrate digestion, as recommended by Teague (11), in the direct photometric determination of available P_2O_5 in the samples of calcium metaphosphate and cottonseed meal, with the results given in Table 13. For

TABLE 13.—*Available P_2O_5 in calcium metaphosphate and cottonseed meal in relation to procedure for digesting the combined water-citrate extract in the direct photometric method*[a]

METHOD AND PROCEDURE	AVAILABLE P_2O_5	
	CALCIUM METAPHOSPHATE[b]	COTTONSEED MEAL[c]
	per cent	*per cent*
Official	62.65	2.17
	62.65	2.20
	62.45	2.17
	62.58	2.18
Photometric:		
HNO_3-$HClO_4$ digestion[d]	64.30	0.71
	64.10	0.75
	63.80	0.67
	64.07	0.71
H_2SO_4-$NaNO_3$ digestion[e]	62.30	2.10
	61.70	2.20
	61.90	2.15
	61.97	2.15

[a] Analyses by J. S. Pittard, R. T. Teague, Jr., and J. G. Jernigan, Analytical Division, North Carolina Department of Agriculture.
[b] Sample 6.
[c] Sample 5.
[d] Digestion as outlined in Instructions to Collaborators.
[e] Digestion as recommended by Teague (11).

the cottonseed meal, the values with sulfuric-nitrate digestion are three times those with nitric-perchloric digestion and are very close to the results by the official method. For the calcium metaphosphate on the other hand, the sulfuric-nitrate values are considerably lower than the nitric-perchloric values, and are also lower than the results by the official method.

CONCLUSIONS

From the results of this and other investigations, it is evident that determination of available P_2O_5 by either volumetric or photometric

analysis of the combined water and citrate extracts of the sample has considerable promise as an alternative to the present official method whereby the available P_2O_5 is determined by difference between the total and the citrate-insoluble P_2O_5. In the present stage of their development, the precision of the direct volumetric method is higher than that of the direct photometric method and the results are in better agreement with those obtained by the official method.

With the direct volumetric method, the results tend to be higher and subject to less variation when the phosphomolybdate is precipitated at 50°C. with continuous agitation for thirty minutes at this temperature (Method III) than when the precipitation is made at 50°C. but heat is not applied during the subsequent agitation (Method II).

With the direct methods, the presence of soluble phosphorus in non-orthophosphate forms, as in calcium metaphosphate and many natural organic materials, introduces the problem of converting this phosphorus to the orthophosphate form in solutions containing large amounts of citrate. Under these conditions the conversion of the soluble organic phosphorus is particularly difficult and time consuming.

The available data do not point conclusively to the superiority of any specific make or type of photometer for use in the photometric method. Comparisons are needed of the various instruments as to their performance under conditions carefully standardized with respect to the controllable variables encountered in the practical execution of the method.

Much additional work must be done before it can be decided whether the direct methods for available P_2O_5 are worthy of designation as official.

SUMMARY

Direct volumetric and photometric methods for available P_2O_5 were compared with the official method by 34 collaborators in some 600 replicated analyses (3 or more replications) of 1 sample each of normal and triple superphosphates, calcium metaphosphate, and cottonseed meal and 2 samples of ammoniated normal superphosphate.

The official method gave the higher result for available P_2O_5 in the majority of the individual comparisons. Thus, on the four superphosphate materials the result by this method was higher in 50 to 57 per cent of the comparisons with the two variations of the direct volumetric method and in 68 per cent of those with the photometric method. For all the comparisons on these samples, the weighted average values by the official method were 0.49 per cent of P_2O_5 higher than by the photometric method and 0.00 to 0.07 per cent higher than by the variations of the volumetric method. Differences of less than 0.25 per cent of P_2O_5 were shown in 64 to 65 per cent of the individual comparisons of the official method with the variations of the volumetric method but in only 36 per cent of the comparisons with the photometric method.

The precision in the analysis of the superphosphate materials, as indicated by the difference between the high and low values in replicate determinations, was best (average, 0.14 per cent of P_2O_5) with the official method and poorest (average, 0.46 per cent) with the photometric method. The difference was less than 0.25 per cent of P_2O_5 in 84 per cent of the individual analyses by the official method, 71 to 74 per cent of the analyses by the variations of the volumetric method, and only 39 per cent of those by the photometric method.

Differences among the results by the photometric method were not decisively correlated with the makes or types of photometric instruments used in this investigation.

In the hands of a number of the collaborators the direct methods gave very low values for available P_2O_5 in the sample of cottonseed meal. Apparently, this was chiefly because of failure in such cases of the procedure for the nitric-perchloric acid digestion of the combined water-citrate extract, as outlined in the Instructions to Collaborators, to effect complete conversion of the soluble organic phosphorus to the orthophosphate form. Similar treatment of the water-citrate extract of calcium metaphosphate was effective in converting the dissolved metaphosphate to the orthophosphate form.

In this investigation the results by the direct volumetric method were generally more precise and in better agreement with those by the official method than were the results by the photometric method.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation of the fine cooperation given by the collaborators and their respective organizations. Special acknowledgment is made to the following persons for the very helpful supplemental data submitted on the samples and methods: Leo J. Faneuf and Stacy B. Randle, Department of Chemistry, New Jersey Agricultural Experiment Station; J. S. Pittard, R. T. Teague, Jr., J. G. Jernigan, and Harry A. Miller, Analytical Division, North Carolina Department of Agriculture; Rhodella Wilkins, Chester Gordon, and Allen B. Lemmon, Bureau of Chemistry, California Department of Agriculture.

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RECOMMENDATIONS*

It is recommended that further study be made of—

(1) Methods for direct determination of available P_2O_5 in fertilizers.

* For report of Subcommittee A and action of the Association, see *This Journal*, 37, 61 (1954).

(2) The use of perchloric acid in preparation of phosphate fertilizer solutions for analysis.

REFERENCES

(1) ALLEN, H. R., *This Journal*, **36**, 872 (1953).
(2) ———, SWIFT, E., HAYES, R., and KAUFMAN, Z. F., *ibid.*, **35**, 764 (1952).
(3) EPPS, E. A., JR., *Anal. Chem.*, **22**, 1062 (1950).
(4) GRIFFIN, E. L., SMITH, J. B., and MARSHALL, C. V., *This Journal*, **36**, 49 (1953)
(5) JACOB, K. D., ARMIGER, W. H., CARO, J. H., and HOFFMAN, W. M., *ibid.*, **34**, 624 (1951).
(6) ———, CARO, J. H., and MAGNESS, R. M., *ibid.*, **35**, 666 (1952).
(7) ———, HOFFMAN, W. M., and SCHRAMM, F. C., *ibid.*, **36**, 632 (1953).
(8) ———, MAGNESS, R. M., and WHITTAKER, C. W., *ibid.*, **33**, 234 (1950).
(9) ———, RADER, L. F., JR., and WHITTAKER, C. W., *ibid.*, **31**, 209 (1948).
(10) ———, WARD, F. N., MAGNESS, R. M., and WHITTAKER, C. W., *ibid.*, **32**, 202 (1949).
(11) TEAGUE, R. T., JR., *ibid.*, **36**, 880 (1953).

REPORT ON NITROGEN IN FERTILIZERS

By H. A. DAVIS (New Hampshire Agricultural Experiment Station, University of New Hampshire, Durham, N. H.), *Associate Referee*

During the 1952 meeting there was considerable informal discussion in regard to the exact details of methods for the determination of nitrogen in fertilizers and it was suggested that a critical survey of the methods for total nitrogen, outlined in *Methods of Analysis*, A.O.A.C., would be desirable. Further suggestion was made that it would be advantageous to designate one of these methods as the "preferred" method to be used particularly in cases when the analysis of a specific product is questioned. As a result of this discussion, the Associate Referee prepared a questionnaire concerning these methods. This questionnaire was submitted for comment to Dr. C. E. Perrin of the Canada Packers, Ltd., and to E. A. Epps, Chief Chemist, Department of Agriculture, State of Louisiana, who had expressed a special interest in the problem. Upon the receipt of their suggestions, a final questionnaire was drawn up.

Briefly, the various methods given in the 7th edition of *Methods of Analysis* are as follows: Section **2.25** is the Kjeldahl method (Section **2.22**) modified to include nitrogen of nitrates. It specifies the use of mercury or its oxide as a catalyst. Section **2.26** is the Gunning Method (Section **2.23**) modified to include the nitrogen of nitrates. It specifies the use of potassium sulfate to increase the boiling point, and copper sulfate as a catalyst. The use of salicylic acid and sodium thiosulfate previous to digestion (for including the nitrogen of nitrates) is common to both sec-

tions **2.25** and **2.26.** Sections **2.22** and **2.23,** together with section **2.24** (Kjeldahl-Wilfarth-Gunning Method) are official methods for nitrogen of protein in feeding stuffs and other materials containing no nitrate nitrogen. The Kjeldahl-Wilfarth-Gunning Method increases the amount of potassium sulfate used in the digestion and specifies copper sulfate or mercuric oxide as the catalyst.

The Kjeldahl-Wilfarth-Gunning Method, section **2.24,** modified by the use of salicylic acid and sodium thiosulfate as used in sections **2.25** and **2.26** to include the nitrogen of nitrates, would probably be satisfactory for total nitrogen in fertilizers. This suggestion was made by Dr. Perrin. It is the opinion of the Referee that most laboratories essentially comply with the official methods; however, there is no doubt but that some chemists prefer one of the official methods over another. It is likely that each chemist has his own particular techniques that appear to be satisfactory under his specific conditions. In brief, if a particular "preferred" procedure can be spelled out, it might have a place when analytical results are questioned.

In order to determine the exact techniques employed in different laboratories in the various steps of the Kjeldahl method, the questionnaire was sent to 47 control laboratories and 21 industrial laboratories. Returns were received from 43 control laboratories and 16 commercial laboratories. This is an excellent return and indicates a definite interest in the problem.

THE QUESTIONNAIRE

To conserve space, the replies to the various questions are summarized as follows:

1. *Weight of sample used.*—19 use 1.0 g; others from 0.25 g–3.5 g depending on the type of material. Factor weights are used in many laboratories.

2. *Size of Kjeldahl flasks.*—17 use 500 ml, 12 use 650 ml, 30 use 800 ml.

3. *Volume concentrated H_2SO_4 added.*—38 use 30 ml, 12 use 35, 5 use 40, the rest varying amounts; 27 use 2 grams of salicylic acid, 19 use 1 gram salicylic acid; the rest, varying amounts.

4. *Standing and mixing.*—50 analysts allow the mixture of acid and sample to stand 30 minutes or more before proceeding with the digestion, 44 agitate sample at 5 to 10 minute intervals, and 15 shake or mix only once or twice. 14 indicate that there was little or no warming at this point.

5. *Use of thiosulfate.*—34 report the addition of 5 grams of $Na_2S_2O_3 \cdot 5H_2O$, others use amounts varying from 2 to 10 grams. 5 let the mixture stand for 30 minutes, 20 let it stand for 5 to 10 minutes, the rest report that they proceed with the digestion immediately. 7 state that the mixture is agitated during the standing period; 25 use no agitation.

6. *Digestion.*—37 use electric heaters, 22 use gas. The mixture is generally heated about 10 minutes before the addition of catalyst; 30 indicate cooling somewhat before addition of the catalyst.

Catalyst.—29 use HgO or Hg, 14 use $CuSO_4$, 2 use selenium. The usual amount of HgO used is 0.7 g. The $CuSO_4$ varies from 0.2 to 1 gram. 12 report the use of antibump aids such as glass beads, chips, etc.

Time of clearing.—This varies considerably (from 10 to 30 minutes). In addition the time for the completion of digestion varies from 15 minutes to 1½ hours. This, of course, will depend on the nature of the sample. Only occasionally is the addition of more acid necessary.

The volume of water added following digestion and cooling varies from 200 to 300 ml. There is no difficulty in caking if the dilution is made while the digestion mixture is still warm.

7. *Distillation.*—33 use electric heaters, 26 use gas. There are considerable differences in the exact strength and volume of sodium hydroxide used for neutralization. There should be no difficulty at this point if sufficient alkali is added to insure an excess during distillation. When mercury is used as a catalyst, sufficient K_2S or $Na_2S_2O_3$ solution is added to precipitate the mercury.

Connecting bulb.—15 use the Kjeldahl bulb, 16 use the Iowa State bulb, 6 use the Davidson scrubber bulb, the rest use various other types.

39 use a straight delivery tube from the condenser into the receiving flask, 4 use a Goessman trap, the rest use various types, such as aeration tubes. With the exception of the Goessman traps, all tubes were immersed in the acid in the receiving flasks. No type of trap other than the Goessman was described in the reports.

Distillation and titration.—The time required for distillation was reported to range from 15 minutes to 1 hour; 44 reported complete distillation in 30 to 45 minutes. Zinc was used as the anti-bump by 51 laboratories, the others used pumice, glass beads, or porcelain chips. The concentrations of the standard acid and base used vary considerably. Many laboratories have their solutions arranged in factor concentrations. Only one laboratory reported the use of boric acid. The concentration of the standard acid is usually 2 to 5 times the concentration of the alkali used.

Indicator.—37 use methyl red as the indicator, 7 use methyl red-methylene blue, 4 use cochineal, 2 use methyl purple. Brom cresol green, sodium alizarine sulfonate, and brom phenol blue were also mentioned.

Additional points.—Comments were requested regarding the use of standardized apparatus, specification of temperature during digestion, and the use of boric acid in the receiver. Very few comments were made in regard to standardized apparatus or to digestion temperatures. (The specification of digestion temperatures is rather difficult.) Dr. Perrin suggested that the burners be regulated so that 250 ml of water in a Kjeldahl flask will be brought to a rolling boil in 4 to 5 minutes. In regard to the use of boric acid, 37 made no comment, 6 stated "yes," 10 "no."

In answer to the question, "*Do you think one 'preferred' method should be so designated in the A.O.A.C. Book of Methods*?" 34 replied "yes," 8 "no," 1 or 2 replied that they were undecided; 1 said "no objection, but is it practical?"

DISCUSSION

A survey of the replies to the questionnaires reveals, in general, that most laboratories conform with the official methods for the determinations of nitrogen. A specific laboratory may have certain points in the procedure that vary from those used in other laboratories. It is the opinion of the Associate Referee that while 34 replies stated that a "preferred" method should be detailed by the A.O.A.C., it is questionable whether such a procedure would be universally used. But in view of the fact that so many answered "yes" to this question, it is believed that a "preferred" choice of procedure should be indicated at certain critical points. Since all of the points mentioned are described in present A.O.A.C. methods,

it is the Associate Referee's opinion that no special collaborative work is required to substantiate these points as outlined below:

1. Optimum weight of sample is variable. 1 gram is the usual weight. Samples high in nitrogen may require as low as 0.7 gram and samples low in nitrogen as much as 3.5 grams. The exact weight of sample used can be left to the judgment of the analyst.

2. An 800 ml Kjeldahl flask is preferred but other sizes are satisfactory.

3. 30 ml of concentrated sulfuric acid containing 2 grams of salicylic acid are specified by the A.O.A.C. and should be used. The addition of the acid to the sample in the flask should be made in such a manner that the sample is completely covered with the acid as rapidly as possible. This mixture should be agitated at 5 or 10 minute intervals for a period of 30 minutes to insure complete reaction of the nitrates with the salicylic acid.

4. Five grams of $Na_2S_2O_3 \cdot 5H_2O$ should be added, shaken, and allowed to stand for at least 15 minutes before proceeding with the digestion.

5. The sample should be heated until the heavy white fumes that first form are removed. The catalyst and K_2SO_4 are then added. Mercuric oxide is preferred. Copper sulfate may be used, but a somewhat longer digestion period is necessary. The time of clearing of the sample and for completion of digestion vary with the sample and must be left to the judgment of the analyst.

6. A total digestion time of 2 hours is recommended. The source of heat—gas or electric—is not critical. It is important to control the heat during the digestion so that an excessive rate of boiling does not take place. In particular, the drying of digest mixture on the sides of the flask must be avoided. Dilution should be made while the digestion mixture is still warm to avoid the difficulty of caking; 250 ml of water is sufficient to use in the dilution. This mixture must be cool before adding the NaOH.

7. In distillation, sufficient sodium hydroxide must be added to completely neutralize the solution and liberate all the ammonia. The type of connecting bulb is important. It must be of such a type that "carry over" of alkali into the receiving flask is prevented. A bulb that will give some "scrubbing" action is desirable. The receiving tube from the condenser into the standard acid should insure no loss of ammonia. Many laboratories use a straight glass tube but the use of a trap of the Goessman type or an aeration tube of some sort is desirable. Distillation may be completed in $\frac{3}{4}$ of an hour or less. The exact concentration of standard acid to be used in the receiver may be left to the individual laboratory. However, the use of 0.5 *N* sulfuric acid and 0.1 *N* sodium hydroxide is recommended.

8. Methyl red indicator is recommended; however, the mixed indicator methyl red-methylene blue is preferred by a number of analysts.

If these points are taken into careful consideration by the analyst,

good agreement in the analytical results between laboratories on the same sample should be obtained without difficulty.

ACKNOWLEDGMENT

The Associate Referee is grateful to the individuals in the control and commercial laboratories who aided in this survey by filling out and returning the questionnaire.

CONCLUSION

The technique involved in the determination of nitrogen in fertilizers has been surveyed by means of a questionnaire. Critical points in the determination have been pointed out and "preferred" practices indicated.

The Associate Referee does not feel that it is advisable at this time to propose or recommend, stepwise, a method for the determination of nitrogen, except to emphasize the points in the above discussion. He therefore has no definite recommendations* to make at this time.

REPORT ON POTASH IN FERTILIZERS

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

In accordance with the recommendations approved by the Association in 1952 (*This Journal*, **36**, 48 (1953)), additional collaborative studies were made on methods for the determination of potash in fertilizers. A copy of the proposed work for 1953 was sent to each chemist who had expressed a willingness to collaborate. Plans and samples were sent to 22 chemists but only 21, representing the fertilizer industry, and commercial and control laboratories, found time to do the work and report to the Associate Referee. One chemist did not report his results because good checks were not obtained.

DIRECTIONS TO COLLABORATORS

1. Prepare a double amount of solution as follows: weigh and transfer 5 grams of each sample to a 500 ml Pyrex volumetric flask. Add 100 ml of ammonium oxalate solution, 250 ml water and digest as in A.O.A.C. method.† When cool, add ammonium hydroxide, make to volume and filter. This should be a sufficient volume of solution for all potash determinations.
2. Using the official method for potash, make 3 individual determinations on each sample on solution prepared as in 1 above.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 61 (1954).
† *Methods of Analysis, A.O.A.C.*, 7th Ed., par. **2.40(a)**, p. 17.

3. Also, using the slightly modified Perrin wet-digestion method, *This Journal*, 35, 61 (1952), make 3 individual potash determinations on each sample on solution prepared as in 1 above.
4. Also, if a flame photometer is available, make 3 different readings on each sample on solution prepared as in 1 above.

Report all results as soon as possible and not later than August 1, 1953, so the report of the General Referee can be completed for the fall meeting.

Please list any comments or criticisms.

The samples sent to you have been ground and prepared for analysis, and *should be analyzed as received.*

COMPOSITION OF SAMPLES

Six samples of mixed fertilizer were sent out for analysis by the three methods mentioned in the Directions. All samples were prepared by grinding in a Micro-Samplmill with the $\frac{1}{8}$ inch screen and were thoroughly mixed before shipment to the collaborators. The six samples were compounded as follows:

1. Mixture of 450 g of an 11-15-24, 450 g of 23-21-19, 50 g of K_2CrO_4, 110 g of $ZnCO_3$, 220 g of $CuCO_3$, 220 g of $Na_2B_4O_7 \cdot 10H_2O$, 50 g of LiCl, 50 g of $Na_2SO_4 \cdot 10H_2O$, 110 g of $MnSO_4 \cdot H_2O$, 50 g of $CoSO_4$. The K_2O level was 12.24% potash.
2. Mixture of 900 g 0-20-20, 110 g of $Na_2B_4O_7 \cdot 10H_2O$, 220 g of $MgSO_4 \cdot 7H_2O$, 220 g of $CuCO_3$, 110 g of MgO, 110 g of $CaSO_4 \cdot 2H_2O$, 110 g of $Na_2SO_4 \cdot 10H_2O$. The K_2O level was 10.25% potash.
3. Mixture of 900 g 0-20-15, 450 g of $(NH_4)_2SO_4$, 220 g of $Na_2SO_4 \cdot 10H_2O$, 50 g of LiCl, 110 g of $CaSO_4 \cdot 2H_2O$. The K_2O level was 7.8% potash.
4. Mixture of 900 g of 5-20-20, 450 g of $(NH_4)_2SO_4$, 110 g of $MnSO_4 \cdot H_2O$, 50 g of $SrCO_3$, 110 g of $CaSO_4 \cdot 2H_2O$, 100 g of $AgNO_3$. The K_2O level was 10.20% potash.
5. Mixture of 220 g of cob-ash (K_2O content 40.6) 450 g of 0-20-25, 220 g of $NaNO_3$, 300 g of $NaC_2H_3O_2 \cdot 3H_2O$, 50 g of $SrCO_3$, 220 g of $CuSO_4 \cdot 5H_2O$, 110 g of $CaSO_4 \cdot 2H_2O$, 120 g of K_2CrO_4, 20 g of $NiCl_2$, 10 g of $CoSO_4$ and 100 g of $MnSO_4 \cdot H_2O$. The K_2O level was 11.63% potash.
6. Mixture of equal parts of two commercial fertilizers, 10-10-10 and 6-12-12. The K_2O level was 11.28% potash.

The K_2O value of all potash ingredients of each sample was determined by the A.O.A.C. method before being added to the mixture. Minor element additives were incorporated in the first five samples at varying levels, while the sixth sample contained only those minor elements which had been incorporated in the original fertilizer from which this sample was compounded. These samples were purposely kept at a relatively low potash level because minor element additives were large.

RESULTS AND DISCUSSION

The collaborators' results are listed in Table 1, and summarized in Table 2.

TABLE 1.—*Per cent potash found by three methods*

COLLABORATOR	NO. OF DETNS.	SAMPLE NO. 1			SAMPLE NO. 2			SAMPLE NO. 3		
		LINDO-GLADDING	PERRIN	FLAME PHOTOMETER	LINDO-GLADDING	PERRIN	FLAME PHOTOMETER	LINDO-GLADDING	PERRIN	FLAME PHOTOMETER
2	3	12.31	13.34	—	10.09	10.44	—	7.83	7.72	—
3	3	11.59	12.83	—	9.72	10.02	—	7.79	7.68	—
4	3	—	—	11.90	—	—	9.69	—	—	7.85
5	3	11.71	11.93	11.73	9.76	10.05	9.69	7.65	7.61	7.82
9	3	11.88	12.55	—	9.63	11.41[a]	—	7.68	7.70	—
10	—	11.56[a]	11.16	—	9.73[a]	10.38[a]	—	7.67	7.48	—
12	3	12.16	11.88	11.78	9.90	9.85	9.75	7.61	7.50	7.62
13	3	12.15	12.19	—	10.09	10.17	—	7.56	7.56	—
15	3	12.50	12.29	—	10.02	9.95	—	7.64	7.60	—
17	6–11	11.48	11.73	11.76	10.04	10.09	9.87	7.53	7.66	7.66
18	3	11.69	11.62	—	9.76	9.66	—	7.52	7.50	—
19	—	11.59	11.51	11.78	10.17	9.69	9.52	7.37	7.37	7.38
22	—	12.50	12.37	12.43	9.76	9.92	9.60	7.80	7.66	8.30
24	3	12.51	12.64	12.17	10.02	12.14	9.92	7.80	7.22	8.15
25	3	11.89	12.34	—	9.76	9.85	—	7.37	7.33	—
26	3	13.03	14.93	—	10.54	11.36	—	8.54	7.71	—
28	3	12.06	12.32	11.96	9.64	9.62	9.66	7.77	7.74	7.68
29	3	12.18	13.73[a]	12.34	9.86	11.05	9.80	8.01	8.13	8.08
32	3	11.87	14.58	11.54	9.87	11.20	9.15	7.66	7.67	7.34
33	3–6	12.08	12.45	12.31	9.72	9.91	10.03	7.35	7.00	7.60
34	3	12.52	12.04	12.21	10.37	10.20	9.57	7.93	7.86	7.63
Calcd from Composition		12.24	—	—	10.25	—	—	7.80	—	—
Average		12.06	12.52	11.99	9.92	10.35	9.69	7.70	7.59	7.76
High		13.03	14.58	12.43	10.54	11.41	10.03	8.54	8.13	8.30
Low		11.48	11.16	11.54	9.63	9.66	9.15	7.35	7.00	7.34
Maximum Variation		1.55	3.42	0.89	0.91	1.75	0.88	1.19	1.13	0.96
Standard Deviation		0.41	0.97	0.29	0.24	0.70	0.22	0.27	0.24	0.29

[a] See comment of the collaborator.

TABLE 1—(*continued*)

COLLABORATOR	NO. OF DETNS.	SAMPLE NO. 4			SAMPLE NO. 5			SAMPLE NO. 6		
		LINDO-GLADDING	PERRIN	FLAME PHOTOMETER	LINDO-GLADDING	PERRIN	FLAME PHOTOMETER	LINDO-GLADDING	PERRIN	FLAME PHOTOMETER
2	3	10.43	10.46	—	12.25	12.03	—	11.68	11.56	—
3	3	10.24	10.88	—	11.63	11.85	—	11.31	11.31	—
4	3	—	—	9.88	—	—	11.80	—	—	11.60
5	3	10.70	10.76	10.17	11.81	12.28	11.81	11.49	11.45	11.39
9	3	9.98	10.37[a]	—	12.17[a]	11.72[a]	—	11.16	11.48	—
10	—	10.02[a]	10.12[a]	—	12.40[a]	11.99	—	11.53	11.47	—
12	3	10.09	10.33	10.33	11.91	12.05	12.05	11.40	11.52	11.42
13	3	10.46	10.19	—	12.49	12.43	—	11.75	11.43	—
15	3	10.69	10.79	—	12.40	12.08	—	11.46	11.42	—
17	6–11	9.78	9.98	10.07	11.49	12.12	11.93	11.35	11.52	11.55
18	3	10.06	9.96	—	11.79	11.99	—	11.55	11.46	—
19	—	9.77	9.81	9.60	10.80	11.55	11.78	11.38	11.16	11.05
22	—	10.45	10.32	9.94	12.00	12.65	12.49	11.69	11.59	11.68
24	3	10.22	9.77	10.46	12.25	11.46	12.11	11.56	11.33	11.49
25	3	9.89	10.02	—	12.03	12.29	—	11.35	11.31	—
26	3	10.91	10.47	—	12.51	12.91	—	12.27	11.57	—
28	3	10.18	10.16	10.16	12.21	12.32	12.18	11.39	11.44	11.29
29	3	10.60	10.61	10.75	12.51	13.00	11.95	11.61	11.84	10.97
32	3	9.96	10.07	9.70	11.37	12.26	12.06	11.26	11.40	10.97
33	3–6	10.78	10.08	9.87	12.76	11.43	12.67	11.81	11.15	11.31
34	3	10.22	9.85	9.57	11.83	11.94	11.79	11.57	11.44	11.31
Calcd from Composition		10.20	—	—	11.63	—	—	11.28	—	—
Average		10.27	10.25	10.50	12.03	12.13	12.05	11.58	11.44	11.34
High		10.60	10.88	10.75	12.76	13.00	12.67	12.27	11.84	11.68
Low		9.77	9.77	9.57	10.80	11.43	11.78	11.16	11.16	10.97
Maximum Variation		0.83	1.11	1.18	1.96	1.57	0.89	1.11	0.68	0.91
Standard Deviation		0.39	0.33	0.34	0.47	0.43	0.28	0.24	0.15	0.23

[a] See comment of the collaborator.

TABLE 2.—*Summary: Per cent K_2O by 3 methods*

SAMPLE	LINDO-GLADDING		PERRIN	FLAME
	MATERIALS	MIXTURE		
1	12.24	12.06	12.52	11.99
2	10.25	9.92	10.35	9.69
3	7.80	7.70	7.59	7.76
4	10.20	10.27	10.25	10.50
5	11.63	12.03	12.13	12.05
6	11.20	11.58	11.44	11.34
Av. of 6 Samples	10.55	10.59	10.71	10.55

A glance at Table 2 might lead one to believe that there is little difference between the three methods; however, if one studies the maximum variations in Table 1 it will be noted that except for Samples 3, 4, and 6, most of the chemists had trouble with the A.O.A.C. and Perrin methods. Sample 5 gave trouble with all methods. Samples 1, 2, and 5 contained minor element additives at 40, 50, and 58 per cent levels—probably higher than any fertilizer on the open market. Many chemists using the leach-back procedure for regular potash work reported large amounts of residue for Samples 1, 2, and 5, part of which was not soluble in the hot water leach-back. This indicates a need for further study of the levels of minor elements that can be added before they affect potash results with the Lindo-Gladding or Perrin methods.

Only one complaint concerning minor element interference was registered for the flame method. The standard deviation values for the flame and Lindo-Gladding methods were of the same order on all samples and for Samples 3 to 6 by the Perrin method. Standard deviations for Samples 1 and 2 by the Perrin method were somewhat higher than those by the flame method.

COMMENTS OF COLLABORATORS

No. 2. We were unable to get satisfactory checks on Samples 1, 2, and 4.

There was difficulty in igniting Nos. 1, 2, and 5 in the official A.O.A.C. method and there seemed to be a heavy residue of some form of copper. The crystallization of these three samples was off color both in the Perrin and the official A.O.A.C. methods and the crucible used in each method showed considerable gain on leach-backs. The gains ranged from 0.0133–0.0051 g for No. 1; 0.0053–0.0123 g for No. 2; and an average gain of 0.0010 g for No. 5. All results reported had leach-back blanks deducted.

On Sample No. 1, the residue was greater than in any other by the Perrin method, much more than by the official method. We found somewhat higher residue on Sample No. 2 by the Perrin than by the official method.

Sample No. 4 had a heavy white precipitate which was soluble in Lindo-Gladding solution.

Conclusions: Usually on regular potash work with platinum dishes, we rarely have much residue left on normal type materials. So if Perrin method is to be used on materials containing certain elements, it appears more study will be necessary.

No. 3. We adhered strictly to the methods in making these determinations, but we suspect that the results obtained by both the A.O.A.C. and the Perrin methods fail to reflect the true potash content of most of these samples.

No. 4. I regret that we were unable to make determinations other than by the flame photometer.

No. 9. Potassium platinic chloride precipitate could not be washed free of impurities.

No. 10. We do not understand why Samples 1, 2, 4 and 5, had such high insoluble residues; neither do we understand the apparent inconsistency when comparing the A.O.A.C. method to the wet digestion method.

No. 12. Perrin method is more efficient than A.O.A.C. official method when trace elements such as chromium and nickel are present. Silver does not seem to interfere.

No. 25. We had considerable trouble, both in the A.O.A.C. method and Perrin method, due to excessive amounts of copper and manganese. In the A.O.A.C. method, these salts contaminated the platinum dishes. In order to obtain consistent results, leaching of crucibles had to be done before calculations for potash were made.

No. 28. Minor elements did not interfere with the determinations of potash and our table shows that good results were obtained by using the Perkin-Elmer flame photometer.

We have been analyzing all potash samples, including potash salts, by the flame photometer. Those found to be under the guaranteed analysis, which are few compared with the number of samples analyzed, are repeated using the official A.O.A.C. method. Most of them check with results obtained by the flame photometer.

In regard to the modified Perrin method, we find that some of the minor elements (maybe too much in sample) seemed to interfere with the analysis, thus yielding high results. By dissolving out the K_2PtCl_6 with hot water, drying and weighing, the results check closer with the other methods. Determinations on samples No. 1, 4, and 5 were tried a second time by the modified Perrin method with the same results. We also tried two determinations of samples No. 1, 4, and 5 using 25 ml aliquots, and found that the minor element interference was eliminated in samples No. 1 and 5. The results on sample No. 1 were 11.90 and 11.90%. Results for sample No. 5 were 12.09 and 12.22%. However, sample No. 4 still showed interference due to the minor elements. The results of sample No. 4 were 11.05 and 11.07%.

No. 29. Sample No. 5. While standing on laboratory shelf, tightly corked, this sample changed from a white salt to a dirty-colored mixture; smelled strongly acetic when weighed.

Samples No. 1 and No. 5 badly discolored the platinum dishes used in the official A.O.A.C. method.

Sample No. 4, after burning off and dissolving in hot water, threw down a white precipitate upon the addition of HCl. Did not notice this effect with Perrin method.

All asbestos pads in crucibles were blue-colored after filtrations of samples containing copper salts. Most of this color was washed out in official method, but little if any of it could be removed in Perrin method.

Perrin method. Considerable greenish white precipitate occurred in samples with heavy copper, and I doubt that very much of this was removed by Gladding wash and alcohol. Precipitates from Sample No. 1 seemed to "gum up" the pads of the filtering crucibles and this made for slow filtration. Sample No. 5 was likewise slow. Samples No. 1 and No. 5 precipitated a bluish white substance that clung

to the Erlenmeyer flask. This was not removable by use of rubber policeman and alcohol. Later it dissolved in strong HCl.

Ever since we have been using the flame photometer for potash in fertilizers we have been running into samples that would run higher or lower than by the official Lindo-Gladding method. We don't know what causes it. With samples No. 5 and 6 of this year's group, a lowering is particularly noticeable.

These samples were evidently purposely overloaded with minor elements to put the methods to an extreme test. This State has been using minor elements in its fertilizers for years, and in increasing amounts, but we have never met anything thus far in the least comparable to samples No. 1, 2 and 5, as judged from the analytical difficulties.

The more I deal with the Perrin method the less favorably impressed I am with it. With fertilizers made up of only the primary plant foods, it seems to work well. When minor elements are added, trouble starts.

No. 32. In samples 1, 2, and 5 excessive amounts of copper appear to cause the Perrin wet combustion method to give high results. The flame results were uncorrected. In samples containing large amounts of metal salts, the preliminary separation should be changed to remove a larger proportion of such salts when the Perrin or Lindo-Gladding methods are used.

No. 33. The Perrin method appears to be very time-consuming and requires close attention while boiling and evaporating. Transfer of the precipitate from a Kjeldahl flask is awkward, and the removal of all precipitate is difficult at times.

COLLABORATORS

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SUMMARY

In the analysis of an ordinary fertilizer mixture (Sample 6) good agreement was obtained among laboratories with each of the three methods, and good agreement was also obtained among methods.

When copper, manganese, cobalt and some other elements were added to fertilizer mixtures in large amounts, the variations with both of the A.O.A.C. methods in results were increased in nearly all cases. Little interference was reported in the flame results, probably due in many cases to removal of interfering ions in the preparation of the solutions.

ACKNOWLEDGMENT

The Associate Referee wishes to express his thanks to the many collaborators for their fine cooperation and to F. W. Quackenbush and E. D. Schall for their suggestions and criticisms in the development of this report.

RECOMMENDATIONS

It is recommended*—

1. That potash studies be made using the Lindo-Gladding and Perrin methods with fertilizer of a wide potash range in which minor elements at various levels have been incorporated.

2. That additional studies be made towards adopting the flame photometer for use in the determination of potash in fertilizers.

3. That some consideration be given to modifying the method of preparation of solution to reduce or to eliminate minor element interference.

No reports were given on acid- and base-forming quality, copper and zinc, free water, inert materials, soil conditioners, or sulfur.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 61 (1954).

REPORT ON PRESERVATIVES AND ARTIFICIAL SWEETENERS

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

Although more literature is published on bringing crops to fruition than on preserving them after they have been harvested, there has been considerable activity in the field of preservatives during the past year.

The largest number of articles on this subject was published by the Japanese. The aspect of greatest interest to them is the prevention of spoilage of soy sauce; they employ hydroxybenzoate as a control (1). Other preservatives investigated by the Japanese include Vitamin K as an antiseptic agent for saké and fish meat paste, and 5-nitro-2-furfural semicarbazone as an antiseptic for milk.

The chief problem encountered by chemists in England and on the European continent appears to be the use of brominated acetic acid derivatives in soft drinks; a fermentation test for these derivatives has recently been devised (2). This test has been found to be a simple and effective means for detecting the presence of active concentrations of preservatives, since once the concentration of bromine has been determined, it can then be established whether or not the bromine is part of an antimicrobial compound. Belgian workers are also using this fermentation inhibition procedure for the detection of bromacetic acid in milk (3). Mention is made in Belgian literature of dehydroacetic acid; a method for its detection in beer, wine, and lemonade is recommended (4).

In the United States, studies have been made of the efficacy of sorbic acid as a mold inhibitor in cheese. Hydrogen peroxide has also been used as a preservative for cheese (5); five hundred p.p.m. has been recommended (6) for spore formers in dairy products, and a patent has been issued to E. I. du Pont de Nemours for the use of one thousand to two thousand p.p.m. of hydrogen peroxide and for its dissipation with the enzyme catalase.

Also recorded is a patent (7) for the addition to flour of α-amylase enzyme derived from *Bacillus mesentericus* or *Bacillus subtilis* in order to retard the staling of bakery products.

A patent has been issued in which is described the use of propylene or butylene glycols (after treatment to remove carbonyl-containing impurities) for coconut which contains 5 to 15 per cent moisture (8).

Workers in the Netherlands have obtained a patent (9) on a durable butter which contains a dithiocarbamic acid or its salt. It is claimed that a cream containing 0.002 per cent of the acid yielded good butter after six months' storage at 12°C.

The use of sulfites was recommended for peeled potatoes (10) and for

fresh grapes (11); however, an article in the German literature (12) contains a claim that sulfur dioxide as a preservative is limited to the acid range and that this agent becomes ineffective as a preservative because of the eventual formation of addition products with aldehydes and sugars.

Among suggestions for uses of antibiotics were the following: from Germany, preservation of mothers' milk with streptomycin (13), and retarding of the growth of *Micrococcus aureus* in custard fillings (14); from Canada, aureomycin for fish (15), and subtilin for milk (16); from Japan, terramycin, chloromycetin, and aureomycin in milk (17); from Great Britain, the use of nisin (18) for the inhibition of growth of anaerobic spore-forming bacteria in gruyere cheese.

A patent has been granted (19) for the preservation of skinless surfaces of fruits and vegetables by a dip which consists of a solution of 8-hydroxyquinoline in combination with a choline compound. Other dips cited were thiourea to preserve citrus fruits (20), and solutions of thioacetamide or thiopropionamide (21) for fresh unpeeled fruits and vegetables. An antiseptic ice containing boric acid, benzoate, and maleic or furmaric acid has been patented (22) for use as a spray for lettuce or fish, a dip for crabs, or clams, or for direct addition to fruit juices.

Also found in Japanese literature are notes on the antimicrobial activity of surface-active substances, such as sodium lauryl sulfonate (23), and on an emulsion of 0.01 per cent wood tar and 0.1 per cent *p*-hydroxybenzoate in water or in salt solution as a preservative for fish (24).

Work on food sterilization by radioactivity has been published by investigators at the University of Chicago (25), at the University of Michigan (26), and in Italy (27).

Because antibiotics and radioactive materials have been used for food preservation, it is possible that their detection and/or determination in foods may be a future problem that will come within the scope of the Referee on Preservatives and Artificial Sweeteners.

During this past year, Associate Referees have studied dimethyldichlorosuccinate, dehydroacetic acid, benzoates and hydroxybenzoates, fluorides, quaternary ammonium compounds, monochloracetic acid, cyclamate sodium, P-4000, and dulcin. Recommendations on these subjects are as follows.

RECOMMENDATIONS

It is recommended*—

(1) That a qualitative test for fluorides, including the quenching of the fluorescence of aluminum oxine as outlined by the Associate Referee be adopted, first action.

(2) That a qualitative method for benzoates and hydroxybenzoates as

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 79 (1954).

described by the Associate Referee in this year's report be submitted to collaborative study.

(3) That the spectrophotometric method as described in the 1952 report of the Associate Referee (28) be compared for speed and specificity with the recently published titration method for the determination of hydroxybenzoates before it is submitted to collaborative study (29).

(4) That the qualitative test for P-4000 as outlined in the report of the Associate Referee be adopted, first action.

(5) That the quantitative method for P-4000 as described in the report of the Associate Referee be further studied.

(6) That further study be made to develop a suitable method for the determination of dulcin (p-phenetyl-urea).

(7) That the method for quaternary ammonium compounds in milk as given in the Associate Referee's report be adopted, first action.

(8) That the method for quaternary ammonium compounds in water solutions as given in the Associate Referee's report be adopted, first action.

(9) That the method for determination of quaternary ammonium compounds as reineckates, described in *This Journal*, **35**, 456 (1952), be adopted, first action.

(10) That the method for the determination of dimethyldichlorosuccinate, *This Journal*, **36**, 538 (1953), be studied collaboratively.

(11) That further work on the determination of monochloracetic acid in fruit juices and beverage bases be discontinued.

(12) That the study of methods for the determination and identification of cyclamate of sodium and calcium be continued.

(13) The Referee also recommends the appointment of Associate Referees on the following subjects:

(a) Detection of hydrogen peroxide in dairy products.

(b) Determination of diphenyl in fruit peel and juice.

(c) Development of a procedure for the separation and estimation of cyclamate sodium and saccharin in the presence of each other.

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REFERENCES

(1) FUJIKAWA, F., TOKUOKA, A., TAKIMURA, M., and MIURA, K., *J. Pharm. Soc. Japan*, **72**, 518 (1952).

(2) MOSSEL, D. A. A., and DE BRUIN, A. S., *Analyst*, **78**, 37 (1953).
(3) THOMAS, SIMONS, and PETIT, *J. Pharm. Belge*, **7**, 217 (1953).
(4) *British Abstracts*, **1474**, May 1953, p. 223.
(5) TRECANI, V., *Ann. microbiol.*, **5**, 17 (1952).
(6) NAMBUDRIPAD, Y. K. N., and IYA, K. K., *Indian J. Dairy Sci.*, **4**, 38 (1951).
(7) STONE, I. (to the Wallerstein Co., Inc.), U. S. Pat. 2,615,810 (Oct. 28, 1952).
(8) KAUFMAN, C. W., and DE MAYA, C. B. (to General Foods Corp.), U. S. Pat. 2,685,812 (February 28, 1952).
(9) TOLLENAAR, F. D., Dutch Pat. 70,854 (Oct. 15, 1952).
(10) *Food Engineering*, May 1953, p. 164.
(11) REYNEKE, J., and PIAGET, J. E. H., *Farming in South Africa*, **27**, 477 (1952).
(12) v. SCHELHORN, M., *Ind. Obst. u. Gemüseverwert.*, **35**, 181 (1950).
(13) CRANE, J. C., STEELE, H. K., and REDFERN, S., *Food Technol.*, **6**, 220 (1952).
(14) GODKIN, W. J., and CATHCART, W. H., *ibid.*, **6**, 224 (1952).
(15) BISSETT, H. M., and TARR, H. L. A., *Fisheries Research Board Can., Progr. Repts. Pacific Coast Stas.*, **No. 93**, 23 (1952).
(16) EVANS, F. R., and CURRAN, H. R., *J. Dairy Sci.*, **35**, 1101 (1952).
(17) INOMOTO, Y., and HOSHIDA, W., *J. Fermentation Technol. (Japan)*, **30**, 287 (1952).
(18) *Food Engineering*, January 1953, p. 152.
(19) GEARY, R. J., U. S. Pat. 2,615,814 (Feb. 28, 1952).
(20) NARA, T., *Japan*, **1149** (1951).
(21) COTHREN, C. D., U. S. Pat. 2,604,409 (July 22, 1952).
(22) FRANSDEN, L. (to Washington Laboratories, Inc.), U. S. Pat. 2,622,032 (Dec. 16, 1952).
(23) SHIBASAKI, I., and TERUI, G., *J. Fermentation Technol. (Japan)*, **31**, 28 (1953).
(24) YAMADA, A. (to Nippon Marine Products Co.), Japanese Pat. 5049 (Sept. 4, 1951).
(25) *Science News Letter*, Feb. 28, 1953.
(26) *Refrig. Eng.*, **61**, 55 (1953).
(27) QUAGLIOZZI, E., and RESCIGNO, A., *Chimica e industria (Milan)*, **34**, 347 (1952).
(28) GAKENHEIMER, H. E., *This Journal*, **36**, 748 (1953).
(29) ETIENNE, H., *Ind. chim. belge*, **17**, 455 (1952).

REPORT ON QUATERNARY AMMONIUM COMPOUNDS IN MILK

By JOHN B. WILSON (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.), *Associate Referee*

In last year's report[1] the bromophenol blue method for quaternary ammonium compounds in milk was recommended as a qualitative test, first action. Since then, Furlong and Elliker[2] have published their "Improved Method for Determining Concentration of Quaternary Ammonium Compounds in Water Solutions and in Milk."

[1] WILSON, J. B., *This Journal*, **36**, 741 (1953).
[2] *J. Dairy Sci.*, **36**, 225 (1953).

The Associate Referee made a study of this method and found that when 5 ml of milk was used as recommended, the quantity of acetylene tetrachloride solution obtained after centrifuging often lacked 0.2 ml or more of the 2 ml specified in the color test, but that satisfactory positive color tests were obtained when the various quaternary ammonium compounds were present at the levels indicated in Table 1.

TABLE 1.—*Various quaternary ammonium compounds giving positive tests in Furlong and Elliker method, with 5 ml samples*

COMPOUND	*p.p.m.*
Lauryl pyridinium chloride	1
Lauryldimethylbenzylammonium chloride	2
Hyamin 10–X	2
Cetab	2
Ethyl Cetab	2
Hyamin 1622	3
Dobenzyl chloride	3
Cetyldimethylbenzylammonium chloride	3
Emulsept 607	5

Furlong and Elliker suggested that their method could be made more sensitive by increasing the quantity of the sample. After several experiments, the Associate Referee decided that a 15 ml sample was convenient and expedient, since it yielded sufficient material for the test. By using this sample and performing the test on 5 ml of ethylene chloride solution, satisfactory positive tests were obtained at the levels given in Table 2.

TABLE 2.—*Various quaternary ammonium compounds giving positive tests in Furlong and Elliker method, with 15 ml of sample and 5 ml of test liquid*

COMPOUND	*p.p.m.*
Laurylpyridinium chloride	1
Hyamin 10–X	1
Cetab	1
Ethyl Cetab	1
Dobenzyl chloride	1
Lauryldimethylbenzylammonium chloride	2
Hyamin 1622	2
Cetyldimethylbenzylammonium chloride	2

For collaborative work, a set of eight solutions was prepared to be added to milk. A quantity of D.C. 12 (lauryldimethylbenzylammonium chloride) was dissolved in water and made up to a definite volume; portions of this solution were diluted to such volumes that, when 5 ml of the final solution was diluted to 500 ml with milk, the milk contained the desired concentration of quaternary.

Each collaborator was furnished with (*a*) a set of the eight solutions of D.C. 12; (*b*) a quantity of redistilled acetylene tetrachloride (solvent); (*c*) some solid eosin yellowish (reagent); (*d*) a 1 per cent Aerosol OT solution (for preparing a titrating solution); and (*e*) some solid D.C. 12 (for preparing standards). Instructions were sent as follows:

PREPARATION OF MILK SAMPLES

Fill a 500 ml volumetric flask about one-half full of milk; with a pipet, add 5ml of the corresponding Q.A.C. solution while rotating the flask; fill to the mark with milk, mix thoroughly, and analyze.

Measure the acetylene tetrachloride and milk as accurately as possible. The author uses 15 ml pipets which are available at supply houses. Since the vapors of acetylene tetrachloride are rated as "extremely hazardous," use suction to pipet the solvent, or measure in a buret. Rinse pipets with alcohol to remove acetylene tetrachloride.

In all cases report the color of the acetylene tetrachloride layer in the test, and if insufficiently colored to warrant titration, report "present" or "absent" as the case may be. If the pink color is not readily visible, examine the tube against a white background or compare with a known blank. Report titration in volume of standard solution and as p.p.m. Q.A.C.

METHOD

APPARATUS

(a) *Centrifuge.*—Clinical high speed type fitted for 50 ml tubes. An International No. 2 centrifuge with head No. 241 at a speed of 2500 r.p.m. is also satisfactory.

(b) *Centrifuge tubes.*—Heavy wall, 40 ml centrifuge tubes. Pyrex, No. 8400 or equivalent.

(c) *Test tubes.*—Pyrex, glass-stoppered, 15×150 mm.

REAGENTS

(a) *Acetylene tetrachloride.*—Should give a distinct pink color in lower layer after sepn, when 5 ml is shaken for 1 min. with 2 ml of buffer soln, (e), 0.5 ml of eosin yellowish soln, and 5 ml of H_2O soln contg 1 p.p.m. of Cetab, Dobenzyl chloride, Ethyl Cetab, Hyamin 10-X, or laurylpyridinium chloride, or 2 p.p.m. of lauryldimethylbenzylammonium chloride. If the reagent does not meet this test, distil under reduced pressure, rejecting the first 10% of the distillate and collecting ca 80% of the vol. placed in the distn flask.

(b) *Lactic acid soln* (50%).—Add 41 g H_2O to 59 g of lactic acid, 85%, reagent grade, and mix.

(c) *Eosin yellowish soln.*—Dissolve 25 mg D&C Red No. 22 in H_2O and dil. to 50 ml.

(d) *Sodium hydroxide soln* (4 *M*).—Dissolve 32 g NaOH in H_2O and dil. to 200 ml.

(e) *Buffer soln* (*p*H 4.5).—Dissolve 25 g citric acid in 75 ml of H_2O and add sufficient (ca 13 ml) of 50% NaOH soln to bring the *p*H to 4.5.

(f) *Aerosol OT soln* (*stock*).—Prep. soln of dioctyl sodium sulfosuccinate to contain 100 mg of the solid chemical per 100 ml.

Det. strength as follows: Pipet 2 ml of a soln contg, in 100 ml, 100 mg of the quaternary ammonium compound to be detd, into glass-stoppered test tube contg 2 ml of acetylene tetrachloride, 2 ml of buffer soln, and 0.5 ml of eosin yellowish

soln. Carefully add from buret the Aerosol OT soln in small amounts, shaking mixt. violently for at least 30 sec. after each addn until after sepn into 2 layers only a light pink color is noticeable when test tube is placed against white background. Now continue the addns in 0.01 or 0.02 ml portions until lower layer is no longer pink.

(g) *Standard aerosol OT soln.*—Dil. to 100 ml such a quantity of the stock soln (f) as will produce a soln, 1 ml of which is equivalent to 0.1 mg of the quaternary ammonium compound to be detd. Standardize against a standard soln 1 ml = 0.1 mg of the quaternary to be detd.

DETERMINATION

Pipet 15 ml of acetylene tetrachloride into centrifuge tube; add 6 ml of lactic acid soln and 15 ml of milk to be tested; stopper and shake for ca 3 min. Add 6 ml of NaOH soln and mix carefully until the curd separates thruout the mixt., then shake for at least 30 sec. Centrifuge at high speed (ca 3200 r.p.m.) for 7 min. Decant serum and discard; puncture layer of curd at 2 points and drain the acetylene chloride ext. into a small beaker. Avoiding any drops of the water soln, transfer with pipet 5 ml of the ext. into a glass-stoppered test tube contg 2 ml of buffer soln and 0.5 ml of the eosin yellowish soln; stopper and shake for ca 2 min. Let stand to sep. and observe color of lower layer. If color is faint, place against a white background. If pink color is observed, a quaternary ammonium compound is present. If deep pink or red color is obtained, titrate with the standard Aerosol OT soln. After each addn of the standard soln, shake the mixt. violently for 0.5 to 1 min., let settle into 2 layers and observe the lower one. Continue addns until no pink color is observed in the lower layer when placed against a white background or compared with blank detn. The titration found represents the quantity of quaternary ammonium compound in 5 ml of sample. Calc. to p.p.m.

Collaborators were also asked to make analyses of water dilutions of each of the solutions made up as with milk, but using distilled water. The procedure for water solutions is given below.

DETERMINATION

Pipet 5 ml of sample into a glass-stoppered test tube contg 2 ml of acetylene tetrachloride, 2 ml of buffer soln, and 0.5 ml of eosin yellowish soln; stopper and shake for 2 min. Let stand to sep. and observe lower layer. If color is faint, examine against a white background. If pink color is observed a quaternary ammonium compound is present. If deep pink or red color is obtained, titrate with the standard Aerosol OT soln as in the method for milk. Calc. to p.p.m.

Results obtained by the several collaborators are given in Table 3.

The data in Table 3 show that the method can be depended upon to disclose the presence of Q.A.C. when 1 to 3 p.p.m. is present in either water or milk. No false positives were given. The quantitative figures are near enough for these small amounts.

When 10 p.p.m. or more is present, the colored compound is easily seen. From the quantitative point of view, there is considerable variation in recoveries, as shown in Table 4.

It will be noted that Collaborator No. 1 obtained the lowest result in each case. It would appear either that some of the Q.A.C. was lost or that the analyst did not actually obtain a colorless endpoint. Since Collaborator No. 1's results for the water solutions were high for three

TABLE 3.—*Determination of Q.A.C.*[a] *by method of Furlong and Elliker*

SOLUTION NO.	VEHICLE	ADDED	FOUND						COLOR OBSERVED				
			1 PERL-MUTTER	2 JOHN-SON	3 TAYLOR	4 THOM-SON	5 WILSON	AVERAGE	1 PERL-MUTTER	2 SCHUR-MAN	3 TAYLOR	4 THOM-SON	5 WILSON
		p.p.m.	*p.p.m.*										
1	Milk	3	1.0	1.0	2.2	2.0	3.0–3.0	2.0	———	Pos.	Pink	Lt Pink	Pink
	Water	3	3.6	3.4	3.2	2.3	2.5–3.0	3.0	———	Pos.	Pink	Lt Pink	Pink
2	Milk	1	0.0	0.5	trace	1.0	1.0–0.8	0.6	———	Pos.	Lt Pk	Vy Lt Pk	Pink
	Water	1	1.4	1.0	1.0	1.0	0.0–0.9	0.6	———	Pos.	Lt Pk	Vy Lt Pk	Pink
3	Milk	0	0.0	0.0	0.0	0.0	0.0–0.0	0.0	———	Neg.	None	Lt Yel	Colorless
	Water	0	0.0	0.0	0.0	0.0	0.0–0.0	0.0	———	Neg.	None	No Col	Colorless
4	Milk	2	1.6	1.0	———	1.7	2.0–2.0	1.7	———	Pos.	———	Lt Pk	Pink
	Water	2	2.0	1.9	———	1.5	1.8–1.8	1.8	———	Pos.	———	Lt Pk	Pink
5	Milk	10	7.6	9.9	8.4	9.6	9.6–9.0	9.0	———	Pos.	Red	Pink	Pink
	Water	10	10.0	12.7	10.6	9.8	9.0–10.4	10.4	———	Pos.	Red	Pink	Pink
6	Milk	36	24.6	29.8	31.6	41.6	29.6–30.6	31.3	———	Pos.	Red	Dk Pk	Str Pk
	Water	36	40.0	40.4	39.4	42.8	36.0–39.7	38.0	———	Pos.	Red	Dk Pk	Str Pk
7	Milk	76	53.4	64.3	67.8	73.4	66.0–64.0	64.8	———	Pos.	Red	Dk Pk	Str Pk
	Water	76	80.0	81.4	82.2	74.5	64.0–76.4	76.4	———	Pos.	Red	Dk Pk	Str Pk
8	Milk	48	38.0	40.4	41.2	49.8	41.6–42.0	42.2	———	Pos.	Red	Dk Pk	Str Pk
	Water	48	50.0	49.3	49.4	50.5	44.0–48.6	48.6	———	Pos.	Red	Dk Pk	Str Pk

[a] D.C. 12 (lauryldimethylbenzylammonium chloride) was the quaternary ammonium compound used in these experiments.

samples, it is possible that the milk contained some substances which may have used up more Q.A.C. than did the other milks.

Collaborator 4 was responsible for three of the four maxima. Although it is possible that the milk used contained some Q.A.C., this fact would

TABLE 4.—*Per cent recoveries of Q.A.C. in milk*

COLLABORATOR NO.	10 P.P.M.	36 P.P.M.	76 P.P.M.	48 P.P.M.	ALL SAMPLES
1	76	68	70	79	
2	99	83	85	84	
3	84	88	89	86	
4	96	116	98	104	
5	96	82	87	87	
5	90	85	84	88	
Maximum	99	116	98	104	116
Minimum	76	68	70	79	68
Average	90	87	84	88	87

surely have been detected if only a few mg of Q.A.C. were present. It is more likely that the titration was carried on too quickly near the end point, so that too much solution was added in the final stages.

The three remaining collaborators' results lie near the average in all cases except one, and since the results for 1 and 4 pair off, the total averages are very good for this type of method.

RECOMMENDATIONS

It is recommended*—

(1) That the method for quaternary ammonium compounds in milk as given in this report be adopted, first action.

(2) That the method for quaternary ammonium compounds in water solutions as given in this report be adopted, first action.

REPORT ON THE DETERMINATION OF QUATERNARY AMMONIUM SALTS AS REINECKATES

By JOHN B. WILSON (Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C.), *Associate Referee*

Collaborative study of the Reineckate Method for quaternary ammonium compounds[1] was initiated last year, but collaborators were not able to report until this spring and summer.

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 79 (1954).

[1] JOHN B. WILSON, *This Journal*, **35**, 455 (1952).

TABLE 1.—*The determination of quaternary ammonium compounds as reineckates*

COMPOUND	FACTOR	MG PRESENT	MG FOUND								RECOVERED (AVERAGE)	
			MINSKER		CARSON		O'NEILL		WILSON			
			PPT.	Q.A.C.	PPT.	Q.A.C.	PPT.	Q.A.C.	PPT.	Q.A.C.	MG.	PER CENT
D.C. 12	0.5458	50.0	89.7	49.0					83.5	44.8	46.9	93.8
Zephiran	0.5582	84.3			155.0 154.9	86.5 86.5					86.5	102.5
Zettyn	0.5834	70.0			117.4 117.1	72.0 71.8	117.7	68.7	114.4 112.2	66.7 65.5	68.9	98.4
Cetab	0.6045	28.0	50.7	30.6					46.6 47.3	28.4 28.6	29.2	104.3
Ethyl Cetab	0.6135	42.5			71.8 71.0	44.0 43.6	71.8	44.0	69.2	42.5	43.5	102.4
Hy 1622	0.6130	78.0	135.4	82.0					122.9 122.5	75.3 76.9	78.1	100.1
Hy 10-X	0.6028	38.0					63.0	32.2	63.7 68.4	38.4 41.4	37.3	98.1
Dobenzyl	0.5916	15.0	31.4	18.6	28.7 26.0	17.0 15.4	28.0	16.6	25.2	14.9	16.5	110.0
L.P.D.	0.5112	95.0	181.4	92.5	180.0 179.1	92.0 91.6	174.2	89.2	171.4	87.6	91.0	95.8
									Grand Average		100.6	Per Cent

Nine solutions of quaternary ammonium compounds, containing from 15 to 95 mg of the compound per 100 ml, were prepared. These solutions were issued to four collaborators in such a manner that each sample was analyzed by at least two of them. As the identity of the compounds was not disclosed, the collaborators were requested to report both the weight of precipitate obtained and the calculations made by the associate referees. The results are given in Table 1.

The results in Table 1 are very good, but tend to be a little high. This tendency may be due to variations in composition. To prevent any excess moisture from increasing the weight of the crystals, they should remain in the desiccator until constant weight is attained.

It is recommended* that the method for "Determination of Quaternary Ammonium Compounds as Reineckates" as described in *This Journal*, **35**, 455 (1952) be adopted, first action.

REPORT ON QUALITATIVE TESTS FOR FLUORINE

By MARY C. HARRIGAN (Food and Drug Administration, 408 Atlantic Ave., Boston, Mass.), *Associate Referee*

The Associate Referee submitted for collaboration a test for minute quantities of fluorine based on the quenching of the fluorescence of aluminum-8-hydroxyquinoline. Details of the method and a fruit juice sirup containing 0.5 mg of fluorine per 150 ml were sent to each of six collaborators. All considered that the test had merit.

L. J. Hardin noted the possibility that sulfuric acid might be contaminated with fluorine; for this reason, directions for running a blank on the acid were added to the procedure. A. G. Buell suggested the addition of acetic acid when barium sulfate and fluoride are precipitated, in order to make filtration easier.

Details of the procedure are as follows:

QUALITATIVE TEST FOR FLUORIDES

REAGENTS

(a) *Aluminum soln.*—Dissolve 2.22 g $AlNH_4(SO_4)_2 \cdot 12H_2O$ in H_2O, add 3 drops HCl, and make up to 250 ml with H_2O.

(b) *Oxine reagent.*—Dissolve sufficient 8-hydroxyquinoline in 2 *N* acetic acid to make a 5% soln. 1 ml of this soln is equivalent to ca 5 ml of the Al soln.

(c) *Ammonium acetate soln.*—Dissolve 77 g of NH_4 acetate in H_2O and make up to 500 ml with H_2O.

(d) *Aluminum 8-hydroxyquinolate.*—Warm 250 ml of Al soln to 50–60° and add excess of oxine reagent. Add NH_4 acetate soln slowly until a permanent ppt forms.

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 79 (1954).

Then add 20–25 ml more to insure complete pptn. Allow ppt to settle and filter thru fritted glass crucible. Wash ppt well with at least 7–8 30 ml portions of cold H_2O and dry at 120–140°. Store in desiccator.

(e) *Chloroform soln of aluminum 8-hydroxyquinolate.*—Dissolve Al-oxine in $CHCl_3$ to prep. a 0.5 mg/ml soln. Make fresh each day.

(f) *Sulfuric acid.*—Concd. If a blank detn reveals the presence of F, purify as in **24.45(c)**, diluting and boiling 3 times.

DETERMINATION

Transfer to beaker 150 ml of a liquid or the equivalent quantity of aq. ext. in the case of solids, and add 5 ml 10% K_2SO_4 soln, 10 ml 10% Ba acetate soln, and 3 ml glacial acetic acid and boil. Allow ppt to settle and decant supernatant liquid thru small filter paper. Transfer ppt to filter paper and wash. Transfer paper and ppt to Pt crucible and ignite. Transfer residue to a small porcelain crucible (5 ml or smaller).

Wet a piece of filter paper with the $CHCl_3$ soln of Al oxine in a spot larger in diam. than the top diam. of the crucible and allow to air dry. Add 2 or 3 drops concd H_2SO_4 to ash and place filter paper spot over crucible. Heat crucible covered with the paper to 50–60°C. for 5 min. Observe paper under ultraviolet light. In the presence of F the fluorescence of the Al oxine will be quenched in the area of the spot over the crucible. The limit of identification is ca 0.05 mg F. Run a blank detn on the H_2SO_4.

It is recommended* that the qualitative test for fluorine by quenching of oxine fluorescence as outlined by the Associate Referee be adopted, first action.

The Associate Referee wishes to thank those who so willingly collaborated:

M. P. Etheredge, Mississippi State Chemical Laboratory, State College, Miss.

L. J. Hardin, University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.

A. G. Buell, San Francisco District, Food and Drug Administration

F. A. Rotondaro, Philadelphia District, Food and Drug Administration

L. C. Weiss, Los Angeles District, Food and Drug Administration

W. C. Woodfin, Buffalo District, Food and Drug Administration

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REPORT ON BENZOATES AND HYDROXYBENZOATES

By HERBERT E. GAKENHEIMER (Food and Drug Administration, Department of Health, Education, and Welfare, Baltimore 2, Md.), *Associate Referee*

Because benzoate compounds have long been used in foods, drugs, and cosmetics for their preservative action and because their use is growing more popular today, an accurate, rapid identification has become desira-

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 79 (1954).

ble. Edwards, Nanji, and Hassan (1) have described the following method for the detection of *p*-hydroxybenzoic acid and its derivatives.

METHOD

Prep. a diazo reagent by dissolving 5 g aniline in a mixt. of 13 ml concd HCl and 26 ml H_2O. Cool to 5°C. and add a cold soln of 4.5 g $NaNO_2$ in 20 ml H_2O. Keep below 5°C. and use within 1 hr.

Ext. benzoate or hydroxybenzoate from sample as directed under **27.45** or **27.48** (2), and transfer 10 ml aliquot to test tube, 20×150 mm, contg 5 ml 15% NaOH soln. Cool to 5°C.; then couple by slowly adding excess of the diazo reagent (ca 25 ml is sufficient). Let stand short time, acidify with 5 ml 10% HCl, and ext with two 20 ml portions of ether. Shake out combined ether exts with 10 ml 10% Na_2CO_3 soln. Separate the ethereal layer and shake with 10 ml 10% NaOH soln. A deep red color develops in the ether layer if benzoates or hydroxybenzoates are present.

The test shows a sensitivity of 0.001%.

The following chemicals have given a positive test: methyl *p*-hydroxybenzoate, *n*-butyl *p*-hydroxybenzoate, *n*-propyl *p*-hydroxybenzoate, *m*-hydroxybenzoic acid, 3,5-dinitrobenzoic acid, ethyl aminobenzoate, benzoic acid, sodium benzoate, and *o*-hydroxybenzoic acid (salicylic acid).

The following preparations which contain benzoates or hydroxybenzoates have given a positive test: Aureomycin troches containing methylparaben and propylparaben; Tracinets containing benzocaine; bacitracin nasal solution containing sodium benzoate; Zylate (Upjohn) containing benzyl benzoate, 36%; benzyl benzoate solution Hynson, Westcott & Dunning, 20%; lemon flavoring containing sodium benzoate; pickles containing sodium benzoate; and oleomargarine containing sodium benzoate.

It is recommended* that work on this subject be continued.

REFERENCES

(1) EDWARDS, F. W., NANJI, H. R., and HASSAN, M. K., *The Analyst*, **62**, 178 (1937).

(2) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950, p. 468.

REPORT ON ARTIFICIAL SWEETENERS

P-4000 AND DULCIN

By WILLIAM S. Cox (Food and Drug Administration, Department of Health, Education, and Welfare, Atlanta, Ga.), *Associate Referee*

In accordance with the recommendation made in the 1952 Report on Artificial Sweeteners,[1] a collaborative study was undertaken in 1953 of

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 79 (1954).

[1] *This Journal*, **35**, 321 (1952); **36**, 749 (1953).

methods which had been developed for the detection and determination of propoxy-2-amino-4-nitrobenzene (P-4000) and which appeared to be suitable. The study is described in the present report.

A set of samples was prepared and submitted to collaborators, as follows:

Sample A consisted of a non-carbonated beverage, made by dissolving together citric acid, flavoring (lemon-lime extract), benzoic acid, and the artificial sweetners P-4000 and dulcin. The resulting solution had the following composition: 40 p.p.m. P-4000, 150 p.p.m. dulcin, 0.1 per cent benzoic acid, 2.0 per cent citric acid, a trace of flavoring, and water.

Sample B was similar to Sample A except that it contained artificial cherry flavoring and color. The resulting solution was composed as follows: 26 p.p.m. P-4000, 200 p.p.m. dulcin, 0.1 per cent benzoic acid, 2.0 per cent citric acid, a trace of flavoring, color, and water. Methods, and directions to collaborators for the qualitative and quantitative methods follow:

P-4000

QUALITATIVE METHOD

To 100 ml sample, add 10% NaOH dropwise, until just alk. (*p*H 7.5–8.0), and ext. 3 times with 25 ml portions of petr. ether, shaking 2 min. each time. Wash combined ether extracts with 5 ml H_2O, discard H_2O, and transfer ether ext. to small beaker or dish, and allow petr. ether to evap. spontaneously to ca 5 ml. Transfer remainder to 25 ml test tube, wash beaker with several small portions of petr. ether, and transfer washings to test tube. Evap. carefully to dryness. Add 2 ml HCl (1+1), then add 2 ml satd $SnCl_2$ soln. Immerse test tube in boiling H_2O bath for 15 min., remove and cool. Add dropwise strong Br-H_2O to excess. If P-4000 is present, the soln will change from nearly colorless to a purplish-red or burgundy red color, and if a considerable excess of Br-H_2O is added, the soln will turn yellow. (NOTE: If the soln is not nearly colorless when removed from H_2O bath, it should be dild until nearly colorless before the Br-H_2O is added.)

If in the qualitative test the petroleum ether is evaporated by using heat, the P-4000 will be found as a dark, gummy residue. However, if the petroleum ether is evaporated spontaneously at room temperature, the P-4000 will be found as a golden-yellow, crystalline residue. The color change produced by the addition of *strong* bromine water is very pronounced, and if a *pronounced* purplish-red color is not produced, the test should be reported as negative. Report the qualitative test as positive or negative.

Ten collaborators reported results on this test. Nine of the collaborators reported positive tests on both samples. The other reported a positive test on Sample A and a negative test on Sample B. In essence, then, positive results were found in all 10 samples containing 4 mg of P-4000 in the test portion, and positive results were found in 9 of 10 samples containing 2.6 mg of P-4000 in the test portion.

QUANTITATIVE METHOD

REAGENTS

(a) *Sodium nitrite soln.*—0.5 g $NaNO_2$ in 100 ml H_2O.

(b) *Hydrochloric acid.*—0.1 *N*.

(c) *1-Naphthol soln.*—0.1 g 1-naphthol in 1 liter 1% Na_2CO_3 soln (prepare fresh soln just before using).

DETERMINATION

Pipet 100 ml sample into 250 ml separatory funnel and make alk. (*p*H 7.5–8.0) with 10% NaOH. Ext. 4 times with 50 ml portions petr. ether, shaking 3 min. each time and cautiously releasing pressure frequently. Wash combined extracts once with 10 ml H_2O. Dil. or conc. petr. ether soln so that each 10 ml contain 0.01–0.08 mg P-4000. Transfer 10 ml to small beaker and remove petr. ether by evapn. Dissolve residue in 10 ml hot H_2O. Cool to 15°, add 0.25 ml $NaNO_2$ soln and mix. Add 0.25 ml 0.1 *N* HCl and mix. After 45 sec., add 4.5 ml 1-naphthol soln and mix. Read, within 1 hr, the % transmission at 515 mμ. Run blank detn upon reagents and use this soln to set colorimeter scale to read 100% transmission. From the standard curve, previously prepd, obtain the amount of P-4000 which corresponds to the % transmission of the sample being analyzed. From diln (or concn.) above, calc. P-4000 present in the original 100 ml sample.

The vial of P-4000 is to be used primarily for the purpose of preparing the standard curve in the range 0.01 to 0.08 milligrams per 10 ml. Purity of the P-4000 may be assumed to be 98.0%.

Since P-4000 is reportedly 4100 times as sweet as sugar, 150 p.p.m. is probably the upper limit in any commercial food product, and 10 p.p.m. is the lower limit.

Quantitative results on the two samples are given in Table 1.

TABLE 1.—*Results expressed in per cent P-4000 recovered by collaborators*

COLLABORATOR	SAMPLE A		SAMPLE B	
	(40 MG/L)	AV.	(26 MG/L)	AV.
A	100		119.2	
	91.3[a]		104.6[a]	
B	76.8		98.8	
C	95.0, 95.0	95.0	92.3, 89.6	91.0
D	76.2, 76.2	76.2	80.8, 85.4	83.1
E	112.5, 105.0	108.3	59.6, 60.8	60.2
F	65.0, 62.5	63.8	76.9, 80.8	78.8
G	87.5, 87.5	87.5	78.1, 78.1	78.1
H	91.0		98.5	
I	83.8		88.5	
J	62.5		76.9	

[a] Repeated determination one month after first determination.

SUMMARIZED COMMENTS OF COLLABORATORS

Most of the collaborators indicated that specific directions should be given for the preparation of the standards.

Most of the collaborators also criticized the wording; "Dil. or conc. petr. ether soln so that each 10 ml contains 0.01 to 0.08 mg of P-4000," and

suggested diluting to a known volume, noting the temperatures, so that duplicate aliquots may be taken if needed.

Several of the collaborators who used the Beckman D.U. Spectrophotometer reported that above 0.06 mg/10 ml, the curve deviated below the straight line obtained up to that concentration. Others found it to be essentially straight up to 0.08 mg/10 ml.

Some collaborators reported fading of colors within 15–30 minutes, while others reported the colors to be essentially stable up to an hour after development.

One collaborator recommended that in preparing the final color solution, the directions should be changed to read: "Dissolve residue in ca 10 ml hot H_2O, and cool. Add two drops 0.1 *N* HCl and mix; then add 2 drops $NaNO_2$ soln, and mix. Add 5 ml 1-naphthol soln, mix, and make up to final vol. of 25 ml." This change would eliminate the tedious measurements of small volumes of the reagents and make volume adjustment a single operation.

While it cannot be said that the results are excellent, the fact that half the collaborators recovered 90 per cent or more of the P-4000 present in each sample shows that the method is promising.

It is believed that the primary source of error of the method as used in this study was the preparation of the standard curve. Some collaborators used water solutions as the standard solution; others used petroleum ether solutions. There were also differences in preparation of the blank solutions.

Two other possible sources of error which should be eliminated or reduced are: (*a*) the changes in concentration of the petroleum ether solutions due to evaporation; and (*b*) the use of the standard curve in the range over 0.06 mg/10 ml, where it no longer follows a straight line.

It is the opinion of the Associate Referee that the method shows promise and justifies further study. The suggestions made by the collaborators should clarify the method and eliminate the differences incurred in the preparation of the standard curve.

DULCIN

A collaborative study of a method for the determination of dulcin was made concurrently with the P-4000 methods study. This method involved the extraction of dulcin with chloroform, the removal of the chloroform by evaporation, the dissolution of the dulcin in water, the bromination of an aliquot with standard bromide-bromate solution, liberation of iodine (by adding KI), and final titration of the excess iodine with standard thiosulfate solution.

Unfortunately, it was found that recovery of the dulcin varied widely, depending upon the concentration of the dulcin, the size of the aliquot,

temperature, and other factors. Since the recoveries were so wide in range, no results are reported herein and the method has been abandoned.

RECOMMENDATIONS

It is recommended*—

(1) That the qualitative test for P-4000 as outlined in this report be adopted, first action.

(2) That the quantitative method for P-4000 as described in this report be further studied.

(3) That further study be made to develop a suitable method for the determination of dulcin (*p*-phenetyl-urea).

ACKNOWLEDGMENT

The Associate Referee desires to express appreciation to the following persons for their assistance in this work: George McClellan, Baltimore; J. H. Bornmann, Chicago; L. G. Ensminger, Cincinnati; M. L. Dow, St. Louis; D. W. McLaren, Buffalo; David Firestone, New York; W. A. Bosin, Minneapolis; J. A. Thomas, New Orleans; F. E. Yarnall, Kansas City, all of the Food and Drug Administration; and to Alphonso Wickroski, Connecticut Agricultural Experiment Station, New Haven, Conn.

No reports were given on Sucaryl®, dimethyldichlorosuccinate and dehydroacetic acid, monochloroacetic acid, or thiourea.

REPORT ON SPICES AND OTHER CONDIMENTS

By E. C. Deal (Food and Drug Administration, Department of Health, Education, and Welfare, New Orleans, La.), *Referee*

Following the last meeting of the Association, Subcommittee C (1) recommended that studies be continued or initiated on ten topics, or subtopics, relating to: Vinegar; Prepared Mustard and Mustard Flour; Mayonnaise and Salad Dressing; Chili Powder; French Dressing; and Spice Oils. Reports were received from three Associate Referees.

VOLATILE OIL IN SPICES

Continuing work along the lines reported last year, Associate Referee Aubrey Carson tried out a new design of trap, together with certain modi-

* For report of Subcommittee D and action of the Association, see *This Journal*, 37, 79 (1954).

fications of technic, in an effort to assure more consistent results. All-glass connections and Glass-col heaters add precision to the method, but results were inconclusive as to the use of the new trap. He recommends that further work be done on the method. The Referee concurs.

PREPARATION OF SAMPLE OF FRENCH DRESSING

A procedure for preparation of sample of french dressing of the separable type was adopted by the Association in 1951. Certain difficulties were reported in the application of the method to large-size (one gallon or more) containers. This year, Associate Referee Aldrich F. Ratay developed a modification of his original method with which he obtained consistent results when he applied it to large-size containers. The Referee feels that no further collaborative work is necessary and recommends that the procedure (2) as adopted in 1951 be amended to include this modification when applied to large-size containers and that no further work be done on this topic.

MAYONNAISE AND SALAD DRESSING

Reports of some difficulties experienced with Methods **28.44** (Total Nitrogen) and **28.46** (Total Fat), as applied to salad dressings, have reached the Referee. Although these methods have official status, as a result of collaborative study and extensive use, it is claimed that difficulties arise with certain samples, particularly those containing gums. Cahn reported (3) that some samples could not be analyzed for nitrogen by method **28.44,** but that this difficulty could be eliminated by making a preliminary treatment with absolute methyl alcohol, followed by extraction with ethyl ether and then with petroleum ether. This is similar to a method previously investigated extensively by Associate Referee Juanita Breit, employing chloroform in the pre-treatment. The results of her work, published earlier (4), showed that little or no advantage was gained by the chloroform treatment. Since the method was modified by directing the use of a greater quantity of sulfuric acid for the digestion, little trouble has been reported.

The second difficulty, that of obtaining complete extraction of the oil from food dressings by method **28.46,** has been reported sporadically over a period of years. In 1949 the Associate Referee made a study of the tentative method and concluded that the difficulty lay in insufficient shaking of the extraction tubes before drawing off the ether. The method was modified to give more specific directions and was subjected to collaborative study. The results appeared to be satisfactory and the method was adopted (5) first action, and made official in 1951.

Recently there have been reported a few instances of salad dressings

from which the fat could not be completely extracted by the three extractions specified in the official method. It would seem desirable to change the method to direct that a fourth extraction be made and tested separately to insure complete extraction of the fat. The Referee believes that the method should also be reworded to provide for removal of the fat from the dried flask, with subsequent taring of the flask, in order to conform to similar fat methods (such as **15.25**) and as a safety measure to guard against weighing non-fatty material.

VINEGAR

It has been suggested that a determination of sorbitol might be useful in detecting the adulteration of wine vinegar with the cheaper and more readily available apple vinegar. Associate Referee Felice A. Rotondaro has been working on the chromatographic separation of sorbitol from mannitol and other sugars. This work should be continued.

RECOMMENDATIONS

It is recommended*—

(1) That studies on the determination of volatile oil in spices be continued.

(2) That studies of methods for detection of caramel in vinegar be continued.

(3) That studies on the determination of tartrates in vinegar be continued.

(4) That methods for the determination of free mineral acids in vinegar be further studied.

(5) That methods for the determination of sorbitol be continued.

(6) That studies of methods for the determination of ash and sugars in prepared mustard be continued.

(7) That studies of methods for the determination of pungent principles in prepared mustard and mustard flour be continued.

(8) That studies of methods for the detection of seeds and stems in ground chili be continued.

REFERENCES

(1) *This Journal*, **36**, 59 (1953).
(2) *Ibid.*, **35**, 232 (1952).
(3) CAHN, F. J., private communication.
(4) BREIT, J., *This Journal*, **34**, 267 (1951).
(5) *Ibid.*, **33**, 86 (1950); **35**, 86 (1952).

* For report of Subcommittee C and action of the Association, see *This Journal*, **37**, 74 (1954).

REPORT ON VOLATILE OIL IN SPICES

By N. AUBREY CARSON (Food and Drug Administration, Department of Health, Education, and Welfare, St. Louis, Mo.), *Associate Referee*

As recommended by Subcommittee C (1) further work has been done on the method for determining volatile oil in spices. The 1952 report of the Associate Referee (2) showed variable collaborative results on nutmeg and allspice. Two of the chief reasons for these variations were the use of tinfoil covered cork joints in part or all of the connections, and the difficulty of measuring the oil in allspice. The volatile oil in allspice contains two fractions, one lighter and one heavier than water, and no trap was available for handling this oil in the usual manner. A new trap was designed to overcome these difficulties. This trap was a combination of Clevenger's lighter- and heavier-than-water traps. A bulb was substituted for the stopcock at the junction of the return tube and graduated tube from Clevenger's lighter-than-water trap. Then the graduated tube and stopcock from Clevenger's heavier-than-water trap were added to the bottom of the bulb. Thus there was a graduated tube both above and below the bulb. A cold finger, extending 3.5 inches below the vapor arm from the distilling flask, was substituted for a condenser. Thus the oil and water in the trap were always cold.

This investigation was undertaken to compare the new trap to Clevenger's trap and to check the precision of the method when all-glass connections are used. Because of the difficulties encountered last year, the same three spices, star anise, nutmeg, and allspice, were employed again. The spices were ground to pass a 20 mesh screen and were stored in a refrigerator. Standard taper glass joints were used on all connections of both traps, and Glass-col heating mantles were the source of heat. Condensers were cooled with ice water.

RESULTS

The advantages of the new trap seemed to be overshadowed by its disadvantages. Even though little or no attention was needed from start to finish with the new apparatus, it was much more difficult to clean and more awkward to handle; lower results were obtained on nutmeg and allspice. Thus the new trap does not seem to show a sufficient superiority over Clevenger's to warrant further investigation.

In allspice, whose volatile oil contains a lighter- and a heavier-than-water fraction, considerable suspension of the oil in the water and of the water in the oil occurred, causing difficulty when the oil was measured. If the volume of oil is determined before it is drawn off, it is still hot and a high reading occurs. If it is drawn off into a graduate and allowed to cool

to room temperature, a suspension occurs, suggesting the need of centrifuging. The oil was drawn off as it came down, and the oil and water were drawn off at the end of the distillation into a graduated conical centrifuge tube and centrifuged. A slightly lower volume of oil was obtained, which indicated that a suspension of water in oil was obtained when centrifuging was not used. In the results listed in Table 1, centrifuging of the oil and water was used on the last three determinations of allspice.

TABLE 1.—*Collaborative results*

SPICE	VOLATILE OIL, % (ML/100G)
Nutmeg	6.2, 6.2, 6.2, 6.2
Star Anise	6.6, 6.6, 6.6, 6.6
Allspice	5.4, 5.4, 5.4, 5.2[a], 5.2,[a] 5.2[a]

[a] These results were measured after centrifuging.

CONCLUSIONS

(1) The Associate Referee found that all-glass connections gave more precise results than any other type.

(2) Glass-col heating mantles are safer, prevent scorching and excessive foaming, and are easier to handle than oil baths.

(3) In spices such as allspice, whose volatile oil contains a lighter- and a heavier-than-water fraction, the oil could be consolidated by centrifuging in a graduated conical centrifuge tube. The volume is then easier to measure.

The Associate Referee recommends* that further work be done on the method.

REFERENCES

(1) *This Journal*, **36**, 59 (1953).
(2) CARSON, N. A., *ibid.*, **36**, 752 (1953).

REPORT ON SORBITOL IN VINEGARS

By FELICE A. ROTONDARO (Food and Drug Administration, Department of Health, Education, and Welfare, Philadelphia, Pa.), *Associate Referee*

This progress report consists of two parts: (1) The quantitative determination of sorbitol and mannitol; and (2) their separation from sugars.

* For report of Subcommittee C and action of the Association, see *This Journal*, **37**, 74 (1954).

The periodate oxidation of sorbitol in neutral solution as adapted by The Toilet Goods Association (TGA method No. 47), as well as the mannitol method given in *New and Nonofficial Remedies*, 1952, p. 601, which utilizes an acid solution, give a satisfactory estimation of either polyhydroxy alcohol isomer. Both methods hasten the reaction by the use of a steam bath or heating to boiling; both are based upon the amount of periodate used by the reaction. The total potassium iodide reduced either in a bicarbonate solution as in the TGA method, or in acid solution as in the NNR method before and after the reaction is determined and the difference is ascribed to the effect of the sample. This does not give any qualitative or purity indication of the substances which effect the reduction of the periodate. By carrying out the reaction in neutral solution and at room temperature, as outlined by Shupe (1) and Bond (2), the determination of the reaction products, formic acid and formaldehyde, in addition to the total periodate used by the reaction, make possible a check on the purity of the sample. In other words, calculation of each of the three determinations gives substantially the same results. Any significant contamination of the sample by such substances as glycerol, ethylene or propylene glycol, glucose, fructose, etc., will be indicated by disagreement of the three results. Even in the case of sorbitol and mannitol, it was found that the speed of the reaction is slightly faster for mannitol than for sorbitol, so that by titrating the formic acid at the end of a relatively short interval of ten to fifteen minutes' reaction time, at room temperature, essentially 100 per cent of mannitol is indicated, whereas only 85–88 per cent of sorbitol appears to be oxidized. The amount of periodate ultimately consumed, however, is the same in both cases and indicates 100 per cent oxidation.

The experience of the Associate Referee with the above method indicates that it may be possible to submit samples for collaborative study this coming year. In the separation of sorbitol from mannitol, glucose, fructose, and/or other sugars, preliminary chromatographic work along the lines indicated by Lew, *et al.*, (3) together with practical pointers suggested by the Research Staff of the Atlas Powder Co., Wilmington, Del., gave promising results.

Attempts to scale chromatographic separations to sample sizes of 2–10 mg of sorbitol, mannitol, glucose, fructose, maltose, and sucrose have been encouraging but not completely satisfactory to date.

A minor practical development in the "streaking" technic of an extruded chromatographic column to locate the various adsorption zones appears worthy of mention at this time. To overcome the difficulty of frequent replacements of the camel-hair brush by reason of the strong deteriorating effect of the alkaline-permanganate streak reagent, and the brittleness of Fiberglass brushes, a light, uniform "streak" was applied by means of capillary drawn out from an 8–10 mm glass tube. The flow of the reagent

is easily controlled by the size of the capillary as well as by the control of the air admitted to the reagent reserve in the upper wide portion of the glass tube, by holding the index finger over the tube pipet-wise. The advantage of the rather flexible capillary end of the tube, together with ease in manipulation gained by a little practice, permits the application of thin uniform streaks admirably suited for the purpose of locating the zones on relatively small chromatographic cores, with the least possible amounts of reagent.

It is expected that practical details for the separation by the above method may be worked out this coming year.

The Associate Referee recommends* that the work be continued.

REFERENCES

(1) SHUPE, I. S., *This Journal*, **26**, 249 (1943).
(2) BOND, H. R., *ibid.*, **33**, 362 (1950).
(3) LEW, B. W., WOLFROM, M. L., and GOEPP, R. M., JR., *J. Am. Chem. Soc.*, **68**, 1449 (1946).

REPORT ON PREPARATION OF SAMPLE AND SAMPLING OF FRENCH DRESSING

By ALDRICH F. RATAY (Food and Drug Administration, Department of Health, Education, and Welfare, Philadelphia, Pa.), *Associate Referee*

The study reported involved the preparation of sample and sampling of french dressing of the separable type in large-size containers of one gallon capacity. Experimental work disclosed that the following procedure is applicable to separable type french dressing in these containers.

FRENCH DRESSING (SEPARABLE)[1]

PREPARATION OF SAMPLE—LARGE CONTAINERS: PROCEDURE

Stir contents of jar thoroly, and add 0.20 g albumin egg powder per 100 g sample. (A mechanical stirrer of the double beater type is satisfactory.) Continue stirring until powder is well dispersed thruout sample. Then add sample in portions to Waring blendor and stir each portion ca 5 min. Transfer emulsified portions to jar of size similar to that of original container. Upon completion of emulsification, stir entire contents of sample to insure uniform mixt.

Transfer portion of prepared sample to a convenient jar (ca 1 pt). Shake ca 20 times and stir with spatula or spoon ca 20 times before each portion is removed for analysis. Make all weighings immediately after prepn of sample. Correct analytical results for added emulsifier.

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* For report of Subcommittee C and action of the Association, see *This Journal*, **37**, 74 (1954).
[1] Cf. *This Journal*, **35**, 86 (1952).

Three one-gallon batches of laboratory-prepared french dressing and two commercial samples were prepared as directed above, and the method was tested by analyzing each sample for fat content.

Results obtained in quadruplicate are given in Table 1.

TABLE 1.—*Fat content of samples*

SAMPLE NO.[a]	TOTAL FAT, %	ARITHMETIC MEAN	ARITHMETIC DEVIATION
1	41.10		−.06
	41.26	41.16	+.10
	41.19		+.03
	41.10		−.06
2	41.16		+.05
	41.16	41.11	+.05
	41.05		−.06
	41.05		−.06
3	40.95		−.02
	40.95	40.97	−.02
	40.92		−.05
	41.06		+.10
4	37.18		−.04
	37.21	37.22	−.01
	37.21		−.01
	37.28		+.06
5	41.28		+.16
	41.00	41.12	−.12
	41.17		+.05
	41.01		−.11

[a] Samples 1, 2, and 3 were prepared in the laboratory and samples 4 and 5 were commercial preparations.

Variations between quadruplicate subdivisions were relatively small, ranging from 0.00 to 0.16 per cent in samples 1, 2, 3, and 4, and from 0.01 to 0.28 per cent in sample 5.

These results disclose that the procedure for preparation of french dressing of the separable type in large-size containers promotes uniform and proper sampling and is satisfactory.

It is recommended* that the method for preparation of sample of french dressing of the separable type in large-size containers as outlined by the Associate Referee be adopted as a procedure, and that no further work be done on this phase of the problem.

No reports were given on seeds and stems in ground chili; sugar, ash, and pungent principles in mustards; or vinegar.

* For report of Subcommittee C and action of the Association, see *This Journal*, **37**, 74 (1954).

REPORT ON CEREALS

By V. E. MUNSEY (Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D.C.), *Referee*

It is recommended*—

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

(2) That the method for sugar in baked products be further studied as recommended by the Associate Referee (essentially **13.30–13.32,** inclusive, for sugars in flour).

(3) That the method for the determination of lactose in bread, *This Journal,* **35.** 697 (1952), be studied further.

(4) That the methods on soybean flour for moisture, **13.63;** ash, **13.64;** nitrogen, **13.65;** with substitution of "15" for "10" so as to read "using 15 g K_2SO_4 or Na_2SO_4," crude fiber, **13.66;** and oil or petroleum ether extract, **13.68,** be made official.

(5) That the method for determination of egg content in bakery products by sterol-digitonin method be adopted, first action and the study continued.

(6) That the Armstrong method of bromate determination in flour be given collaborative study.

(7) That the procedure for lipoids and method I for lipoid P_2O_5 (*This Journal,* **36,** 760 (1953)), remain first action and the study be continued.

(8) That the study of methods for choline in noodles be discontinued.

(9) That the sterol-digitonin method for determination of egg content of noodles be made first action and study be continued.

(10) That the study of moisture determinations be continued.

(11) That the official method for lipoid, **13.35,** and lipoid P_2O_5, **13.36,** be deleted, final action.

REPORT ON POTASSIUM BROMATE IN FLOUR

By W. L. RAINEY (Commander-Larabee Milling Co., Minneapolis, Minn.), *Associate Referee*

INTRODUCTION

Marjorie Howe (4) lists various methods, prior to her own, that have been used for potassium bromate determination. These methods may be summarized in the following manner:

* For report of Subcommittee D and action of the Association, see *This Journal,* 37, 76 (1954).

(A) Meyer (7) employed an iodometric titration on a water extract of flour. There are certain substances in the extract which interfere with this titration, necessitating a correction curve.

(B) Geddes and Lehberg (3) tried leaching a sample of flour with water, ashing in the presence of potassium hydroxide, oxidizing the bromide to bromine, and titrating with 0.001 *N* sodium thiosulfate. This method is adaptable to the determination of bromate in the presence of chlorates, iodates, and persulfates, but the complexity of the method makes it inadequate as a control procedure. Also, bromide residues from methyl bromide used as a fumigant could be a source of interference in this method.

(C) Geddes (2), in his report as Associate Referee on the determination of bromate in flour, made use of a carbon tetrachloride flotation procedure to separate the bromate, which was then determined iodometrically. In this report, a complete method has also been described for the determination of potassium bromate in the presence of potassium persulfate and potassium iodate. This method is also too time-consuming for use as a control procedure.

(D) Hoffer and Alcock (5) extracted flour with 25 per cent potassium chloride in acetic acid and measured the absorbance of the amylose-iodine solution.

(E) Johnson and Alcock (6) employed the spectrophotometer with the above technic.

In addition to the above procedures may be mentioned the method of Towner and Koehn (8), who used the carbon tetrachloride flotation technic modified by the use of standard centrifuge tubes instead of the special metal tubes given in the procedure by Geddes (2).

Howe (4) proposed a method which involved dissolving bromate in a water suspension of flour and clarifying the solution by the addition of zinc sulfate and sodium hydroxide. Iodide is added to the resulting solution and is oxidized by the bromate to iodine, which is then titrated with sodium thiosulfate. This method has the disadvantage of an extremely difficult end-point determination.

Armstrong (1) has recently published a revision of the Howe method in which the concentration of zinc sulfate and sodium hydroxide are increased to effect better protein separation, and excess sodium thiosulfate is added to the iodine solution which is then back-titrated with potassium iodate solution.

COLLABORATIVE STUDIES

Three different samples of flour containing 5.0, 25.0, and 50.0 p.p.m. of potassium bromate were sent out in March 1951 to collaborators who

were asked to determine the bromate by the Towner and Koehn (8) and the Marjorie Howe (4) procedures. The data are summarized in Table 1. The results of both methods, with the exception of those obtained by collaborator No. 5, were fairly good.

It was suggested that the size of the sample used by the Howe method should be increased from 10 to 100 grams in order to minimize variations due to non-uniform distribution of bromate in the flour sample. A procedure was set up whereby 100 grams of flour, 3–4 grams of filtercel, and

TABLE 1.—*Determination of potassium bromate in flour by the Towner and Koehn method and by the Howe method*

KBr ADDED TO FLOUR	KBr FOUND, P.P.M.					METHOD USED
	COLLABORATOR NO.					
p.p.m.	1	2	3	4	5	
5.0	5.0	7.22	4.6	4.80	9.8	Towner–Koehn
	4.0	6.56	4.8	5.11	9.9	Howe
25.0	24.0	26.86	20.3	24.01	41.7	Towner–Koehn
	22.0	28.17	24.5	24.85	40.6	Howe
50.0	49.0	47.78	42.6	48.90	80.1	Towner–Koehn
	45.0	50.87	46.3	50.00	81.2	Howe

1000 ml of distilled water were agitated for ten minutes. Quantities of 125 ml of 0.18 *M* zinc sulfate and 125 ml of 0.18 *N* sodium hydroxide were added, followed by stirring for two minutes. After the solution was allowed to settle for ten minutes it was filtered and 50 ml of the clarified solution was taken as an aliquot and treated by the Howe procedure.

Three samples of flour containing 3.0, 20.0, and 45.0 p.p.m. of potassium bromate were sent out to collaborators in February, 1952, along with copies of the revised Howe procedure, to be checked by this one method. The data are summarized in Table 2.

The results of collaborator No. 6 should probably not be considered, since they are very low and vary considerably within each group. Results of this nature suggest that the samples of flour were not agitated sufficiently. Flour forms lumps very readily, so that it is often difficult to disperse it thoroughly in suspension.

On the basis of the results of the other six collaborators, standard errors were calculated as shown in Table 2. The per cent standard error is much greater at low bromate concentrations. This is partly due to the difficulty of determining the end-point in the titration when low concentrations of bromate are used.

TABLE 2.—*Determination of potassium bromate in flour by the revised Howe method*

KBr ADDED TO FLOUR	KBr FOUND, P.P.M.							AV. FOUND FOR ALL COLLABORATORS	AV. RECOVERY	S.E.	S.E.
	COLLABORATOR NO.										
p.p.m.	1	2	3	4	5	6	7	*p.p.m.*	*per cent*	*p.p.m.*	*per cent*
3.0	2.0 1.7	4.18 3.65 3.00	4.5 4.9	3.6	2.33 2.18	2.8 1.2 1.2	2.81 3.20				
Av.	1.85	3.61	4.7		2.26	1.7	3.00	3.2	105.7	1.03	32.2
20.0	19.8 19.1	23.24 23.94 22.50	20.7 20.7 20.7	20.0	19.14 19.06	18.8 14.9	20.04 19.80				
Av.	19.45	23.23	20.7		19.10	16.85	19.92	20.4	102.0	1.58	7.7
45.0	43.0 42.7	51.20 50.50 50.22	46.3 45.6	43.6	45.08 45.24	29.0 37.7	44.79 44.79				
Av.	42.85	50.64	45.95		45.16	33.35	44.79	45.5	101.1	2.94	6.4

ARMSTRONG'S METHOD FOR DETERMINATION OF BROMATE IN FLOUR

Since the last set of collaborative samples, Armstrong (1) has published a procedure for the determination of potassium bromate in flour, which is a revision of the Howe method. This revised method, with some simplifying features, is described below.

METHOD

REAGENTS

(a) *Zinc sulfate soln.*—Dissolve 20 g $ZnSO_4 \cdot 7H_2O$ in H_2O and dil. to 1 l.

(b) *Sodium hydroxide, 0.4 N.*—Dissolve 17 g NaOH in H_2O and dil. to 1 l.

(c) *Sulfuric acid, 4 N.*—Add 112 ml concd H_2SO_4 to H_2O and dil. to 1 l.

(d) *Potassium iodide soln.*—Dissolve 25 g KI in H_2O and dil. to 50 ml. Store in amber colored bottle in cool place.

(e) *Ammonium molybdate soln.*—Dissolve 3 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in H_2O and dil. to 100 ml.

(f) *Starch soln.*—Pour suspension of 1 g soluble starch in 5 ml H_2O into 100 ml briskly boiling H_2O. Boil for 2 min. and cool rapidly. Prepare fresh soln daily.

(g) *Standard potassium bromate soln.*—Prep. stock soln by dissolving 5.000 g $KBrO_3$ and dil. to 1 l. Prep. standard $KBrO_3$ soln by diluting 25 ml of the stock soln to 250 ml.

(h) *Potassium iodate, 0.0898 N.*—Dissolve 3.204 g KIO_3 and dil. to 1 l.

(i) *Potassium iodate, 0.00359 N.*—Dil. 10 ml 0.0898 *N* KIO_3 soln to 250 ml. Prep. fresh diln daily.

(j) *Sodium thiosulfate stock soln.*—Dissolve 22.5 g $Na_2S_2O_3 \cdot 5H_2O$ and 0.06 g anhyd. Na_2CO_3 and dil. to 1 l. Dil. 10 ml of this stock soln to 250 ml.

Transfer 5 ml of this dil. soln to a 250 ml conical flask. Add 100 ml H_2O, 10 ml 4 *N* H_2SO_4, and 1 ml KI soln. Add 5 ml starch soln and titrate with 0.00359 *N* KIO_3 from a 5 ml buret graduated in 0.01 ml. Adjust the titer of stock Na thiosulfate so that a 10:250 diln is 0.00359 *N*. Store stock soln in amber-colored bottle in cool place.

(k) *Sodium thiosulfate, 0.00359 N.*—Dil. 10 ml of stock Na thiosulfate soln to 250 ml. Prep. fresh soln daily. Check the titer of the dild soln as above at least monthly.

DETERMINATION

Add 50 g of flour to be tested to 200 ml $ZnSO_4$ soln in 600 ml beaker. Stir thoroly with a mechanical stirrer for at least 10 min., making sure that all lumps are broken up so that all particles of flour are thoroly wetted. Add 50 ml 0.4 *N* NaOH from a buret and continue to stir thoroly for another 2 min. Allow to stand for 10 min. and then centrifuge for 5 min. Filtering of the liquid to remove floating particles will give a clear colorless soln. Transfer a 50 ml aliquot of this filtrate to a 250 ml flask. Add 50 ml distd H_2O, 10 ml 4 *N* H_2SO_4, 1 ml KI soln, and 1 or 2 drops NH_4 molybdate soln.

With steady mixing, add from a pipet an excess of 0.00359 *N* Na thiosulfate (5 or 10 ml). Add 5 ml starch soln and titrate the excess of thiosulfate with 0.00359 *N* KIO_3 to the first permanent faint purple tinge of the liquid.

$$\text{P.p.m. } KBrO_3 \text{ in the flour} = \frac{X(Y - titration)}{5}$$

where X = vol. of the aliquot in ml; Y = vol. of thiosulfate used in ml.

EXPERIMENTAL RESULTS BY THE ARMSTRONG METHOD

The Armstrong method (1) does not obtain complete recovery of the potassium bromate from the flour sample except at low bromate concentrations. Results obtained in this laboratory by Armstrong's procedure without any modification are shown in Table 3. Except for one isolated case at very low bromate concentration, all the results show less than

TABLE 3.—*Recovery of potassium bromate from flour by the Armstrong method with no modifications*

NO. OF SAMPLES	SAMPLE RANGE	RANGE, %	AVERAGE, %
	p.p.m.		
4	3–8	93.3–110.0	100.8
4	20–30	93.0– 96.3	94.8
19	45–50	90.0– 99.1	96.2
3	95	91.8– 96.3	94.1

100 per cent recovery, a fact which indicates a loss somewhere in the procedure. Several modifications were tried in order to improve the yield, and various factors were studied to determine if there were some critical points in the analysis. These factors were as follows:

(1) *Effect of rate of agitation of flour in the zinc sulfate solution.*—Some experimental evidence indicated that variable results were obtained which could be traced partly to incomplete dispersion of flour in the zinc sulfate solution. Flour forms small lumps in suspension which are not wetted by the solution. These lumps could prevent some bromate from being dissolved. Flour containing 50 p.p.m. of potassium bromate was stirred by two different agitators for ten minutes. The results are given in Table 4.

The high speed stirrer gave no significant increase in recoveries, but the results were more consistent. A Waring blendor was tried, but it produced an emulsion which could not be separated by centrifugation.

TABLE 4.—*Comparison of recoveries obtained with two types of agitation*

SAMPLE NO.	$KBrO_3$ RECOVERED, P.P.M.	
	SLOW SPEED STIRRER	HIGH SPEED STIRRER
1	49.5	48.6
2	47.7	48.8
3	48.6	48.4
4	47.5	48.0
Average:	48.4	48.5

TABLE 5.—*Effect of temperature variation upon recovery of potassium bromate*

SAMPLE NO.	PROCESSING TEMP., °F.	$KBrO_3$ ADDED	$KBrO_3$ RECOVERED	
		p.p.m.	*p.p.m.*	*per cent*
1	40	50	47.5	95.0
2	75	50	46.3	92.6
3	136	50	48.0	96.0

(2) *Effect of temperature on ease of dissolving bromate out of flour.*—Certain temperatures were maintained through the procedure as far as the filtration step, after which they were allowed to reach their own level in a room maintained at normal temperatures (72°–80°F.). Data in Table 5 indicate that temperature has no significant effect upon recovery.

(3) *Effect of time required for individual steps.*—No significant results were obtained by varying the time of agitation, except that the sample should be agitated for at least ten minutes. There were no large losses of bromate even after four hours of agitation. Small losses with clear flours may occur over long periods of agitation (Table 6). Results obtained in this laboratory are indicative, rather than conclusive.

The effect of allowing the filtered solution to stand prior to adding sulfuric acid and the remaining reagents is shown in Table 7, part 1. The data indicate that there is an insignificant loss when the filtered solution is allowed to stand prior to the balance of the analysis.

Table 7, part 2, gives the effect of allowing the aliquot of filtered sample to stand with the addition of 50 ml of distilled water and 10 ml of sulfuric

TABLE 6.—*Effect upon recovery of varying time of agitation*

AGITATION TIME FOR FLOUR IN $ZnSO_4$ SOLUTION	$KBrO_3$ ADDED	$KBrO_3$ RECOVERED	
min.	*p.p.m.*	*p.p.m.*	*per cent*
		Patent Flour	
2	45	43.6	96.9
10	45	44.6	99.2
30	45	44.2	98.2
60	45	45.9	102.0
240	45	43.3	96.2
		Clear Flour	
10	50	48.8	97.6
240	50	46.8	93.6

TABLE 7.—*Effect of allowing filtered sample to stand prior to addition of other reagents*

TIME OF STANDING	$KBrO_3$ ADDED	$KBrO_3$ RECOVERED
min.	*p.p.m.*	*p.p.m.*
1. Sample alone		
2	50	46.8
60	50	46.0
180	50	45.8
2. Sample + 50 ml distd H_2O + 10 ml H_2SO_4		
½	50	47.0
10	50	46.4
30	50	45.6
60	50	45.6
3. Sample + 50 ml distd H_2O + 10 ml H_2SO_4 + 1 ml KI		
½	50	47.3
10	50	47.2
30	50	48.0
60	50	48.2
4. Sample + 50 ml distd H_2O + 10 ml H_2SO_4 + 1 ml KI + 1–2 drops $(MN_4)_2MoO_4$		
½	50	47.4
10	50	49.2
40	50	48.6
70	50	51.0

acid, but before potassium iodide and the remaining reagents are added. There is a slow loss of bromate if the analysis is delayed at this point.

In Table 7, part 3, is shown the effect of allowing the aliquot of filtered sample to stand, with the addition of 50 ml of distilled water, 10 ml of sulfuric acid, and 1 ml of potassium iodide, but before adding 1 to 2 drops of ammonium molybdate and subsequent reagents. No significant trends were noted.

Data in Table 7, part 4, give the effect of allowing the aliquot of filtered sample to stand, with the addition of 50 ml of distilled water, 10 ml of sulfuric acid, 1 ml of potassium iodide, and 1 to 2 drops of ammonium molybdate, but before adding thiosulfate and the remainder of the reagents. Recoveries show a slow increase with time.

(4) *Effect of the concentrations of zinc sulfate and sodium hydroxide upon*

TABLE 8.—*Comparison of bromate recoveries using Howe and Armstrong concentrations*

CONCENTRATION USED	$ZnSO_4$ ADDED FOR MOLAR CONCN OF:[a]	NaOH ADDED FOR MOLAR CONCN OF:[b]	$KBrO_3$ ADDED	$KBrO_3$ RECOVERED	
			p.p.m.	*p.p.m.*	*per cent*
Howe	0.0180	0.0180	50	47.4	94.8
Howe	0.0180	0.0180	50	46.2	92.4
Howe	0.0300	0.0350	50	47.1	94.2
Howe	0.0400	0.0500	50	49.0	98.0
Howe	0.0480	0.0650	50	47.5	95.0
Armstrong	0.0557	0.0800	50	48.6	97.2
Armstrong	0.0557	0.0800	50	49.0	98.0
Howe	0.0180	0.0180	30	26.4	88.0
Armstrong	0.0557	0.0800	30	28.5	95.0
Howe	0.0180	0.0180	8	6.0	75.0
Armstrong	0.0557	0.0800	8	8.0	100.0
Howe	0.0180	0.0180	95	87.3	91.9
Howe	0.0180	0.0180	95	90.0	94.7
Armstrong	0.0557	0.0800	95	89.4	94.1
Armstrong	0.0557	0.0800	95	91.5	96.3

[a] Assuming all $ZnSO_4$ added to be present in solution as $ZnSO_4$.
[b] Assuming all NaOH added to be present in solution as NaOH.

recovery of bromate.—Results of the effect upon bromate recovery, when the concentrations recommended by Armstrong (1) and Howe (4) were used, were first compared.

Howe added zinc sulfate and sodium hydroxide in such quantities that if each were still present as such in the final solution, the concentration of each would be 0.018 *M*. Armstrong used more concentrated solutions, so that the final solution would be 0.0557 *M* with respect to zinc sulfate and 0.0800 *M* with respect to sodium hydroxide. Data were obtained using both Howe's and Armstrong's concentrations, plus some intermediate concentrations (Table 8).

The filtered solutions, using Howe's concentrations, had a slight turbidity; those in which Armstrong's concentrations were used were water-clear, which indicated better protein clarification by the latter method. The data clearly show that better recoveries of bromate can be obtained by using the concentrations of zinc sulfate and sodium hydroxide recommended by Armstrong.

Results of doubling the potassium bromate concentration, but halving the sample of flour, were next compared. The concentrations of zinc sulfate and sodium hydroxide were those of Armstrong. Data are given in Table 9.

TABLE 9.—*Recoveries of potassium bromate, using Armstrong concentrations, doubling bromate concentration, and halving flour sample*

$ZnSO_4$ ADDED FOR MOLAR CONCN OF:[a]	NaOH ADDED FOR MOLAR CONCN OF:[b]	*p*H OF FILTERED SOLUTION	$KBrO_3$ ADDED	$KBrO_3$ RECOVERED	
			p.p.m.	*p.p.m.*	*per cent*
0.0557	0.0800	7.15	50	47.6	95.2
0.0557	0.0800	7.25	50	46.2	92.4
0.0557	0.0560	6.72	50	45.3	90.6
0.0557	0.0400	6.60	50	45.8	91.6
0.1114	0.0800	6.50	50	45.6	91.2
0.0835	0.0800	6.71	50	47.2	94.4
0.0557	0.0800	7.18	50	47.7	95.4

[a] Assuming all $ZnSO_4$ added to be present in solution as $ZnSO_4$.
[b] Assuming all NaOH added to be present in solution as NaOH.

The net result was to yield the same amount of bromate as in the previous tests, but with only half as much flour present in the solution.

The data indicate that the concentrations of zinc sulfate and sodium hydroxide used by Armstrong give the best results; optimum *p*H should be about 7.15.

CONCLUSIONS

(1) The titration end-point by the Armstrong procedure is very good and no difficulties are encountered. The end-point in the Howe procedure is poor and the procedure is quite difficult, especially for an inexperienced analyst. Complete recovery of potassium bromate has not been obtained by the Armstrong procedure. Armstrong notes this fact (1) and this laboratory has corroborated his results by many determinations. Various modifications in the procedure did not increase the bromate recovery.

Armstrong has suggested a method (1) for determining the recovery of bromate from flour by his procedure; it consists essentially of adding a known amount of potassium bromate to flour in a zinc sulfate solution and determining the amount of bromate recovered. He then has calculated a factor to be used in correcting the results obtained by his method. The difficulty with this procedure is that the per cent recovery varies with almost every determination. However, he found that recoveries averaged about 94 to 95 per cent; this laboratory found that recoveries ranged from 90 to 100 per cent and averaged about 95 to 96 per cent.

(3) After solutions have been prepared and standardized, analysis by the Armstrong procedure is fairly rapid.

(4) Armstrong states that iodate interferes, but benzoyl peroxide, chlorine, chlorine dioxide, and persulfate do not interfere. That statement was not tested in this laboratory.

It is believed that the Armstrong procedure is the easiest method yet

proposed by which an inexperienced analyst can determine bromate in flour. The accuracy of the results are comparable with those by other methods that have previously been tried. Further investigation* will probably reveal the cause for the losses in bromate during analysis, and will indicate appropriate remedies to correct for these losses. At present, however, fairly close estimates of bromate can be made by using the Armstrong procedure and dividing the results obtained by the factor 0.95.

REFERENCES

(1) ARMSTRONG, A. W., *Analyst*, **77**, 460 (1952).
(2) GEDDES, W. F., *This Journal*, **31**, 260 (1948).
(3) ———, and LEHBERG, F. H., *Cereal Chem.*, **15**, 49 (1938).
(4) HOWE, M., *ibid.*, **28**, 132 (1951).
(5) HOFFER, A., and ALCOCK, A. W., *ibid.*, **23**, 66 (1946).
(6) JOHNSON, L. R., and ALCOCK, A. W., *ibid.*, **25**, 266 (1948).
(7) MEYER, A. W., *ibid.*, **8**, 482 (1931).
(8) TOWNER, A. A., and KOEHN, R. C., *ibid.*, **23**, 64 (1946).

REPORT ON EGG CONTENT OF BAKERY PRODUCTS

By V. E. MUNSEY (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.), *Referee*

The Association has repeatedly recommended the study of methods applicable to the determination of egg content of bakery products. A method based on the extraction of the sterols and precipitation with digitonin, described in the report on egg content of noodles,[1] has given good results on bakery products of known composition. Two egg breads containing 1.77 and 3.48 per cent, respectively, of egg solids on a moisture-free basis (m.f.b.) showed 105 and 103 per cent recoveries. A cake mix containing 4.49 per cent egg solids gave recoveries of 103, 93 and 97 per cent. Yellow cake mix No. 1, containing 5.06 per cent egg solids (m.f.b.) and egg bread No. 2, containing 4.00 per cent egg solids (m.f.b.) were analyzed with recoveries of 93, 95, 95, and 103 and 106 per cent, respectively based on the determined composition of the raw materials. In view of these satisfactory results and the collaborative results with the same method on noodles, these samples, together with digitonin and the method, were sent to nine collaborators. Each collaborator was directed to use 32 mg of digitonin per determination and report per cent sterol on a moisture-free basis, using the values for solids of 95.8 per cent on yellow cake mix No. 1 and 91.8 per cent on egg bread No. 2. The results are given in Table 1.

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 77 (1954).
[1] *This Journal*, **37**, 92 (1954).

TABLE 1.—*Per cent sterol and egg content in cake mix and egg bread on m.f.b.*

COLLABORATOR	CAKE MIX NO. 1		AV.	EGG BREAD NO. 2		AV.
	STEROL	EGG SOLIDS		STEROL	EGG CONTENT	
	per cent	*per cent*		*per cent*	*per cent*	
1	0.138	4.70	4.75	0.133	3.96	4.00
	0.140	4.80		0.135	4.05	
2	0.138	4.70	4.77	0.136	4.10	4.17
	0.140	4.80		0.139	4.24	
	0.140	4.80				
3	0.133	4.46	4.11	0.119	3.30	3.44
	0.118	3.75		0.125	3.58	
4	0.137	4.64	4.64	0.132	3.91	3.89
	0.137	4.64		0.131	3.86	
5	0.128	4.22	4.18	0.121	3.39	3.25
	0.126	4.13		0.115	3.11	
6	0.140	4.78	4.78	0.130	3.82	3.82
	0.140	4.78		0.130	3.82	
7	0.129	4.27	4.53	0.128	3.72	3.66
	0.144	4.78		0.128	3.72	
				0.124	3.53	
8	0.143	4.93	4.97	0.133	3.96	3.96
	0.145	5.01		0.133	3.96	
9	0.117	3.70	3.92	0.117	3.21	3.30
	0.126	4.13		0.121	3.39	
10	0.122	3.94	4.06	0.131	3.86	3.72
	0.127	4.17		0.125	3.58	
		Max.	4.97			4.17
		Min.	3.92			3.25
		Av.	4.47			3.72

The formulas used for calculation of the egg content of these samples were based on the analysis of the raw materials used and are as follows:

$$\text{Cake mix: } \%\text{ egg solids} = \frac{\%\text{ sterol (m.f.b.)} - 0.038}{2.17 - 0.038},$$

$$\text{Egg bread: } \%\text{ egg solids} = \frac{\%\text{ sterol (m.f.b.)} - 0.049}{2.17 - 0.049}.$$

In the use of a general formula, consideration must be given to the correction for non-egg sterol. Five commercial cake mixes, A, B, C, D, E, containing no egg, had 0.049, 0.061, 0.055, 0.038 and 0.037 per cent, respectively, of sterol (m.f.b.). In the case of bread, the variation in blank is illustrated by the analysis of 9 commercial breads and one laboratory bread containing no added egg (Table 2).

TABLE 2.—*Per cent sterol (m.f.b.) in commercial bread*

FIRM	STEROL	FIRM	STEROL
	per cent		*per cent*
1	.050	6	.038
2	.051	7	.046
3	.052	8	.047
4	.045	9	.052
5	.047	Laboratory bread	.049
	Max.	0.052	
	Min.	0.038	
	Av.	0.048	

Referring back to Table 1, 4 chemists on the cake mix and 3 on the egg bread reported considerably too low but on the whole the results may be considered quite good. The method should find application to various cereal products containing egg. Caution is in order in regard to interpretation of results because of variation in blank from non-egg sterols and variation in type of egg used (yolk or whole egg or mixture).

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It is recommended* that the method be adopted, first action, and that study be continued.

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 77 (1954).

REPORT ON EGG CONTENT OF NOODLES

By V. E. MUNSEY (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.), *Referee*

This report is a continuation of the collaborative study on methods for the determination of the egg solids in noodles. For the past two years, reports on this subject based on the determination of lipoid P_2O_5 have been made to the Association; last year the reports were based on the determination of choline (1). Neither of these studies was entirely satisfactory. This year the determination has been based on the extraction of the sterol and its precipitation with digitonin. The advantages of the sterol determination have been recognized in the development of the official sterol method, **13.113.** This official method is relatively complex and time-consuming. In an attempt to obtain a simpler and more rapid procedure, the official extraction for sterols has been modified and combined with a modification of the digitonin precipitation method of Hadorn (2). The resulting procedure is relatively short, specific, and capable of giving good estimation of egg solids. The method as submitted to twelve collaborators was published in *This Journal*, **37**, 92 (1954).

COLLABORATIVE STUDY

The samples of noodles sent the collaborators consisted of: No. 1, yolk noodles containing 5.56 per cent yolk solids (moisture-free basis) and No. 3, whole egg noodles containing 5.74 per cent whole egg solids (moisture-free basis), on the basis of raw material used in laboratory preparation. The sterol content of the durum flour used in No. 1 and No. 3 was 0.050–0.053 per cent (m.f.b.) and 0.053–0.053 per cent, respectively; that of the dried egg yolk in No. 1 was 2.88–2.87 per cent (m.f.b.); and that of the dried whole egg in No. 3 was 2.17–2.17 per cent (m.f.b.).

Digitonin from the same source was sent to most of the collaborators. The results were computed to the moisture-free basis by all collaborators using the same conversion factor. The results for per cent sterol and egg solids content appear in Table 1.

The formula used for calculation of egg content is as follows:

$$\text{Per cent yolk solids} = \frac{\%\ \text{sterols, m.f.b.} - .052}{2.88 - .052}$$

$$\text{Per cent whole egg solids} = \frac{\%\ \text{sterols, m.f.b.} - .053}{2.17 - .053}.$$

For derivation of a general formula, data are presented in Table 2 on the composition of semolina and durum flours and in Tables 3 and 4 on yolk and eggs.

TABLE 1.—*Per cent of sterol and egg content in noodles, m.f.b.*

COLLABORATOR	YOLK NOODLE NO. 1		WHOLE EGG NOODLE NO. 3	
	STEROL	YOLK SOLIDS	STEROL	WHOLE EGG SOLIDS
	per cent	*per cent*	*per cent*	*per cent*
1	0.205	5.42	0.171	5.58
	0.204	5.38	0.168	5.44
	0.208	5.60	0.170	5.54
			0.175	5.77
2	0.206	5.45	0.166	5.35
	0.206	5.45	0.166	5.35
3[a]	0.194	5.02	0.147	—
	0.196	5.10	0.166	5.35
	0.171	—	0.167	5.40
4	0.206	5.45	0.169	5.49
	0.209	5.56	0.175	5.77
	0.211	5.63	0.176	5.82
5	0.204	5.38	0.164	5.25
	0.203	5.34	0.162	5.16
5[a]	0.189	4.85	0.146	4.40
	0.187	4.78	0.144	4.31
6[a]	0.193	4.99	0.168	5.44
	0.192	4.96	0.166	5.34
7	0.196	5.10	0.162	5.16
	0.200	5.24	0.160	5.06
8	0.210	5.60	0.174	5.72
	0.210	5.60	0.174	5.72
9	0.198	5.16	0.167	5.39
	0.198	5.16	0.167	5.39
9[a]	0.184	4.67	0.143	4.26
	0.184	4.67	0.149	4.54
10	0.168	4.11[b]	0.148	4.50
	0.190	4.89	0.150	4.59
11	0.192	4.96	0.161	5.06
	0.196	5.10	0.159	5.16
12[c]	0.198	5.16	0.170	5.52
	Max.	5.60		5.72
	Min.	4.67		4.35
	Av.	5.20		5.23

[a] Collaborators used their own digitonin.
[b] Not included in average.
[c] Received too late for inclusion in average.

TABLE 2.—*Per cent sterol, m.f.b., by digitonin method in durum flour and semolina*

FIRM	PRODUCT	CROP YEAR	STEROL
B	Durum patent flour	1950	.057
D	Durum patent flour	1950	.054
H	Durum patent flour	1950	.048
F	Durum patent flour	1950	.053
C	Durum patent flour	1950	.055
A	Durum patent flour	1950	.054
I	Semolina	1950	.052
E	Semolina	1950	.054
A	Semolina	1952	.048
B	Semolina	1952	.049
C	Semolina	1952	Av. .050
D	Semolina	1952	Av. .053
E	Semolina	1952	Av. .055
D	Durum patent flour	1952	Av. .051
F	Durum patent flour	1952	.047
B	Durum patent flour	1952	.049
C	Durum patent flour	1952	.054
E	Durum patent flour	1952	.052
G	Bread flour	1952	.048
E	Bread flour	1952	.055
	Durum flour blend	1952	.057
F	Durum patent flour	1953	.052
H	Durum patent flour	1953	.050
B	Durum patent flour	1953	.052
D	Durum patent flour	1953	.055
E	Durum patent flour	1953	.054
E	Semolina	1953	.056
H	Semolina	1953	.051
B	Semolina	1953	.050
C	Semolina	1953	.049
D	Semolina	1953	.055

For 31 samples: Max. .057; Min. .047; Av. .052

The commercial frozen yolks were collected at egg-breaking plants in various parts of the country by inspectors of the Food and Drug Administration and sent to Washington, D. C. in frozen condition.

The results of this study are good with the exception of those obtained by one or two collaborators. In the calculation, it will be noted that the sterol content of durum flour by the digitonin method is about double the value for durum flour by the official sterol method, **13.113,** while the values for the yolk and whole egg solids are in close agreement (see *This Journal,* **34,** 119 (1949)). However, in Table 4, the frozen yolks are higher by the digitonin method by about 0.2 per cent sterol than by the official method;

TABLE 3.—*Per cent sterol (m.f.b.) by digitonin method on eggs*

SOURCE	PRODUCT	YEAR	STEROL, %
A	Dried whole egg #2	1952	2.07
A	Dried whole egg #8	1952	2.02
A	Dried whole egg #4	1952	2.08
A	Dried whole egg #5	1952	2.06
A	Dried whole egg #7	1952	2.07
A	Dried whole egg #1	1952	2.14
A	Dried whole egg #9	1952	2.07
B	Dried whole egg Drum 4–43	1952	2.17
			2.08 (Av. for 8 samples)
A	Dried yolk—Chicago	1952	2.79
A	Dried yolk—New York	1952	2.85
A	Dried yolk—Missouri	1952	2.88
A	Dried yolk—Nebraska	1952	2.96
A	Dried yolk—Missouri	1952	2.83
A	Dried yolk—Minneapolis	1952	2.80
B	Dried yolk, Drum 93	1952	2.98
B	Dried yolk, Drum 6218	1952	2.91
—	Dried yolk, Bag 418	1952	Av. 2.88
			2.87 (Av. for 9 samples)

the official procedure was used for extraction in both methods.

For comparison, the Referee determined egg solids by the official sterol method, **13.113,** to be 5.19 and 5.33 per cent yolk solids (m.f.b.) on No .1, and 5.25 and 5.40 per cent whole egg solids (m.f.b.) on No. 3, using average values of raw material for conversion. Also, sterols extracted by the official sterol method, **13.113,** followed by precipitation with digitonin, gave 5.60 per cent yolk solids in No. 1 and 5.77 per cent whole egg solids on No. 3 (both m.f.b.). These values are in good agreement for the two different procedures.

The digitonin procedure has other applications to egg-containing products. It is necessary to consider the egg level in order to have the proper ratio of digitonin to sterol and at the same time not use excessive amounts of an expensive reagent. Likewise, correction for a blank is necessary for the non-egg sterols.

ACKNOWLEDGMENT

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J. J. Winston, Jacobs-Winston Laboratories, New York, N. Y.

TABLE 4.—*Per cent sterol by digitonin and official methods*

SOURCE	PRODUCT	YEAR	STEROL, %		SOLIDS %
			DIGITONIN	OFFICIAL	
Iowa-C	Frozen yolk, 20-349 L	1953	2.94	2.69	45.2
			2.96	2.72	45.2
Iowa-D	Frozen yolk, 20-350 L	1953	2.90	2.64	42.2
			2.88	2.64	42.0
Wash. E	Frozen yolk, 40-968 L	1953	2.93	2.75	43.1
			2.98	2.89	43.5
Wash. F	Frozen yolk, 40-969 L	1953	3.06	2.71	43.7
			3.03	2.77	43.7
Nebr. G	Frozen yolk, 61-384 L	1953	2.96	2.68	44.8
			2.95	2.70	44.8
Missouri-H	Frozen yolk, 62-641 L	1953	2.90	2.68	44.5
			2.90	2.65	44.4
Missouri-I	Frozen yolk, 62-642 L	1953	2.96	2.79	46.8
			2.94	2.79	46.8
Illinois-J	Frozen yolk, 59-262 L	1953	2.87	2.67	45.0
			2.85	2.75	45.0
	Frozen yolk, 70-498 L	1953	2.84	2.61	44.4
			2.92		44.4
	Frozen yolk, 61-383 L	1953	2.87	2.62	45.0
			2.85		45.3
	Frozen yolk, 43-128 L	1953	3.03	2.82	45.2
			3.03		45.2
	Frozen yolk, 59-261 K	1953	2.88	2.65	43.2
			2.78		43.2
	Frozen yolk, 57-075 L	1953	2.95	2.71	44.7
			2.92		44.6
	Min.		2.83	2.61	42.1
	Max.		3.05	2.82	46.8
	Av. (13 samples)		2.93	2.71	44.5

Frank J. Kokoski, State Food Laboratory, Albany, N. Y.

John E. Despaul, Chicago Quartermaster Inspection Service Field Office, Chicago, Ill.

Oscar R. Wiger, Public Health Service, Los Angeles, Calif.

Roland L. Giusti, Department of Public Health, Berkeley, Calif.

Mark Zenk, Grain Branch, PMA, U. S. Department of Agriculture, Beltsville, Md.

Curt Rupke, Sylvia Shendleman, Harold E. Theper, Harry Conroy, and Catherine Cunningham, all of the Food and Drug Administration.

It is recommended* that the method be adopted, first action, and that the study be continued.

* For report of Subcommittee D and action of the Association, see *This Journal*, 37, 77 (1954).

REFERENCES

(1) MUNSEY, V. E., *This Journal*, **36**, 766 (1953).
(2) HADOWN, V. H., *Mitt. Lebensm. Hyg.*, **43**, 41 (1952).

REPORT ON SOYBEAN FLOUR

By T. C. LAW (Law and Company, Atlanta, Georgia), *Associate Referee*

The methods for the determination of moisture **(13.63)**; ash **(13.64)**; nitrogen **(13.65)**; crude fiber **(13.66)**; and oil or petroleum ether extract **(13.67)** for the analysis of soybean flour have had first action status for five years. These methods are the same as those adopted as official by the American Oil Chemists' Society. They were originally proposed to these two organizations for adoption after collaborative study and agreement of 19 laboratories which were interested in their use. Insofar as possible, they specify technics already included in methods designated as official for the analysis of other products.

During recent months, individuals in 14 laboratories which were known to have an interest in the use of these methods were asked to indicate if their use has proved satisfactory in the analysis of soybean flour. Eight of the users stated that the methods are satisfactory. The other six had little experience in their use or had not employed them, and consequently refrained from commenting.

The methods appear to have been used with satisfaction by both inspection and industrial laboratories for five years. The technics involved are well standardized. There seems to be little or no need for further collaborative study.

RECOMMENDATIONS

It is recommended* that the methods for the analysis of soybean flour, listed above, be made official. It is further recommended that 15 instead of 10 grams of potassium sulfate or sodium sulfate be specified in the method for the determination of nitrogen, thus making it specific and within the range of the sulfate specified in *Official Methods of Analysis*, **2.24**, for the determination of nitrogen by the Kjeldahl–Wilfarth–Gunning Method.

If these recommendations are adopted, the method for the determination of nitrogen in soybean flour **(13.65)** should read as follows:

"Proceed as directed in **2.24**, using 15 g K_2SO_4 or Na_2SO_4 and 0.7 g HgO or its equivalent in Hg (Na alizarin sulfonate may be used as indicator)."

* For report of Subcommittee D and action of the Association, see *This Journal*, 37, 76 (1954).

REPORT ON STARCH IN CEREAL PRODUCTS

By EDWARD F. STEAGALL (Food and Drug Administration, Department of Health, Education, and Welfare, Los Angeles 15, Calif.), *Associate Referee*

After disappointing collaborative results were obtained two years ago with the diastase method (1), further consideration was given the Munsey modification of the Rask-McVey method (2). It was felt that by making the method more specific and by eliminating some of the variables, good results could be obtained. Although the method is simple and is the most rapid of any available, it is very empirical.

Three main changes that were made in the method are noted below:

(1) *Cold 5.7 N Hydrochloric Acid.*—It was found that the use of cold 5.7 *N* hydrochloric acid would forestall any possibility of subsequent hydrolysis of the starch due to warming and would still give accurate results.

(2) *Stirring.*—Mechanical stirring of the sample with the acid causes a more complete solution of the starch.

(3) *Hyflo Super-Cel.*—It was found that this filter aid possesses the best combined qualities of retention and speed of filtration; it was also discovered that it would give an appreciable blank unless digested in 1+1 hydrochloric acid.

Other minor changes were made in the method but these were either mechanical in nature or for purposes of editorial clarification only.

The revised method was then tested by chemists in this laboratory for the determination of starch in prepared samples of flour, bread, and graham crackers. They were asked to make duplicate determinations and, upon completion of the analysis, to comment on how the method might further be improved. Their results are recorded in Table 1.

TABLE 1.—*Per cent starch by the modified Rask-McVey method*

ANALYST	FLOUR	BREAD	GRAHAM CRACKERS
1	70.06	53.02	49.20
	70.08	52.92	49.18
2	69.24	51.64	48.72
	69.16	51.64	48.60
3	68.66	53.17	49.05
	69.76	53.68	47.05
Official Method (3)	70.26	53.21	49.20
	70.26	52.99	49.26

These results show fairly close agreement and are a decided improvement over past results. All results come within a maximum variation (from low to high) of 5 per cent.

The comments of these analysts were then incorporated into the method, and the revised method is as follows:

DETERMINATION OF STARCH

APPARATUS

(a) *Mechanical stirrer.*—Any type of stirrer, electric or air driven, fitted with a glass stirring rod. Flatten one end of the rod lengthwise to form a paddle just wide enough to fit in the neck of a Kohlrausch flask. The older type of Kohlrausch flasks have very narrow necks whereas the newer type flasks have necks 15–18 mm O.D. The newer type flasks are preferable.

(b) *Prepared Gooch crucible.*—A crucible contg an asbestos pad and loosely filled ⅔ full of dry, acid-washed Hyflo Super-Cel. These crucibles should be prepd well ahead of the detn and dried to constant wt, ca 2 hrs at 130°C.

REAGENTS

(a) *Ethyl alcohol, 95%.*—Reagent grade.

(b) *Alcoholic potassium hydroxide soln.*—Dissolve 4 g KOH in 100 ml 95% ethyl alcohol.

(c) *Hydrochloric acid, 5.7 N.*—1+1 HCl, mixed, cooled, and adjusted to exact normality by titration. Store in refrigerator and use cold.

(d) *Hyflo Super-Cel.*—To 250 g Hyflo Super-Cel (Johns-Manville), add 100 ml 1+1 HCl and digest on steambath for 2 hrs. Filter on paper in Buchner funnel and wash with distd H_2O until neutral to litmus. Dry at 130°C. Break up and store in tightly closed jar.

DETERMINATION[1]

Accurately weigh 1 g sample, ground to pass 40 mesh sieve, into 125 ml Erlenmeyer flask, add 2.0 ±0.1 g Hyflo Super-Cel, add 50 ml alcoholic KOH, mix well, and heat on steam bath for 30 min. with flask fitted to reflux condenser. Shake at ca 5 min. intervals. Fit filter paper (S & S No. 589 white ribbon or equivalent), conveniently cut out with bork borer, into Gooch crucible. Transfer sample with aid of stream of alcohol, police flask twice, and neglect thin film of filter aid remaining. Continue to wash until the total vol. in suction flask amounts to 100 ml. Continue to dry few min. by suction to remove most of the alcohol from the sample. Invert crucible over 150 ml beaker and transfer sample. (A gentle tap on the bottom of the inverted crucible is usually sufficient to dislodge the sample, and if sample has been sucked dry enough, any portion remaining in the crucible may be brushed out.) Carefully remove filter disc from bottom of the filter cake and brush any adhering material into beaker. Thoroly break up caked sample with stirring rod. Pour 60 ml of cold 5.7 *N* HCl into 100 ml graduate. Add 8–10 ml of this acid to sample, stirring to form thick, smooth paste completely free from lumps. (It is absolutely imperative that all lumps be broken up so that all the sample can come in contact with the acid.) Continue to add small amounts of acid and stir to thin suspension by using total of ca 25 ml. Finally pour suspension into 100 ml Kohlrausch flask. Rinse out beaker with remainder of original 60 ml of acid, using small successive portions. This mixing and transferring should take ca 5 min. Stir suspension in

[1] Due to the nature of the method, it is essential that it be done in one day up to the point of weighing the starch.

flask for 10 min. by means of mechanical stirrer. Rinse down stirrer and make nearly to vol. with H_2O. The soln at this point should be essentially at room temp. If not, adjust to room temp. promptly in order to minimize hydrolysis. Immediately make to vol. with H_2O and add 1 ml more H_2O from Mohr pipet to correct for vol. of filter-aid. Immediately mix well and filter into 250 ml suction flask through Gooch crucible fitted with filter paper, as above, on which is a thin pre-coat of filter-aid. Immediately pipet 50 ml of filtrate into 115 ml alcohol in 250 ml beaker and transfer the filter-aid carefully by inverting the prepd Gooch crucible. Mix well with stirring rod and transfer pptd starch and filter-aid to same Gooch crucible by use of suction, keeping mixture agitated for entire time filtration is in progress. After nearly all the sample is transferred, turn off suction and empty flask. Using suction, wash contents of crucible with 100 ml of alcohol, guided by a previously measured 100 ml marker on the suction flask. Dry at 130°C. for 2 hrs. Cool ca 10 min. in desiccator charged with fresh H_2SO_4, (or freshly ignited CaO or other efficient desiccant) and weigh quickly. (Starch is hygroscopic and should not be exposed longer than necessary.) Report results in duplicate as per cent starch.

DISCUSSION

Analyst R. A. Baxter makes the following comment:

"Since thorough mixing of the dry filter-cake with the acid is essential, it may be advantageous to use a small glass mortar and pestle instead of a beaker and glass rod for this purpose. This would assure a more thorough mixing in less time. This suggestion was tried on bread run No. 3 and a slightly higher result—54.45 per cent starch—was obtained."

This comment deserves further consideration and may improve the results still more. It is intended to submit the method to collaboration during the next year after a study of the use of the glass mortar and pestle.

It is recommended* that this study be continued.

ACKNOWLEDGMENT

The advice and assistance of the following, all of the Los Angeles District of the Food and Drug Administration, are greatly appreciated: H. M. Bollinger, L. C. Weiss, R. A. Baxter, and D. Menschenfreund.

REFERENCES

(1) STEAGALL, E. F., *This Journal*, **35**, 691 (1952).
(2) MUNSEY, V. E., *ibid.*, **34**, 270 (1951).
(3) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., p. 348, **22.35.**

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 76 (1954).

REPORT ON METHODS OF YEAST ANALYSIS

By A. L. BRANDON (Anheuser-Busch, Inc., St. Louis, Mo.),
Associate Referee

In last year's report to the Association (1), the Associate Referee outlined the need for the standardization of methods of analysis of yeast, and recommended that (*a*) collaborative studies of methods for the determination of moisture, total nitrogen, and P_2O_5 in yeast be continued until satisfactory methods are developed; and (*b*) collaborative studies of methods for the determination of ash, ether extract, crude fiber, and crude carbohydrate be postponed until the studies on moisture, total nitrogen, and P_2O_5 are completed.

The study was initiated early in 1952 with seventeen laboratories expressing a desire to participate. In order to determine the extent of experience of the collaborators in analyzing yeast, questionnaires were sent to all, asking them if they analyzed yeast regularly, occasionally, or not at all for the various constituents usually determined in a complete analysis. A summary of the answers to this questionnaire is shown in Table 1 and data indicate that moisture and total nitrogen are the only two constituents regularly determined by a majority of the collaborators. Five of the collaborators answered that they analyze yeast for any of the constituents only occasionally, and two not at all.

SAMPLES

Four yeasts, consisting of a primary bakers' yeast, a non-debittered brewers' yeast, a debittered brewers' yeast, and a Torula yeast, grown on sulfite waste liquor, were obtained in 100-lb. drums and stored at 40°F. Samples were distributed to the collaborators in 4-oz. screw-capped bottles which had been sealed with paraffin to prevent moisture changes.

TABLE 1.—*Summary of answers to questionnaire sent to collaborators*[a]

ANALYSIS	ANALYZED YEAST		
	REGULARLY	OCCASIONALLY	NOT AT ALL
Moisture	10	5	2
Total nitrogen	9	5	3
P_2O_5	6	3	8
Ash	5	9	3
Ether extract	2	7	8
Crude fiber	1	6	10
Crude carbohydrate	0	4	13

[a] Total number of collaborators = 17.

MOISTURE

The summary of methods used by the collaborators for the moisture determination is shown in Table 2, and a tabulation of the values reported is given in Table 3.

Although oven methods were used exclusively, there was considerable variation in the methods with respect to sample size, oven temperature, and time of drying, which may account for the high coefficients of variation of 7.79%, 7.32%, 7.31%, and 26.44%, for Samples No. 1 to 4, respectively. However, the fact that the value of 26.44% for Sample No. 4 was so much higher than the values for Samples No. 1 to 3 indicates that sample variation as well as method variation was the cause of the high variances.

Examination of the sample stocks showed high moisture values for Samples No. 2 and No. 4. In order to obtain samples having a constant moisture content, 5-pound lots of the four samples were air-dried at room temperature for forty-eight hours, and the dried samples were stored in large, screw-capped bottles. The moisture content of the samples was determined before and after air-drying and again one and three weeks after air-drying to determine if the moisture content of the samples remained constant when they were stored in the laboratory in screw-capped bottles. The values are recorded in Table 4 and show that the moisture content remained constant over the three-week period with the exception of Sample No. 4, which showed a slight increase in moisture.

TABLE 2.—*Moisture methods used by collaborators*

COLLABORATOR NO.	SAMPLE SIZE	TYPE OF OVEN	TEMPERATURE, °C	DRYING TIME, HRS.	REMARKS
	gram				
1	1	Vacuum 15–16 mm. Hg.	100	3	
2, 12	5	Vacuum 25–30 in. Hg.	70–72	16	
3	10	Air forced draft	100	5	
4	5	Vacuum 23 in. Hg.	100	5	
5	10	Water	Boiling water	7	
6	5	Air convection	105 ± 0.5	5	A.S.B.C. method
7	2–5	Air convection	100	16½	
7	10	Brabender	125	½	
8	2	Air convection	105	15	
9, 11, 16	10	Air convection	100	5	U.S.P. method
10	2	Air convection	100	16	
13	2	Air convection	100 ± 0.5	16	
14	5	Air convection	103–105	4	
15	5	Air convection	103	4	

TABLE 3.—*Average values and statistical analysis of moisture data submitted by collaborators*

COLLABORATOR NO.	MOISTURE, %			
	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3	SAMPLE NO. 4
	PRIMARY BAKERS'	NON-DEBITTERED BREWERS'	DEBITTERED BREWERS'	TORULA
1	4.555	9.155	4.155	8.015
2	4.550	8.550	4.150	11.800
3	4.670	8.565	4.290	9.445
4	4.880	8.500	4.240	9.310
5	4.015	8.330	3.635	9.465
6	4.200	8.300	3.970	9.400
7	4.760[a]	9.146[a]	4.616[a]	11.173[a]
7	4.750[b]	9.100[b]	4.400[b]	11.200[b]
8	4.930	9.760	4.985	9.795
9	5.800[c]	7.200[c]	4.500[c]	5.300[c]
10	4.936[d]	9.613[d]	4.677[d]	9.480[d]
10	4.860[d]	9.666[d]	4.613[d]	9.537[d]
11	5.230	8.875	4.555	10.535
12	4.630[e]	8.373[f]	4.025[e]	11.283[f]
13	4.715	8.485	4.455	8.530
14	4.905	9.740	4.480	16.705
15	4.530	8.655	3.945	9.450
16	4.685	8.875	4.345	9.080
Mean ($\bar{X}$)	4.756	8.827	4.335	9.972
Std. Dev. (s)	0.379	0.646	0.317	2.637
Coeff. of Var. (%)	7.97	7.32	7.31	26.44
Range	1.785	2.560	1.350	11.405
3 s	1.137	1.938	0.951	7.911

[a] Three values—air oven.
[b] Brabender.
[c] One value.
[d] Two analysts—three values.
[e] Four values.
[f] Three values.

The four samples were then transferred to 4-oz. screw-capped bottles, the moisture content of each sample was determined, and the jars were sealed with adhesive tape to prevent any change in the moisture content. The samples were stored in a cool place, and, after four to six weeks, the moisture content was determined on five of the samples selected at random, after which they were distributed to the sixteen collaborators who had reported analyses on the first set of samples. The data of the above moisture analyses, recorded in Table 5, indicate that the moisture content remained constant over the four-to-six week period.

The collaborators were instructed to analyze the samples for moisture only, immediately upon receipt of the samples (or as soon as possible thereafter) and again two or three weeks later by one of the two methods submitted, in order to determine if there was any change in the moisture when the samples were stored in the collaborators' laboratories.

TABLE 4.—*Moisture values of yeast samples before and after air drying*[a]

SAMPLE NO.	MOISTURE, %							
	BEFORE AIR DRYING		AFTER AIR DRYING		1 WK AFTER AIR DRYING		3 WKS AFTER AIR DRYING	
		AVERAGE		AVERAGE		AVERAGE		AVERAGE
1	6.06	6.030	4.87	4.895	5.14	5.145	4.83	4.810
	6.00		4.92		5.15		4.79	
2	13.12	13.340	6.50	6.475	6.65	6.660	6.37	6.310
	13.56		6.45		6.67		6.25	
3	5.41	5.335	4.69	4.630	4.85	4.850	4.35	4.390
	5.26		4.57		4.85		4.43	
4	15.32	15.295	6.17	6.185	6.46	6.470	6.87	6.810
	15.27		6.20		6.48		6.75	

[a] Dried for 16 hrs at 105°C. in an air oven.

METHODS

PREPARATION OF SAMPLE

Before opening sample for analysis, alternately invert and roll the jar 25 times, or more if necessary, to secure a homogeneous mixture. *Do not remove sample from jar for mixing.* Avoid extreme temperatures and humidities when opening the jar for analysis and close and seal immediately after removing sample. Keep sample tightly sealed at all times.

TABLE 5.—*Statistical analysis of moisture values of yeast samples in 4-oz. jars*[a]

	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4	
	INITIAL	AFTER 4–6 WKS	INITIAL	AFTER 4–6 WKS	INITIAL	AFTER 4–6 WKS	INITIAL	AFTER 4–6 WKS
Range	4.887–5.091	5.019–5.083	5.767–6.137	6.115–6.249	4.186–4.591	4.411–4.527	6.162–6.482	5.944–6.048
Mean ($\bar{x}$)	5.001	5.056	5.968	6.200	4.412	4.487	6.275	6.004
Std. Dev. (s)	0.066	0.034	0.093	0.061	0.123	0.045	0.087	0.044
Coeff. of Var. (%)	1.31	0.67	1.56	0.98	2.79	1.00	1.39	0.73
No. of samples (n)	17	6	20	5	20	5	20	5

[a] Dried for 16 hrs at 105°C. in an air oven.

MOISTURE

Method No. 1—Vacuum Oven Method

APPARATUS

(a) *Metal dish.*—Diam. ca 55 mm, height about 15 mm, provided with inverted step-in cover fitting tightly on inside.

(b) *Metal dish.*—Diam. ca 65 mm, height about 20 mm, provided with slip-over cover fitting tightly on outside.

(c) *Air-tight desiccator.*—Reignited CaO, $CaCl_2$, or Drierite are satisfactory drying agents.

(d) *Vacuum oven.*—Connect with pump or vacuum system capable of maintaining pressure equivalent to 50 mm or less of Hg, and provided with thermometer passing into oven in such a way that the bulb is near samples. Connect H_2SO_4 gas-drying bottle with oven for admitting dry air to sweep moisture from oven and when releasing vacuum.

DETERMINATION

Weigh accurately ca 2 g well-mixed sample in covered dish previously dried at 98–100°C., cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat at 98–100°C. 5 hrs at pressure of 50 mm Hg or less. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover on dish, transfer to desiccator, and weigh soon after room temp. is attained.

Method No. 2—Air Oven Method

APPARATUS

(a) *Metal dish.*—same as for Method No. 1.

(b) *Metal dish.*—same as for Method No. 1.

(c) *Desiccator.*—same as for Method No. 1.

(d) *Air oven.*—Should be regulated to 100°C. ±1°C.

DETERMINATION

Weigh accurately ca 2 g well-mixed sample in covered dish previously dried at 100°C. ±1°C., cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat for 5 hrs at 100°C. ±1°C. Tighten cover on dish, transfer to desiccator, and weigh soon after attaining room temp.

At the writing of this report, thirteen of the sixteen collaborators had submitted results on the moisture analysis of the second set of samples. Only two collaborators reported data on Method No. 1; their results are recorded in Table 6. These data were not analyzed statistically, since there were too few values.

Data on Method No. 2, summarized in Table 7, were reported by twelve collaborators, and show considerably less variation of values from the second set of samples than from the first. All of the collaborators except one (Collaborator No. 6) found little change in the moisture content of the samples in the second analysis, and indicate that the moisture content remains fairly stable over a period of time. It is interesting to note that the analysis of the data which excludes the values of Collaborator No. 6 shows considerably less variation than when these data are in-

TABLE 6.—*Individual and average values of moisture data submitted by collaborators on second set of yeast samples: Method No. 1–vacuum oven at 100°C. for 5 hours*

COLLABORATOR NO.	MOISTURE, %															
	SAMPLE NO. 1				SAMPLE NO. 2				SAMPLE NO. 3				SAMPLE NO. 4			
	1ST ANAL.		2ND ANAL.		1ST ANAL.		2ND ANAL.		1ST ANAL.		2ND ANAL.		1ST ANAL.		2ND ANAL.	
	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.
1	5.44	5.445	5.83	5.845	7.43	7.435	7.96	8.005	5.23	5.275	5.73	5.720	6.83	6.765	7.18	7.200
	5.45		5.86		7.44		8.05		5.32		5.71		6.70		7.22	
1[a]			5.26	5.290			7.14	7.170			4.93	4.950			6.55	6.605
			5.32				7.20				4.97				6.66	
5	5.23	5.165	5.31	5.310	6.39	6.390	6.36	6.370	4.74	4.655	4.78	4.800	6.28	6.275	6.33	6.310
	5.10		5.31		6.39		6.38		4.57		4.82		6.27		6.29	

[a] 3 hrs drying period.

cluded. The writer is unable to account for the differences found by this collaborator. It is also interesting to note that Collaborator No. 1 found practically no difference in the moisture content in the two analyses by Method No. 2 but did find a difference by Method No. 1.

COMMENTS OF COLLABORATORS

Collaborator No. 1: As explained in my report 6/24/52, we found that the 3-hour period gave us constant weight. We used for constant weight the definition of *Official Methods of Analysis*, 7th Ed., **29.3,** i.e., dry at one hour intervals until change in weight between successive weighings is less than 2 mg.

Finally, I understand that the loss in weight which one reports as moisture is really varying proportions of: (1) loss of water; (2) loss of other volatile material; and (3) loss due to decomposition. I thought of this concept when I noted that the "100°C. in air for 5 hours" values were quite comparable to the "100°C. *in vacuo* for 3 hours" values. On the other hand, the "100°C. *in vacuo* for 5 hours" values averaged something like $\frac{3}{4}$ per cent higher.

Collaborator No. 6: We would like to point out the rapid moisture pickup which occurred during the first forty-eight hours after removing the plastic seals from the jars, even though the lids remained tightly closed.

Collaborator No. 8: I would like to point out that Sample No. 3, which showed the greatest variation on standing, had no lining in the screw cap.

Collaborator No. 10: It should be pointed out that our tests were conducted in a Freas mechanical convection air oven. Our experience has shown that higher results are usually obtained with an oven equipped with mechanical convection than with an oven where the atmosphere is static.

NITROGEN

A summary of the nitrogen methods is shown in Table 8 and of the collaborative data in Table 9. In general, the Kjeldahl method was used with modifications, and the data showed good correlation between the collaborators. This is well illustrated by the small spread of the coefficients of variance which were 3.00 per cent, 3.28 per cent, 2.05 per cent, and 4.54 per cent for Samples Nos. 1 to 4, respectively. In order for the nitrogen values to be evaluated properly they had to be calculated on a dry-solids basis, and the low coefficients of variance obtained are further evidence that the high variance found in the moisture values (Table 3) is caused mainly by sample variation.

PHOSPHORIC ACID

A summary of the methods used for P_2O_5 is shown in Table 10 and the collaborative data in Table 11. Considerable variation was noted in the

TABLE 7.—*Individual and average values and statistical analysis of moisture data submitted by collaborators on second set of yeast samples—Method No. 2: air oven at 100°C. for 5 hours*

COLLABORATOR NO.	MOISTURE, %															
	SAMPLE NO. 1				SAMPLE NO. 2				SAMPLE NO. 3				SAMPLE NO. 4			
	1ST ANAL.		2ND ANAL.		1ST ANAL.		2ND ANAL.		1ST ANAL.		2ND ANAL.		1ST ANAL.		2ND ANAL.	
	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.	DUP.	AV.
1	5.29 5.29	5.290	5.25 5.19	5.220	6.93 6.94	6.935	7.12 6.95	7.040	4.96 4.75	4.855	5.30 5.10	5.200	6.41 6.35	6.380	6.63 6.58	6.605
2	5.21 5.14	5.175			6.73 6.78	6.755			4.91 4.91	4.910			6.73 6.62	6.675		
3	5.08 5.07	5.075	5.27 5.19	5.230	6.48 6.64	6.560	6.43 6.38	6.405	4.68 4.79	4.735	4.98 4.93	4.955	6.23 6.25	6.240	6.10 6.22	6.160
6	3.49 3.61	3.550	4.71 4.85	4.780	4.64 4.95	4.795	6.08 6.32	6.200	3.31 3.47	3.390	4.38 4.53	4.455	4.00 4.98	4.490	6.19 6.12	6.155
7	5.26 5.35	5.305	5.38 5.38	5.380	6.70 6.55	6.625	6.75 6.85	6.800	4.90 4.75	4.825	5.05 5.05	5.050	5.93 6.05	5.990	5.99 5.89	5.940
8	4.94 4.86	4.900	5.34 5.21	5.275	6.10 6.20	6.150	6.07 6.33	6.200	4.87 4.87	4.870	5.53 5.51	5.520	6.00 5.80	5.900	5.68 5.90	5.790
10	5.75 5.75	5.750			6.99 6.89	6.940			5.31 5.27	5.290			6.74 6.69	6.715		

11	5.33 5.28	5.305	5.34 5.36	5.350	6.57 6.58	6.575	6.75 6.73	6.740	4.93 4.90	4.915	5.09 5.11	5.100	6.63 6.62	6.625	6.73 6.72	6.725
12	4.81 4.99	4.900	5.59 5.55	5.570	5.87 5.73	5.800	6.41 6.35	6.380	4.59 4.60	4.595	4.99 4.98	4.985	5.89 5.96	5.925	6.32 6.36	6.340
13			5.47 5.52	5.495			6.61 6.55	6.580			5.02 5.02	5.020			6.38 6.43	6.405
13[a]	5.18 5.19	5.185	5.46 5.50	5.480	6.41 6.41	6.410	6.68 6.70	6.690	4.84 4.86	4.850	5.00 5.06	5.030	6.13 6.14	6.135	6.39 6.41	6.400
14	5.00 5.02	5.010	5.36 5.47	5.415	6.65 6.71	6.680	6.80 6.64	6.720	4.71 4.68	4.695	4.97 5.01	4.990	6.40 6.33	6.365	6.33 6.45	6.390
15	5.23 5.32	5.275	5.09 5.05	5.070	6.35 6.46	6.405	6.11 6.24	6.175	4.73 4.80	4.765	4.62 4.58	4.600	6.16 6.15	6.155	5.87 5.94	5.905

Excluding Data from Collaborator No. 6

Mean ($\bar{x}$)	5.199	5.334	6.543	6.560	4.846	5.047	6.297	6.251
Std. Dev. (s)	0.251	0.153	0.351	0.291	0.186	0.241	0.307	0.323
Coeff. of Var. (%)	4.830	2.870	5.360	4.440	3.840	4.780	4.880	5.170
Range	0.850	0.500	1.140	0.865	0.595	0.920	0.815	0.930
$3s$	0.753	0.459	1.053	0.873	0.558	0.723	0.921	0.969

Including Data from Collaborator No. 6

Mean ($\bar{x}$)	5.049	5.279	6.384	6.524	4.713	4.988	6.133	6.242
Std. Dev. (s)	0.551	0.227	0.623	0.298	0.473	0.294	0.618	0.307
Coeff. of Var. (%)	10.910	4.300	9.760	4.570	10.040	5.890	10.080	4.920
Range	2.200	0.790	2.145	0.840	1.900	1.065	2.225	0.935
$3s$	1.653	0.681	1.869	0.894	1.419	0.882	1.854	0.921

[a] Drying period of 16 hours.

TABLE 8.—*Summary of nitrogen procedures used*

COLLABORATOR NO.	METHOD	SAMPLE SIZE	DIGESTION			DISTILLATION	
			DIGESTION MIXTURE	H_2SO_4	DIGESTION TIME	ACID	INDICATOR
		grams		*ml*	*min.*		
1	Modified A.O.A.C. Proc. **2.23**	1.0	K_2SO_4+$CuSO_4$ (D.C. Tab)	25	150	*N*/7 HCl	Methyl Purple
2	Modified U.S.P.	0.3	K_2SO_4+$CuSO_4$	35	Clear+20 min.	*N*/10 H_2SO_4	Methyl Purple
3	Modified A.O.A.C. Proc. **2.24**	0.5	K_2SO_4+$CuSO_4$	25	50	*N*/10 HCl	Methyl Red
4	Modified U.S.P.	*ca* 0.7	K_2SO_4+HgO+H_2SeO_3 (D.C. Tab)	25	60	*N*/10 H_2SO_4	Methyl Red
5	Modified A.O.A.C. Proc. **2.24**	*ca* 0.8	K_2SO_4+Hg	25	120	0.093 *N* Acid	Methyl Red
6	Modified A.S.B.C.	1.0	Na_2SO_4+$CuSO_4$+$SeOCl_2$	25	Clear+20 min.	*N*/10 H_2SO_4	Methyl Red
7	A.O.A.C. Proc. **2.24**						
8	Micro-Kjeldahl (3)	0.1	K_2SO_4+$CuSO_4$	4	Clear+20 min.	2% Boric Acid	Methyl Red+Brom Cresol Green
9	U.S.P.	1.0	K_2SO_4+$CuSO_4$	20	Clear+30 min.	0.5*N*	Methyl Red
10	A.O.A.C. Proc. **2.23**	*ca* 0.7	K_2SO_4+$CuSO_4$	20	120	*ca* 0.3*N* H_2SO_4	Methyl Red
11	Semi-micro Kjeldahl (4)	0.03	Na_2SO_4+$CuSeO_3$	2	Clear+30 min.	*N*/50 H_2SO_4	Brom Cresol Green
12	Modified U.S.P.	0.3	K_2SO_4+$CuSO_4$	35	Clear+20 min.	*N*/10 H_2SO_4	Methyl Purple
13	Modified Kjeldahl	0.6	Na_2SO_4+Hengar Granule	20	30	Boric Acid	Methyl Purple
14	Modified Kjeldahl	0.7	Na_2SO_4+$CuSO_4$	25	Clear	*N*/10 H_2SO_4	Methyl Red
15	Modified Kjeldahl	0.8	K_2SO_4+Hengar Granule	25			
16	Modified A.O.A.C. Proc. **2.23**	1.0	Na_2SO_4+$CuSO_4$	20	100	*N*/10 H_2SO_4	

TABLE 9.—*Average values and statistical analysis of total nitrogen data submitted by collaborators*

COLLABORATOR NO.	TOTAL NITROGEN—DRY BASIS, %			
	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3	SAMPLE NO. 4
1	8.230	7.480	8.430	9.465
2	7.830	7.150	8.050	9.425
3	8.110[a]	7.216[a]	8.310[e]	9.480[b]
4	8.170	7.320	8.285	9.460
5	7.820[b]	6.980[b]	8.090[a]	9.213[a]
6	8.070	7.220	7.990	9.405
7	7.693[a]	6.483	7.933[a]	8.266[a]
8	8.120	7.330	8.305	9.380
9	8.068[c]	7.133[c]	8.241[c]	9.218[c]
10	7.993[d]	7.177[d]	8.290[d]	9.337[d]
10	8.107[d]	7.283[d]	8.320[d]	9.410[d]
11	7.210	6.790	8.470	8.195
12	7.910	7.180	8.100	9.490
13	8.010	7.245	8.380	9.645
14	8.200	7.335	8.440	9.735
15	7.765	7.010	8.010	9.180
16	7.980	7.280	8.215	9.540
Mean ($\bar{x}$)	7.958	7.154	8.227	9.285
Std. Dev. (s)	0.248	0.235	0.169	0.422
Coeff. of Var. (%)	3.00	3.28	2.05	4.54
Range	1.020	0.997	0.437	1.540

[a] Three values.
[b] Four values.
[c] One value.
[d] Two analysts; three values.
[e] Five values.

methods used, as well as in the values reported.

Examination of Table 11 shows that Collaborators Nos. 4, 7, 9, and 15 obtained values considerably lower than those reported by the rest. It was noted that Collaborators Nos. 4 and 15 used a dry-ashing procedure, where the samples were ashed above 500°C. without the addition of magnesium nitrate, and collaborators Nos. 7 and 9 used a wet-ashing procedure with an HCl–HNO_3 mixture.

In the P_2O_5 determination, it is essential that all of the phosphorus in yeast, which is predominantly organic, must be converted to orthophosphate before it can be determined by the usual methods. When yeast is dry-ashed at high temperatures (500°C. or above) in the presence of $Mg(NO_3)_2$, pyrophosphoric acid is formed, but can be converted to the ortho form by hydrolysis in weak acid. Collaborator No. 3, who performed the dry-ashing at 575°C. without the addition of Mg $(NO_3)_2$, first heated the sample in strong acid and then in dilute acid, thereby converting the pyrophosphate to the ortho form.

TABLE 10.—*Summary of P_2O_5 methods used*

COLLABORATOR NO.	METHOD	SAMPLE SIZE (*grams*)	ASHING PROCEDURE		DETERMINATION	
			TYPE OF ASHING	ASHING MEDIUM	TYPE OF DETERMINATION	DETERMINED AS
1	Modified A.O.A.C. Proc. 2.8	2.0	Wet	H_2SO_4–HNO_3	Gravimetric	$Mg_2P_2O_7$
2	Modified A.O.A.C. Proc. 2.13	0.2	Dry (525°C)	$Mg(NO_3)_2$	Titrimetric	$(NH_4)_3PO_4 \cdot 12MoO_3$
3	Modified A.O.A.C. Proc. 2.39, 2.40	2–3	Dry (575°C)	None	Colorimetric	Molbydenum Blue
4	Modified A.O.A.C. Proc. 2.13	4	Dry (500°C)	None	Titrimetric	$(NH_4)_3PO_4 \cdot 12MoO_3$
5	Modified A.O.A.C. Proc. 2.8 to 2.10	ca 2	Wet	H_2SO_4–HNO_3	Gravimetric	$Mg_2P_2O_7$
6	Modified A.O.A.C. Proc. 2.13	0.8	Dry (500°C)	$Mg(NO_3)_2$	Titrimetric	$(NH_4)_3PO_4 \cdot 12MoO_3$
7	A.O.A.C. Proc. 2.8, 2.9, 2.10, 2.11, 2.12, 2.13		Wet	HCl–HNO_3	Gravimetric Titrimetric	$Mg_2P_2O_7$ $(NH_4)_3PO_4 \cdot 12MoO_3$
8	Micro-Method (2)	0.1	Wet	H_2SO_4–H_2O_2	Colorimetric	Molybdenum Blue
9	A.O.A.C. Proc. 2.8, 2.9, 2.10		Wet	HCl–HNO_3	Titrimetric	$(NH_4)_3PO_4 \cdot 12MoO_3$
10	Micro-Method	ca 0.125	Wet	H_2SO_4–HNO_3	Colorimetric	Molybdivanado-phosphoric acid
11	A.O.A.C. Proc. 2.8, 2.9, 2.10	2	Wet	H_2SO_4–HNO_3	Gravimetric	$Mg_2P_2O_7$
12	Modified A.O.A.C. Proc. 2.13	0.2	Dry (525°C)	$Mg(NO_3)_2$	Titrimetric	$(NH_4)_3PO_4 \cdot 12MoO_3$
13	Micro-Method (2)	0.01	Wet	H_2SO_4–$HClO_4$	Colorimetric	Molbydenum Blue
14	Modified A.O.A.C. Proc. 2.13	1.0	Dry (482°C)	None	Volumetric	$(NH_4)_3PO_4 \cdot 12MoO_3$
15	Modified A.O.A.C. Proc. 2.13	ca 1.0	Dry (621°C)	None	Gravimetric	$(NH_4)_3PO_4 \cdot 12MoO_3$
16	A.O.A.C. Proc. 2.8, 2.9 (d) and 2.10	2.0	Wet	H_2SO_4–$NaNO_3$	Gravimetric	$Mg_2P_2O_7$

TABLE 11.—*Average values and statistical analysis of P_2O_5 data submitted by collaborators*

COLLABORATOR NO.	P_2O_5—DRY BASIS, %			
	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3	SAMPLE NO. 4
1	2.610	4.325	4.190	4.600
2	2.730	4.335	4.080	4.500
3	2.410	4.320	4.170	4.480
4	1.375	1.870	4.190	4.260
5	2.650	4.350	4.455	4.550
6	2.405	4.365	4.265	4.460
7	1.930[a]	1.995[a]	1.890[a]	2.125[a]
7	1.635[b]	1.840[b]	1.640[b]	1.800[b]
8	2.400	4.520	4.365	4.670
9	0.730[c]	1.422[c]	1.351[c]	1.890[c]
10	2.680[d]	4.587[d]	4.423[d]	4.707[d]
10	2.600[d]	4.550[d]	4.363[d]	4.607[d]
11	2.280	4.195	4.300	3.510
12	2.380	4.385	4.135	4.515
13	2.425	4.360	4.135	4.745
14	2.520	4.480	4.255	4.610
15	1.975	3.560	3.380	3.835
16	2.435	4.180	4.085	4.410
Mean ($\bar{x}$)	2.232	3.752	3.760	4.015
Std. Dev. (s)	0.524	1.133	1.012	1.005
Coeff. of Var. (%)	23.49	30.20	26.93	25.03
Range	1.998	3.165	3.033	2.855

[a] Gravimetric.
[b] Titrimetric.
[c] Single value.
[d] Two analysts—three values.

In wet-ashing procedures, it is necessary that the temperature of the digest be high enough not only to destroy the organic matter but also to convert organic phosphorus to the inorganic ortho form. The temperature of digests containing HCl–HNO_3 mixtures is only slightly over 100°C., while that of digests containing H_2SO_4 is over 300°C. It is very doubtful that all of the organic phosphorus can be converted to the inorganic ortho form when mixtures of HCl–HNO_3 are used.

A summary of collaborative data, excluding values from Collaborators Nos. 4, 7, 9, and 15, is shown in Table 12. The coefficients of variation are 5.56 per cent, 2.83 per cent, 3.01 per cent, and 6.93 per cent for Samples Nos. 1 to 4, respectively, and definitely indicate that the methods used by Collaborators Nos. 4, 7, 9, and 15 are not applicable to yeast. Again Sample No. 4 showed the greatest variation.

CONCLUSIONS

Collaborative studies of methods for the determination of moisture in yeast have shown that extreme care must be taken in the preparation of

TABLE 12.—*Average values and statistical analysis of P_2O_5 data submitted by collaborators*[a]

COLLABORATOR NO.	P_2O_5—DRY BASIS, %			
	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3	SAMPLE NO. 4
1	2.610	4.325	4.190	4.600
2	2.730	4.335	4.080	4.500
3	2.410	4.320	4.170	4.480
5	2.650	4.350	4.455	4.550
6	2.405	4.365	4.265	4.460
8	2.400	4.520	4.365	4.670
10	2.680	4.587	4.423	4.707
10	2.600	4.550	4.363	4.607
11	2.280	4.195	4.300	3.510
12	2.380	4.385	4.135	4.515
13	2.425	4.360	4.135	4.745
14	2.520	4.480	4.255	4.610
16	2.435	4.180	4.085	4.410
Mean ($\overline{X}$)	2.502	4.381	4.248	4.490
Std. Dev. (s)	0.139	0.124	0.128	0.311
Coeff. of Var. (%)	5.56	2.83	3.01	6.93
Range	0.450	0.407	0.343	1.235

[a] Excluding data reported by collaborators 4, 7, 9 and 15.

the samples and in the storage of the samples during the study if reliable data is to be obtained in evaluating moisture methods. Since some types of dried yeast are very hydroscopic, they must be protected from changes in moisture content due to varying humidity.

Collaborative studies have indicated that drying yeast at 100°C. in an air oven for five hours will give reproducible results with a minimum of variance. However, more work needs to be done with this particular procedure. Evidence pointing to a shorter drying period in a vacuum oven procedure should be studied.

The Kjeldahl procedure appears to be the most logical method for the determination of nitrogen in yeast. Further collaborative studies should be carried out to make the method or methods conform to A.O.A.C. procedures **2.22, 2.23,** or **2.24** (5).

Collaborative studies have indicated that both dry-ashing and wet-ashing procedures are applicable to the determination of P_2O_5 in yeast. The dry-ashing procedure should include the addition of $Mg(NO_3)_2$ and the wet-ashing procedure should include the use of sulfuric acid.

COLLABORATORS

1. LaMar N. BeMiller, Mead Johnson and Co., Evansville, Ind.
2. Stanley A. Bobrowski, Jr., Yeast Plant #2, Anheuser-Busch, Inc., Old Bridge, N. J.

3. E. F. Budde, Quaker Oats Co., Research Laboratories, Chicago, Ill.
4. H. Delo, Gerber Products Co., Fremont, Mich.
5. J. Fetkovich, H. J. Heinz Co., Pittsburgh, Pa.
6. L. S. Gamer, Olympia Brewing Co., Olympia, Wash.
7. F. W. Handelong, National Biscuit Co., New York, N. Y.
8. H. A. Jett and H. J. Buehler, Central Research Division, Anheuser-Busch, Inc., St. Louis, Mo.
9. E. O. Krueger, Abbott Laboratories, North Chicago, Ill.
10. W. E. Maynard, The Fleischmann Laboratories, New York, N. Y.
11. D. A. Overbye, Brooklyn Quality Control Laboratories, E. R. Squibb and Sons, Brooklyn, N. Y.
12. G. E. Reinhardt, Yeast Plant #1, Anheuser-Busch, Inc., St. Louis, Mo.
13. F. J. Rudert, Red Star Yeast and Products Co., Milwaukee, Wis.
14. A. Sigal, Pabst Brewing Co., Wilwaukee, Wis.
15. E. K. Spotts, Ward Baking Co., Bronx, N. Y.
16. Miss E. Stegemeyer, Laboratories Division, The Kroger Food Foundation, Cincinnati, Ohio.

ACKNOWLEDGMENT

The Associate Referee wishes to thank the above collaborators for their excellent cooperation and assistance in this study and Miss Dorothy Albright for her technical assistance and help in preparing the samples.

RECOMMENDATIONS

It is recommended*—

(1) That collaborative studies of methods for the determination of moisture in yeast be continued and that both vacuum-oven and air-oven procedures be studied.

(2) That collaborative studies of methods for the determination of nitrogen in yeast by the Kjeldahl method be continued.

(3) That collaborative studies of methods for the determination of P_2O_5 in yeast be continued, and that a dry-ashing procedure using $Mg(NO_3)_2$ and a wet-ashing procedure using H_2SO_4 be studied.

(4) That collaborative studies of methods for the determination of ash, ether extract, crude fiber, and crude carbohydrate in yeast be postponed until the studies on moisture, total nitrogen, and P_2O_5 are completed.

REFERENCES

(1) BRANDON, A. L., *This Journal*, **36**, 781 (1953).
(2) FISKE, C. H., and SUBBAROW, Y., *J, Biol. Chem.*, **66**, 375 (1925).
(3) MA, T. S., and ZUAZAGA, G., *Ind. Eng. Chem. Anal. Ed.*, **14**, 280 (1942).
(4) MARKHAM, R., *J. Biol. Chem.*, **36**, 790 (1942).
(5) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chem-

* For report of Subcommittee D and action of the Association, see *This Journal*, **37**, 75 (1954).

ists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950, p. 345, (**2.22, 2.23,** and **2.24**).

No reports were given on sugars in baked products, milk solids and butterfat in bread, or moisture.

REPORT ON GELATIN, DESSERT PREPARATIONS, AND MIXES

By S. C. Rowe (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.), *Referee*

Last year the General Referee recommended (1) that work on methods for sucrose and dextrose, **21.13, 21.14,** and **21.15,** be continued; and (2) that the first action methods for jelly strength, **21.6** and **21.12,** be made official. These recommendations were approved by Committee C.

No report was received this year from the Associate Referee. It is recommended* that final action on methods under **21.13, 21.14,** and **21.15** not be taken until further work is done to evaluate them.

REPORT ON STANDARDIZATION OF MICRO-CHEMICAL METHODS

By C. L. Ogg (Eastern Utilization Research Branch,† Philadelphia 18, Pennsylvania), *Referee*

This report gives the recommendations of the Associate Referee and Referee based on collaborative studies of micromethods of analysis for alkoxyl and acetyl groups, and for nitrogen by the Dumas method. The studies on methods for alkoxyl and acetyl groups were designed to determine which of the currently used micromethods gives more accurate and precise results and also to determine the effects of variations in apparatus and procedure on the results obtained by each method. The results of the study are being made available to the Committee on Standardization of Microchemical Apparatus which functions under the American Chemical Society's Analytical Division so that this committee can recommend specifications for the most promising apparatus. A tentative Dumas

* For report of Subcommittee C and action of the Association, see *This Journal*, 37, 70 (1954).

† One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Service, U. S. Department of Agriculture.

method, based on the results of last year's study of the method, was tested collaboratively, and in addition more information was obtained on the newer, more rapid modifications of the Dumas method.

From a statistical analysis of the data obtained in these studies, it is recommended—*

(1) That additional collaborative work be done to evaluate the methods for determining acetyl groups.

(2) That additional alkoxyl samples be submitted to the collaborators for analysis to determine whether or not the results on methyl and ethyl esters will be similar to those obtained on the methyl and ethyl ethers.

(3) That, if the results with the esters are in accord with the findings on the ethers, a procedure be written for the Clark alkoxyl method and submitted for collaborative study.

(4) That the tentative Dumas method be revised in an attempt to improve its inter-laboratory precision and that the revised method be tested collaboratively.

(5) That a rapid method such as, or similar to, the Shelberg or Zimmermann procedures be submitted for collaborative study.

(6) That work to develop a Kjeldahl procedure for the analysis of materials containing N-N and N-O groups be discontinued until the studies on the Dumas method show whether or not there is urgent need for such a method.

(7) That, since the Carius method for chlorine and bromine has been adopted, first action, further studies of the catalytic combustion method be discontinued until methods have been developed for the more common elements and groups for which no standard microchemical methods exist.

(8) That further collaborative work be done to develop a gravimetric method for sulfur because the present volumetric method cannot be used to analyze materials containing phosphorus.

REPORT ON MICROANALYTICAL DETERMINATION OF ACETYL AND ALKOXYL GROUPS

By A. STEYERMARK, *Associate Referee*, and ELEANOR E. LOESCHAUER
(Hoffmann–La Roche Inc., Nutley, N. J.)

Questionnaires sent to the collaborators of previous studies (5–7, 16–18) indicated that the majority wished next to study the acetyl and alkoxyl determinations. This coincided with the plans of the Committee for the Standardization of Microchemical Apparatus of the Division of Analytical Chemistry, American Chemical Society, which will eventually

* For report of Subcommittee C and action of the Association, see *This Journal*, 37, 73 (1954).

recommend specifications for the apparatus used (2, 10, 12–15). Consequently, this year it was decided by the Referees to begin the study of these determinations.

For the acetyl determination, two samples[1] were selected: acetanilid and β-dextrose pentaacetate, which were representative of compounds in which the acetyl groups are attached to nitrogen and oxygen atoms, respectively. For the alkoxyl determination, the two samples selected were *p*-ethoxybenzoic acid and vanillin, for the determination of ethoxyl and methoxyl groups, respectively. In both of these compounds, there are ether linkages. Ethoxyl and methoxyl determinations on compounds in which the groups are present in the form of esters were not included in this report because the Referees felt that this would require too much work by the collaborators. Consequently, two additional samples, in which the ethoxyl and methoxyl groups are present in the form of esters, will be sent out at a later date and constitute the second part of the work on the alkoxyl determination. As with previous studies, for the preliminary work the collaborators were asked to do the determinations in whatever manner they wished and to fill in questionnaires giving in detail the apparatus and procedures involved. In this way, not only may different methods be compared, but differences in the apparatus and procedures of one method can likewise be observed.

ACETYL DETERMINATION

N-Acetyl values were obtained by the collaborators who used the methods of Roth–Kuhn (4, 8), Elek and Harte (4, 11), and Clark (3). O-Acetyl values were obtained by the collaborators with the three methods mentioned above as well as that of Alicino (1).

N-Acetyl.—Acetanilid was analyzed by four collaborators who used the Roth-Kuhn method and reported a total of 17 values; two collaborators who used the Clark method and reported a total of 8 values; and six collaborators who used the Elek and Harte method and reported a total of 24 values. These results are shown in Table 1. In all tables, n is the number of values reported by each collaborator, $\bar{x}$ is the mean of his data, and s is the standard deviation of his data which is calculated by the formula:

$$s = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}},$$

[1] The samples submitted were: Acetanilid, β-dextrose pentaacetate, *p*-ethoxybenzoic acid, and vanillin. Before being sent to the various collaborators, these samples were analyzed with the following results:
Acetanilid: (C_8H_9ON, U.S.P. Powder, Merck & Co., Inc.): Calcd: C, 71.09; H, 6.70; N, 10.36. Found: C, 71.02, 71.01, 70.96, 71.32; H, 6.64, 6.64, 6.45, 6.66; N, 10.52.
β-Dextrose pentaacetate ($C_{18}H_{22}O_{11}$, Eastman Kodak Co.): Calcd: C, 49.23; H, 5.68. Found: C, 49.46, 49.49, 49.67, 49.19; H, 5.57, 5.87, 5.38, 5.38.
p-Ethoxybenzoic acid ($C_9H_{10}O_3$), Eastman Kodak Co. twice recrystallized from ethanol: Calcd: C, 65.05; H, 6.07. Found: C, 65.00, 65.06; H, 5.98. 6.20.
Vanillin ($C_8H_8O_3$, U.S.P., Merck & Co., Inc.): Calcd: C, 63.15; H, 5.30. Found: C, 63.44, 63.17, 63.37, 63.32; H, 5.63, 4.94, 5.54, 5.50.

where x is the individual value. Also given in the tables are the values for $\bar{x}-theory$, $\bar{\bar{x}}$ which is the mean of the $\bar{x}$'s, $\bar{\bar{x}}-theory$, $\bar{s}$ which is the mean of the s's, and $s_{\bar{x}}$ which is the standard deviation of the $\bar{x}$'s. Table 1 shows the values obtained by the Roth-Kuhn method. For this method, $\bar{\bar{x}}=31.02$, $\bar{s}=0.241$, $\bar{\bar{x}}-theory=-0.83$, and $s_{\bar{x}}=1.590$.

Table 1 also shows the results obtained by the six collaborators who analyzed acetanilid by the method of Elek and Harte. For this method, $\bar{\bar{x}}=32.16$, $\bar{s}=0.279$, $\bar{\bar{x}}-theory=+0.31$, and $s_{\bar{x}}=1.022$.

Table 1 further gives the results obtained by the two collaborators who analyzed acetanilid by the method of Clark. This work gave $\bar{\bar{x}}=31.60$, $\bar{s}=0.080$, $\bar{\bar{x}}-theory=-0.25$, and $s_{\bar{x}}=0.226$.

Wherever possible, comparisons were made, and in all these cases the Student's t values were calculated, using the formula:

$$t = (\bar{x}_a - \bar{x}_b)\sqrt{\frac{n_a n_b(n_a + n_b - 2)}{(n_a + n_b)[\Sigma(x_a - \bar{x}_a)^2 + \Sigma(x_b - \bar{x}_b)^2]}}$$

where n_a and n_b are the number of values in groups a and b respectively, x_a and x_b are individual values for the two groups, and $\bar{x}_a$ and $\bar{x}_b$ are the means of the values for the two groups. If the calculated t value was greater than the critical value ($t_{0.05}$)

TABLE 1.—*N-Acetyl; sample: acetanilid (31.85% N-acetyl)*

COLLABORATOR NO.	n	$\bar{x}$	s	$\bar{x}-Theory$	$\bar{\bar{x}}$	$\bar{\bar{x}}-Theory$	$\bar{s}$	$s_{\bar{x}}$
				Roth-Kuhn Method				
35	4	31.77	0.290	−0.08				
59	4	31.85	0.325	−0.00				
62	3	28.63	0.231	−3.22				
79	6	31.81	0.116	−0.04				
					31.02	−0.83	0.241	1.590
				Elek and Harte Method				
1	4	32.38	0.179	+0.53				
17	1	34.07	——	+2.22				
37	7	31.80	0.374	−0.05				
49	4	31.80	0.423	−0.05				
71	4	31.27	0.363	−0.58				
77	4	31.64	0.055	−0.21				
					32.16	+0.31	0.279	1.002
				Clark Method				
30	5	31.76	0.040	−0.09				
73	3	31.44	0.120	−0.41				
					31.60	−0.25	0.080	0.226

obtained from a table of Student's t's (9, 19), the difference between the two means was significant at the 5% level and the procedure whose mean was nearer the theoretical value was considered to be the better. If the t value was not greater than the critical value, the variance ratio, F, was calculated by the equation:

$$F = \frac{(s_{\bar{x}})_a^2}{(s_{\bar{x}})_b^2}$$

where $(s_{\bar{x}})_a^2$ is always the larger value. If the calculated F value was greater than the critical value ($F_{0.05}$) obtained from the table of F values (9, 19), the difference in precision between the two groups of data was significant at the 10% level and the procedure with the lower $s_{\bar{x}}$ was the more precise. The data are significant at the 10% level, and not at the 5% level, due to the fact that the numerator has been chosen.

It was felt that no comparisons should be made of the Clark method with others, inasmuch as only two collaborators used this procedure. A comparison was made between the Roth–Kuhn and Elek and Harte methods (see Table 5). Calculation of the Student's t value was not found to be critical, where $t = 1.402$ and $t_{0.05} = 2.306$. Calculation of the F value also was not found to be critical, where $F = 2.52$ and $F_{0.05} = 5.41$, showing that there is no choice between the two methods. Figure 1 is a plot of the values obtained using the Roth–Kuhn and the Elek and Harte methods, showing the distribution of the points.

Since no one method proved more accurate or precise than the others, an attempt was made to determine whether or not something in common among the procedures would prove to be significant. Table 2 lists variations that became apparent while studying the completed questionnaires returned by the collaborators. For example, alkali was used by some for the hydrolysis; others used p-toluene sulfonic acid, while some used other acids. Also, some used a standard alkali in the final titration while others used the iodometric procedure, titrating with thiosulfate. Some collaborators used samples weighing less than 10 mg while others worked with those of more than

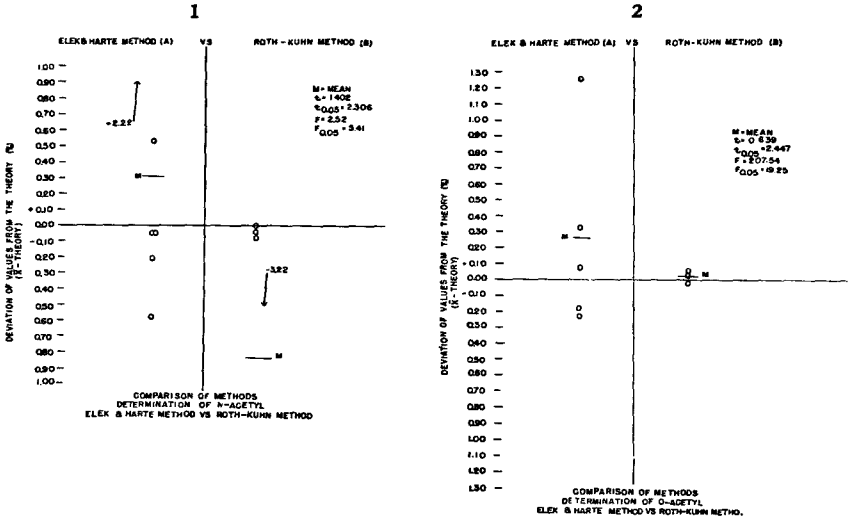


FIG. 1.—Comparison of methods in the determination of N-acetyl.
FIG. 2.—Comparison of methods in the determination of O-acetyl.

TABLE 2.—*Variations in acetyl determination*

VARIATION		COLLABORATOR NUMBER											
		1	17	30	35	37	49	59	62	71	73	77	79
Silver sulfate	Yes		*	*		*	*			*			
	No	*											
Hydrolyzing agent	*p*-Toluene sulfonic acid	*	*	*		*	*	*	*	*		*	*
	Other acid				*								*
	Alkali										*		
Iodine used to absorb acetic acid	Yes		*				*			*			
Standardized solution used	Thiosulfate		*				*			*		*	
	Alkali	*		*	*	*		*	*		*		*
Time of hydrolysis	Less than 1 hr				*			*					*
	More than 1 hr	*	*	*		*	*		*	*	*	*	
Sample weight Range	Less than 10 mg	*	*	*	*		*		*			*	
	More than 10 mg				*	*		*		*	*		*

TABLE 3.—*Effect of variations on accuracy and precision of the N-acetyl determination; sample: acetanilid (31.85% N-acetyl)*

VARIATION		NUMBER OF COLLABORATORS	$\bar{\bar{x}}$	$\Sigma(\bar{x}-\bar{\bar{x}})^2$	$(s_{\bar{x}})^2$	t	$t_{0.05}$	F	$F_{0.05}$
Titrating agent	Standard alkali	8	31.43	9.4248	1.346	1.053	2.228	1.20	4.35
	Sodium thiosulfate	4	32.20	4.8354	1.612				
Sample weight range	Less than 10 mg	7	31.72	15.5231	2.587	0.090	2.201	44.45	4.95
	More than 10 mg	6	31.66	0.2908	0.0582				

10 mg. However, no attempt was made to compare the results obtained unless four or more collaborators were involved in each of the variations. Table 3 shows the comparisons made. In this table, the symbols used are the same as described above. It can be seen that there is no choice in using standard alkali or thiosulfate, since calculation of the t value was not found to be critical; $t=1.053$ and $t_{0.05}=2.228$. Also, calculation of the F value was not found to be critical ($F=1.20$ and $F_{0.05}=4.35$). Comparing the results for sample size, sample less than 10 mg versus sample greater than 10 mg, calculation of the t value was not found to be critical ($t=0.090$ and $t_{0.05}=2.201$). Calculation of the F value was found to be critical ($F=44.45$ and $F_{0.05}=4.95$) and in favor of the sample greater than 10 mg. (The opposite will be seen to be true later in connection with the O-acetyl.)

O-Acetyl.—β-Dextrose pentaacetate was analyzed by eleven collaborators who used the various methods, namely the Roth–Kuhn (4, 8), Elek and Harte (4, 11), Clark (3), and Alicino (1) methods. Three collaborators analyzed the sample by the Roth–Kuhn method doing a total of 14 analyses. Table 4 shows the results obtained by this method: $\bar{\bar{x}}=55.15$, $\bar{\bar{x}}-theory=+0.02$, $\bar{s}=0.220$, and $s_{\bar{x}}=0.042$. Five

TABLE 4.—*O-Acetyl; sample: β-dextrose pentaacetate (55.13% O-acetyl)*

COLLABORATOR NO.	n	$\bar{x}$	s	$\bar{x}-Theory$	$\bar{\bar{x}}$	$\bar{\bar{x}}-Theory$	$\bar{s}$	$s_{\bar{x}}$
				Roth-Kuhn Method				
35	4	55.10	0.395	−0.03				
59	4	55.16	0.188	+0.03				
79	6	55.18	0.078	+0.05				
					55.15	+0.02	0.220	0.042
				Elek and Harte Method				
17	8	56.38	2.587	+1.25				
37	6	54.95	0.584	−0.18				
49	4	55.20	0.119	+0.07				
71	4	55.45	0.520	+0.32				
77	5	54.90	0.194	−0.23				
					55.38	+0.25	0.801	0.603
				Clark Method				
30	4	55.00	0.147	−0.13				
73	4	63.83	0.980	+8.70				
					59.42	+4.29	0.564	6.243
				Alicino Method				
1	4	55.45	0.198	+0.32				

collaborators analyzed the sample by the Elek and Harte procedure, reporting a total of 27 analyses. Table 4 also gives the results: $\bar{\bar{x}} = 55.38$, $\bar{\bar{x}} - theory = +0.25$, $\bar{s} = 0.801$, and $s_{\bar{x}} = 0.603$. In addition, Table 4 shows the results obtained by the two collaborators who used the Clark method and reported a total of 8 determinations. These were $\bar{\bar{x}} = 59.42$, $\bar{\bar{x}} - theory = +4.29$, $\bar{s} = 0.564$, and $s_{\bar{x}} = 6.243$. One collaborator used the Alicino method, reporting 4 determinations, the results of which are given in Table 4. Not enough collaborators used the various methods to give sufficient data to make a comparison of them. Even so, such a comparison was made for the Roth–Kuhn method versus the Elek and Harte method. Table 5 shows the data obtained in this comparison. Calculation of the t value was not found to be critical ($t = 0.639$ and $t_{0.05} = 2.447$). Calculation of the F value was found to be critical ($F = 207.54$ and $F_{0.05} = 19.25$) and in favor of the Roth–Kuhn method. This would indicate that the Roth–Kuhn method is more precise at the 10% level, but should not be stressed. Figure 2 is a plot of the data obtained with these two methods.

An attempt was made to determine whether or not some part of the procedures would show a significant difference for the O-acetyl determination. The same comparisons were made that were done for the N-acetyl. Table 6 gives the results. Comparing the use of standard alkali in the titration of the acetic acid obtained to the use of the iodometric method and titrating with standard thiosulfate, calculation of the t value was not found to be critical ($t = 0.530$ and $t_{0.05} = 2.262$). Calculation of the F value was found to be critical ($F = 30.43$ and $F_{0.05} = 8.94$) and in favor of the use of the iodometric procedure. (With N-acetyl, there was no choice.) A comparison was made regarding sample size, sample of less than 10 mg versus sample greater than 10 mg. Calculation of the t value was not found to be critical ($t = 0.869$ and $t_{0.05} = 2.228$). Calculation of the F value was found to be critical ($F = 42.33$ and $F_{0.05} = 5.05$) and in favor of samples of less than 10 mg. This is exactly the opposite of what was true for the N-acetyl.

ALKOXYL DETERMINATION

Ethoxyl determinations were made by the methods of Elek (4, 11), Clark (3), Pregl (8), and Steyermark (11). The same methods were used for the determination of methoxyl as listed above for the determination of ethoxyl.

Ethoxyl.—Twelve collaborators analyzed *p*-ethoxybenzoic acid by the Elek method, making a total of 63 determinations. The results of these, shown in Table 7, were $\bar{\bar{x}} = 27.12$, $\bar{\bar{x}} - theory = 0.00$, $\bar{s} = 0.156$, and $s_{\bar{x}} = 0.187$. Six collaborators used the Clark method, reporting a total of 32 determinations. The results, shown in Table 7, were $\bar{\bar{x}} = 27.17$, $\bar{\bar{x}} - theory = +0.05$, $\bar{s} = 0.074$, and $s_{\bar{x}} = 0.079$. Two persons analyzed the sample by the Pregl method, reporting a total of 16 determinations, 10 of which were done by the volumetric procedure and the other 6 by the gravimetric (see Table 7). For these results, $\bar{\bar{x}} = 26.87$, $\bar{\bar{x}} - theory = -0.25$, $\bar{s} = 0.248$, and $s_{\bar{x}} = 0.149$. One person analyzed the sample by the Steyermark method, making a total of 12 determinations. The results of these are shown in Table 7. Because of the small number of collaborators involved in the Pregl and Steyermark methods, no comparisons involving these procedures were made. Comparison between the Clark and the Elek methods was possible, the results of which are shown in Table 5, and the individual points are plotted as shown in Figure 3. Calculation of the t value was not found to be critical ($t = 0.620$ and $t_{0.05} = 2.120$). However, calculation of the F value was found to be critical and in favor of the Clark method ($F = 5.59$ and $F_{0.05} = 4.70$).

TABLE 5.—*Comparison of methods*

DETERMINATION OF:	COMPARISON OF:	NO. OF COLLABORATORS	$\bar{\bar{x}}$	$\Sigma(\bar{x}-\bar{\bar{x}})^2$	$(s_{\bar{x}})^2$	t	$t_{0.05}$	F	$F_{0.05}$
N-Acetyl (Acetanilid) Theory = 31.85%	Elek and Harte	6	32.16	5.0182	1.0036	1.402	2.306	2.52	5.41
	Roth–Kuhn	4	31.02	7.5876	2.5292				
O-Acetyl (β-Dextrose Pentaacetate) Theory = 55.13%)	Elek and Harte	5	55.38	1.4526	0.3632	0.639	2.447	207.54	19.25
	Roth–Kuhn	3	55.15	0.0035	0.00175				
Ethoxyl (*p*-Ethoxybenzoic Acid) Theory = 27.12%	Clark	6	27.17	0.0313	0.00626	0.620	2.120	5.59	4.70
	Elek	12	27.12	0.3851	0.0350				
Methoxyl (Vanillin) Theory = 20.40%	Clark	7	20.30	0.0407	0.0068	0.380	2.101	10.62	4.00
	Elek	13	20.26	0.8662	0.0722				

TABLE 6.—*Effect of variations on accuracy and precision of the O-acetyl determination*

VARIATION		NO. OF COLLABORATORS	$\bar{\bar{x}}$	$\Sigma(\bar{x}-\bar{\bar{x}})^2$	$(s_{\bar{x}})^2$	t	$t_{0.05}$	F	$F_{0.05}$
Titrating agent	Standard alkali	7	56.38	64.8835	10.81	0.530	2.262	30.43	8.94
	Sodium thiosulfate	4	55.48	1.0657	0.3552				
Sample weight range	Less than 10 mg	6	55.34	1.4801	0.2960	0.869	2.228	42.33	5.05
	More than 10 mg	6	56.61	62.6571	12.53				

TABLE 7.—*Ethoxyl; sample: p-ethoxybenzoic acid (27.12% ethoxyl)*

COLLABORATOR NO.	n	$\bar{x}$	s	$\bar{x}$—*Theory*	$\bar{\bar{x}}$	$\bar{\bar{x}}$—*Theory*	$\bar{s}$	$s_{\bar{x}}$
				Elek Method				
1	4	27.18	0.166	+0.06				
17	8	26.78	0.159	−0.34				
29	4	27.43	0.106	+0.31				
30	4	27.10	0.106	−0.02				
40	4	27.13	0.101	+0.01				
46	5	27.12	0.159	0.00				
60	8	27.08	0.100	−0.04				
71	4	27.40	0.136	+0.28				
73	6	26.96	0.304	−0.16				
74	5	27.18	0.240	+0.06				
77	5	26.88	0.131	−0.24				
79	6	27.17	0.169	+0.05				
					27.12	0.00	0.156	0.187
				Clark Method				
2	4	27.03	0.050	−0.09				
8	4	27.19	0.080	+0.07				
37	4	27.19	0.127	+0.07				
45	8	27.14	0.082	+0.02				
50	8	27.25	0.057	+0.13				
57	4	27.23	0.050	+0.11				
					27.17	+0.05	0.074	0.079
				Pregl Method				
35 (Vol.)	10	26.76	0.321	−0.36				
59 (Grav.)	6	26.97	0.175	−0.15				
					26.87	−0.25	0.248	0.149
				Steyermark Method				
49	12	27.70	0.347	+0.58				

An attempt was made to determine whether or not certain variations in the procedure would give significant results. Table 8 lists the variations which were noted from the questionnaires returned by the collaborators. Wherever possible, variations were compared, as for example: whether a water or an air condenser was used, whether or not propionic or acetic anhydride was used, etc. The results of these comparisons are shown in Table 9. Comparison was made between the results

TABLE 8.—*Variations in alkoxyl determination*

VARIATION		COLLABORATOR NUMBER																						
		1	2	8	17	27	29	30	35	37	40	45	46	49	50	57	59	60	62	71	73	74	77	79
Hydriodic acid	Pre-treated	*		*	*	*	*	*		*	*	*	*	*	*					*	*		*	
	Untreated								*							*			*			*		*
Rate of sweeping	2 or less bubbles/sec.	*	*	*	*	*		*	*		*		*	*	*		*	*	*		*	*	*	*
	More than 2 bubbles/sec.																							
Type of condenser	Water							*			*			*	*	*		*	*	*		*	*	*
	Air		*	*	*	*	*		*	*		*	*				*				*			
Sample weight range	Less than 10 mg.	*			*	*	*	*	*	*	*	*		*	*	*	*	*		*	*	*	*	*
	More than 10 mg.		*	*						*			*		*									
Gas used	Carbon dioxide	*		*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*
	Nitrogen		*			*																*		
Solvent includes:	Propionic anhydride	*			*			*	*		*		*	*			*					*	*	*
	Acetic anhydride						*											*	*		*			
	Neither		*	*		*				*		*			*	*				*				
Scrubber mixture contains:	Red phosphorus	*				*		*	*		*	*	*									*		
	$CdSO_4$–$Na_2S_2O_3$		*		*		*							*		*	*	*	*	*	*		*	*
	Other									*					*									
Tin foil	Yes													*										
	No	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*
Reaction mixture boiled	Less than 45 min.	*				*	*			*							*	*	*					
	More than 45 min.		*	*	*			*	*		*	*	*	*	*	*				*	*	*	*	*

TABLE 9.—*Effect of variations on accuracy and precision of the ethoxyl determination*

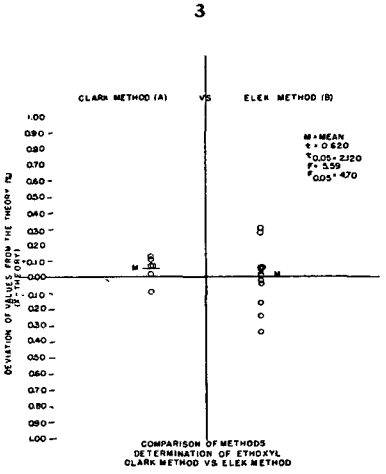
Sample: *p*-Ethoxybenzoic Acid (27.12% Ethoxyl)

VARIATION		NO. OF COLLABORATORS	$\bar{\bar{x}}$	$\Sigma(x-\bar{\bar{x}})^2$	$(s_{\bar{x}})^2$	t	$t_{0.05}$	F	$F_{0.05}$
Splitting agent—Hydriodic acid	Pre-treated	12	27.14	0.4005	0.0364	0.704	2.110	1.50	4.03
	Untreated	7	27.08	0.1459	0.0243				
Condenser	Air	10	27.06	0.3681	0.0409	1.598	2.101	1.15	3.18
	Water	10	27.21	0.4250	0.04722				
Sample weight range	Less than 10 mg	18	27.14	0.9003	0.05296	0.188	2.080	7.46	5.83
	More than 10 mg	5	27.16	0.0284	0.0071				
Solvent includes:	Propionic anhydride or acetic anhydride	14	27.10	0.7912	0.06086	1.011	2.093	4.80	3.98
	Neither	7	27.20	0.0761	0.01268				
Scrubber mixture contains:	$CdSO_4$–$Na_2S_2O_3$	11	27.16	0.7333	0.07333	0.989	2.131	2.95	4.74
	Red phosphorus (in aqueous $CdSO_4$ or other)	6	27.04	0.1244	0.02488				
Boiling time	Less than 45 minutes	5	27.17	0.1162	0.02905	0.357	2.093	1.82	5.86
	More than 45 minutes	16	27.13	0.7918	0.05279				

TABLE 10.—*Methoxyl sample: vanillin (20.40% methoxyl)*

COLLABORATOR NO.	n	$\bar{x}$	s	$\bar{x}$—*Theory*	$\bar{\bar{x}}$	$\bar{\bar{x}}$—*Theory*	$\bar{s}$	$s_{\bar{x}}$
				Elek Method				
1	4	20.42	0.387	+0.02				
17	4	20.36	0.095	−0.04				
29	4	20.45	0.198	+0.05				
30	4	20.58	0.164	+0.18				
40	4	20.32	0.032	−0.08				
46	4	20.45	0.101	+0.05				
60	5	20.25	0.091	−0.15				
62	2	19.75	0.212	−0.65				
71	4	20.25	0.573	−0.15				
73	6	20.02	0.330	−0.38				
74	5	20.51	0.445	+0.11				
77	6	20.22	0.112	−0.18				
79	6	19.74	1.093	−0.66				
					20.26	−0.14	0.295	0.269
				Clark Method				
2	4	20.35	0.057	−0.05				
8	4	20.28	0.068	−0.12				
27	5	20.33	0.081	−0.07				
37	4	20.30	0.064	−0.10				
45	8	20.34	0.044	−0.06				
50	8	20.38	0.061	−0.02				
57	4	20.13	0.126	−0.27				
					20.30	−0.10	0.072	0.082
				Pregl Method				
59	4	20.15	0.123	−0.25				
				Steyermark Method				
49	4	20.37	0.227	−0.03				

of those collaborators who used pre-treated or untreated hydriodic acid. Only the results for the volumetric procedure were used, since there is evidence which indicates that only the pre-treated acid should be used for the gravimetric determination (11). There is no difference in the accuracy or precision when either pre-treated or untreated acid is used, since calculation of the t and the F values were not found to be critical ($t=0.704$, $t_{0.05}=2.110$, $F=1.50$, and $F_{0.05}=4.03$). Likewise, there was no significant difference in the results when a water or an air condenser was used. Calculations show that $t=1.598$, $t_{0.05}=2.101$, $F=1.15$, and $F_{0.05}=3.18$. The results



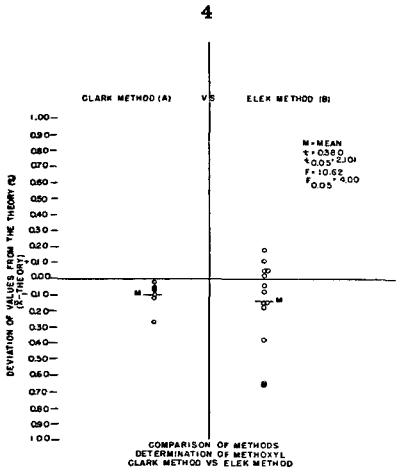


FIG. 3.—Comparison of methods in the determination of ethoxyl.
FIG. 4.—Comparison of methods in the determination of methoxyl.

obtained with samples of less than 10 mg were compared with those obtained with samples of greater than 10 mg. Calculation of the t value was not found to be critical ($t = 0.188$ and $t_{0.05} = 2.080$). However, calculation of the F value was found to be critical and in favor of a sample weight greater than 10 mg ($F = 7.46$ and $F_{0.05} = 5.83$). Too much stress should not be placed upon this, since a comparable condition did not exist with the methoxyl determination as will be shown later. Comparison of the results obtained when propionic or acetic anhydride was used as a solvent, and when neither was used, did not yield a t value which was critical ($t = 1.011$ and $t_{0.05} = 2.093$). Calculation of the F value did give a critical value and is in favor of the use of neither ($F = 4.80$ and $F_{0.05} = 3.98$). Some collaborators used a mixture of cadmium sulfate and sodium thiosulfate in the scrubber while others used an aqueous suspension of red phosphorus, some of which contained cadmium sulfate, and some did not. A comparison of the results of these variations did not yield critical values for t or F ($t = 0.989$, $t_{0.05} = 2.131$, $F = 2.95$, and $F_{0.05} = 4.74$). A comparison of the results obtained when the reaction mixture was boiled for less than 45 minutes and when the mixture was boiled for more than 45 minutes yielded no critical values for either t or F ($t = 0.357$, $t_{0.05} = 2.093$, $F = 1.82$, and $F_{0.05} = 5.86$).

For those variations where critical values of F were obtained, it cannot be determined whether it is the apparatus or the variations in the procedure that give the better results, since these variations are present in the Clark method.

Methoxyl.—Thirteen collaborators used the Elek method, reporting a total of 58 determinations. The results, shown in Table 10, were: $\bar{\bar{x}} = 20.26$, $\bar{\bar{x}} - theory = -0.14$, $\bar{s} = 0.295$, and $s_{\bar{x}} = 0.269$. Seven collaborators used the Clark method, reporting a total of 37 values. These results are also shown in Table 10: $\bar{\bar{x}} = 20.30$, $\bar{\bar{x}} - theory = -0.10$, $\bar{s} = 0.072$, and $s_{\bar{x}} = 0.082$. One collaborator used the Pregl method, reporting 4 values, shown in Table 10, and one collaborator used the Steyermark method, also reporting 4 values, shown in Table 10. Due to the fact that only one collaborator used the Pregl method and one the Steyermark, no comparisons of the data could be made. A comparison between the Clark and Elek methods was pos-

TABLE 11.—*Effect of variations on accuracy and precision of the methoxyl determination*

Sample: Vanillin (20.40% Methoxyl)

VARIATION		NO. OF COLLABORATORS	$\bar{\bar{x}}$	$\Sigma(\bar{x}-\bar{\bar{x}})^2$	$(s_{\bar{x}})^2$	t	$t_{0.05}$	F	$F_{0.05}$
Splitting agent—Hydriodic acid	Pre-treated	13	20.32	0.1564	0.01303	1.284	2.101	8.74	3.00
	Untreated	7	20.19	0.6834	0.1139				
Condenser	Air	10	20.30	0.1549	0.0172	0.741	2.093	4.26	3.13
	Water	11	20.23	0.7325	0.07325				
Sample weight range	Less than 10 mg	18	20.28	0.6280	0.03694	0.789	2.080	1.24	2.96
	More than 10 mg	5	20.35	0.0183	0.04575				
Solvent includes:	Propionic anhydride	10	20.31	0.5094	0.0566	1.258	2.179	1.61	3.86
	Acetic anhydride	4	20.12	0.2727	0.0909				
Solvent includes:	Propionic anhydride or Acetic anhydride	14	20.26	0.8903	0.06848	0.418	2.086	11.10	3.55
	Neither	8	20.30	0.0432	0.00617				
Scrubber mixture contains:	$CdSO_4$–$Na_2S_2O_3$	12	20.17	0.5781	0.05255	1.751	2.120	1.48	4.70
	Red Phosphorus (in aqueous $CdSO_4$ or other)	6	20.36	0.1770	0.0354				
Boiling time	Less than 45 min.	7	20.24	0.3365	0.0561	0.507	2.086	1.33	2.85
	More than 45 min.	15	20.29	0.5921	0.0423				

sible. Table 5 lists the results and Figure 4 is a plot of the individual values. Calculation of the t value was not found to be critical ($t=0.380$ and $t_{0.05}=2.101$). However, the F value was found to be critical and in favor of the Clark method ($F=10.62$ and $F_{0.05}=4.00$). Comparisons were made between the variations in procedure when possible. (See Table 8 in which the variations in the alkoxyl determination are shown.) Table 11 shows the data for the comparisons made. The data submitted by those who used pretreated hydriodic acid were compared with the data of those who used the untreated. Only volumetric results were used (see under *Ethoxyl* for the reason the gravimetric results are not included). Calculation of the t value was not found to be critical ($t=1.284$ and $t_{0.05}=2.101$). However, calculation of the F value was found to be critical and in favor of pre-treated hydriodic acid ($F=8.74$ and $F_{0.05}=3.00$), but it should be remembered that there was no difference for the ethoxyl. The results obtained when propionic anhydride was used as a solvent were compared to those obtained when acetic anhydride was used. Neither a critical t value nor a critical F value was obtained ($t=1.258$, $t_{0.05}=2.179$, $F=1.61$, and $F_{0.05}=3.86$). The comparison when propionic or acetic anhydride was used, and when neither was used, did not yield a t value which was critical ($t=0.418$ and $t_{0.05}=2.086$). However, the F value was found to be critical and in favor of the use of neither ($F=11.10$ and $F_{0.05}=3.55$). A comparison of the results for samples of less than 10 mg and samples of more than 10 mg did not yield critical values ($t=0.789$, $t_{0.05}=2.080$, $F=1.24$, and $F_{0.05}=2.96$). A study of the use of an air condenser compared to the use of a water condenser did not yield a critical t value ($t=0.741$ and $t_{0.05}=2.093$). However, the F value was found to be critical and in favor of the use of the air condenser ($F=4.26$ and $F_{0.05}=3.13$), although it will be remembered no difference was noted with the ethoxyl. The use of cadmium sulfate-sodium thiosulfate mixture in the scrubber, as compared to the aqueous suspension of red phosphorus, failed to yield critical values, $t=1.751$, $t_{0.05}=2.120$, $F=1.48$, and $F_{0.05}=4.70$. There was no significant difference when the reaction mixture was boiled for more than or less than 45 minutes, $t=0.507$, $t_{0.05}=2.086$, $F=1.33$, and $F_{0.05}=2.85$. It should be noted that in the methoxyl determination, as was the case in the ethoxyl determination, the variations which appear to give better results are the same as those found in the Clark method, so that it cannot be stated whether the improvement is due to these particular conditions or to the apparatus.

CONCLUSIONS

(1) *N-Acetyl.* There is no choice between the Roth–Kuhn or the Elek and Harte methods. Although comparisons were made regarding variations in procedure, the results were not in agreement with the same comparisons of the O-acetyl and consequently are not stressed.

(2) *O-Acetyl.* There was hardly sufficient data to compare methods, but a limited comparison indicated that the Roth–Kuhn method was more precise than the Elek and Harte procedure. This point, too, should not be stressed. Regarding comparison of the variations as stated above, the findings should not be stressed.

(3) *Ethoxyl.* (A) Clark method versus Elek method: Comparison did not yield a critical t value but did give a critical F value in favor of the Clark method. (B) The use of propionic or acetic anhydride versus the use of neither did not yield a t value which was critical. The calculated F value was critical and in favor of the use of neither. (C) Scrubber mix-

ture: The use of an aqueous suspension of red phosphorus versus the use of cadmium sulfate–sodium thiosulfate showed that neither the *t* nor the *F* values were critical. (D) Boiling time of reaction mixture: Boiled for less than 45 minutes versus boiled for more than 45 minutes. Neither the *t* nor the *F* values were critical.

It will be noted that the above critical variation is found in the Clark method, and consequently, it is not known whether it is the method itself or the variation which is significant.

(4) *Methoxyl.* (A) Clark method versus Elek method: The calculated *t* value was not critical, but the *F* value was critical and in favor of the Clark method. (B) The use of propionic or acetic anhydride versus the use of neither did not yield a *t* value which was critical. The calculated *F* value was critical and in favor of the use of neither. (C) Scrubber mixture: The use of aqueous suspension of red phosphorus versus the use of cadmium sulfate–sodium thiosulfate showed that neither the *t* nor the *F* values were critical. (D) Boiling time of reaction mixture: Boiled for less than 45 minutes versus boiled for more than 45 minutes. Neither the *t* nor the *F* values were critical. It will be noted that the above critical variation is found in the Clark method, and consequently, it is not known whether it is the method itself or the variation which is significant.

RECOMMENDATIONS

The Associate Referee recommends*—

Acetyl—

(1) That additional collaborative work be done on the determination of acetyl groups.

Alkoxyl—

(1) That additional samples be sent out, namely an ethyl and a methyl ester, to determine whether or not the results will be any different from those when the groups are present in the form of ethers.

(2) That if the results with the esters are in accord with the findings with the ethers, a procedure should be written for the Clark method and be submitted for further collaborative study.

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COLLABORATORS

The collaborators on the acetyl and alkoxyl analyses were:

* For report of Subcommittee C and action of the Association, see *This Journal*, 37, 74 (1954).

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REFERENCES

(1) ALICINO, J. F., *Anal. Chem.*, **20**, 590 (1948).
(2) *Anal. Chem.*, **21**, 651 (1949).
(3) CLARK, E. P., *Semimicro Quantitative Organic Analysis*, Academic Press, Inc., New York, 1943.
(4) NIEDERL, J. B., and NIEDERL, V., *Micromethods of Quantitative Organic Analysis*, 2nd ed., New York, John Wiley & Sons, 1942.
(5) OGG, C. L., *This Journal*, **35**, 305 (1952).
(6) *Ibid.*, **36**, 335 (1953).
(7) *Ibid.*, **36**, 344 (1953).
(8) ROTH, H., *F. Pregl Quantitative Organische Mikroanalyse*, Fünfte Auflage, Wien, Springer-Verlag, 1947.
(9) SNEDECOR, G. W., *Statistical Methods*, 4th ed., The Iowa State College Press, Ames, Iowa, 1946, pp. 82 and 218.
(10) STEYERMARK, A., *Anal. Chem.*, **22**, 1228 (1950).
(11) ———, *Quantitative Organic Microanalysis*, The Blakiston Company, Philadelphia, 1951.
(12) ———, ALBER, H. K., ALUISE, V. A., HUFFMAN, E. W. D., JOLLEY, E. L., KUCK, J. A., MORAN, J. J., and WILLITS, C. O., *Anal. Chem.*, **23**, 1689 (1951).
(13) ———, ALBER, H. K., ALUISE, V. A., HUFFMAN, E. W. D., KUCK, J. A., MORAN, J. J., and WILLITS, C. O., *Anal. Chem.*, **21**, 1283 (1949).
(14) *Ibid.*, **21**, 1555 (1949).
(15) *Ibid.*, **23**, 523 (1951).
(16) STEYERMARK, A., and FAULKNER, M. B., *This Journal*, **35**, 291 (1952).
(17) ———, and GARNER, M. W., *ibid.*, **36**, 319 (1953).
(18) WILLITS, C. O., and OGG, C. L., *ibid.*, **32**, 56 (1949).
(19) YOUDEN, W. J., *Statistical Methods for Chemists*, pp. 119–120, John Wiley & Sons, New York, 1951.

REPORT ON MICROANALYTICAL DETERMINATION OF NITROGEN BY THE DUMAS METHOD

By C. L. Ogg (Eastern Utilization Research Branch,* Philadelphia 18, Pa.), *Referee*

The Dumas method for determining nitrogen was studied in 1948 (5), 1952 (1), and again this year. The first study showed only that the combustion temperature should be above 650°C. to prevent low and erratic results. Last year each collaborator again used the method normally employed in his laboratory except that temperatures above 650°C. were specified. The results were analyzed statistically to obtain indications as to which variables in the procedures were preferred because they produced better accuracy or precision.

A procedure was written to include those variables indicated to be best by last year's study. This tentative procedure, and samples of the same two materials analyzed last year, were sent to collaborators with the request that they follow the revised procedure as closely as possible. If modification of the procedure by the collaborator was necessary, the changes made were to be called to the attention of the Referee when the results were submitted.

Since the tentative method permitted the analyst a number of choices in the apparatus to be used, the card supplied for reporting results also contained a form to be checked to provide information concerning the apparatus used for each set of results.

The 1952 study indicated that the method of Shelberg (2), or that of Zimmermann (6) which is similar to Shelberg's, might be superior to the conventional micro Dumas method. Because there was not sufficient data available from the 1952 study to evaluate these newer methods properly, those collaborators now using the Shelberg or Zimmermann methods were asked to analyze the samples this year by either of these procedures rather than by the tentative Dumas method.

The micro Dumas procedure submitted to the collaborators for the 1953 study was as follows:

TENTATIVE DUMAS NITROGEN PROCEDURE

REAGENTS

(a) *Potassium hydroxide soln.*—Dissolve 50 g KOH in 50 ml H_2O.

(b) *Mercury.*—Previously used or slightly dirty mercury is preferred.

(c) *Copper oxide; Coarse.*—Wire form, about 1 mm in diam. and 2–4 mm long. *Fine.*—CuO wire ground to pass 40 mesh but not 100 mesh sieve. Pre-ignite both at 700–800°C. for 30 min. in nickel or steel crucible and store in glass bottles having 1–2″, 7 mm O.D. glass pour-out tubing.

* One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Service, United States Department of Agriculture.

(d) *Copper (metal).*—Clean, degreased copper turnings or wool.
(e) *Dry ice.*—Solid CO_2. Must give micro bubbles ca 0.2 mm diam. in nitrometer.
(f) *Asbestos (fiber).*—Acid wash and pre-ignite to 800°C.

APPARATUS

(a) *Dewar flask.*—1 liter, for carbon dioxide generator, fitted with 2-hole rubber stopper contg 2 gas delivery tubes, one with stopcock and ball joint leading to app., and other to bottom of cylinder contg 15–18″ of H_2O or with one hole stopper containing standard mercury valve (7).

(b) *Combustion tube.*[1]—Quartz or Vycor, 520–530 mm long, 11 mm O. D., 8 mm I.D., with 7/15 S.T. inner joint on tip.

(c) *Needle valve.*[2]—Standard (7), stainless steel with 7/15 S.T. glass outer joint cemented to tip with Krönig or deKhotinsky cement and with 12/5 glass ball joint cemented to nitrometer end.

(d) *Nitrometer.*—Either Stehr or Pregl type (7), 1.5 ml graduated in hundredths, calibrated in intervals of at least 0.2 ml and preferably 0.1 ml and with magnifying lens attachment.

(e) *Furnaces; Sample burner.*—Electric,[3,4] 13–14 mm I.D., by 3–4″ long, operated at a temp. of 775–800°C. *Long furnace.*—Electric, 13–14 mm. I.D. 8″ long, operated at temp. of 750–775°C.

PREPARATION OF APPARATUS

Dewar flask.—Break into pieces ½″ or smaller sufficient dry ice to fill flask, place in flask, insert stopper containing tubes, open stopcock on delivery tube, place pressure tube in cylinder of water, and allow to stand 1 hr for removal of trapped air. Close and open stopcock occasionally during hour. (One filling should supply CO_2 for one week.)

Combustion tube, permanent filling.—Place enough acid-washed, pre-ignited asbestos fibers in clean tube to form a 3–4 mm loose plug in end of tube. Use a glass rod to push asbestos in place. Holding the tube in vertical position, introduce in succession 10–12 cm of pre-ignited coarse CuO (tap tube to settle CuO), 2–4 mm loose asbestos plug, 3–4 cm Cu turnings or wool, 2–4 mm loose asbestos plug, 5–6 cm coarse CuO (tap tube to settle CuO), 2–4 mm loose asbestos plug, and 3–4 cm Cu turnings or wool; cap with 4–6 mm loose asbestos plug. Place tube in long furnace so that tip and ca 5 cm of CuO filling protrudes. Connect CO_2 source by placing one-hole rubber stopper in tube and moistening and inserting tapered CO_2 delivery tube. Sweep 10 min. with CO_2, bring long furnace to temp. with slow stream of CO_2 through tube and after 1 hr make simulated sample burning. Turn off burner and long furnace and allow tube to cool with rapid CO_2 flow.

Nitrometer.—Fill nitrometer with mercury to midway between capillary side arm opening and opening of tubulation for connecting leveling bulb. Place few mg Hg_2Cl_2 on mercury to prevent sticking of gas bubbles. Fill nitrometer leveling bulb with 50% KOH. Connect needle valve to nitrometer side arm through ball and socket joint held together by pressure clamp.

Control analysis.—Make control analysis by the following procedure using standard pure compound and calculate blank correction. Repeat until calculated blanks check to ±0.003 ml.

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[1] Standard tube (7) without joint may be used.
[2] Precision stopcock and rubber tubing connections may be used.
[3] Mechanically operated sample burner preferred.
[4] A gas sample burner may be used but specified temperature must be obtained.

PROCEDURE

Weigh in micro porcelain boat[5] sufficient sample to give 0.4–0.8 ml nitrogen. Use micro balance if sample weight is less than 10 mg, otherwise semimicro balance may be used. Disconnect cooled combustion tube, remove from furnace and introduce temporary filling of 7–8 cm of coarse CuO, 1–2 cm fine CuO, boat containing sample, 4–5 cm fine CuO, and 2–3 cm coarse CuO.[6] Holding tube at angle, rotate and tap to mix sample and CuO. Replace tube in combustion furnaces, connect CO_2 supply, and flush tube with rapid CO_2 stream 3–5 min. Turn on long furnace, or if split type furnace is used pull furnace into position over combustion tube. Connect needle valve–nitrometer assembly (with valve open) by placing small amount of Krönig cement on 7/15 inner joint of combustion tube, warming joint and cement with small flame, and connecting 7/15 outer joint of needle valve. Have leveling bulb in lower position and nitrometer cock open during this operation. When joint has cooled, close valve, raise leveling bulb until KOH level is in bulb above stopcock, close stopcock, and place bulb in lower position. Open valve so that CO_2 flow is 3–5 bubbles/sec. and continue sweeping until micro bubbles (diam. 0.2 mm, or equal to width of calibration lines) are obtained. Reduce CO_2 flow to 1–2 bubbles/sec. when testing for micro bubbles. Displace any N in nitrometer, close stopcock on delivery tube, open needle valve, place sample burner 4″ from long furnace, bring burner to temperature, 775–800°C., and move burner over sample at rate of 0.5 cm/min. When gas flow into nitrometer slows or stops, close needle valve, open delivery stopcock, then open needle valve to allow flow of 2 bubbles/sec. Turn off sample burner 3–5 min. after it reaches the long furnace. Continue sweeping at 2 bubbles/sec. until bubble size approaches micro, turn off or remove long furnace, and speed up flow to 3–5 bubbles/sec. When micro bubbles are obtained (check with flow of 1–2 bubbles/sec.), force any KOH that may have leaked through stopcock back into cup by carefully opening stopcock with leveling bulb raised above stopcock, hang bulb in upper position, and let stand 10 min. Gently heat combustion tube joint, disconnect from needle valve, remove tube from furnace when cool, remove temporary filling, and prepare for next sample. Adjust nitrometer leveling bulb so that KOH in bulb and tube are level and read volume of nitrogen using magnifying lens attached to nitrometer. Determine and record temperature of air adjacent to nitrometer, and barometric pressure in room. Calculate % N in sample as follows:

$$\text{Per cent N} = V_c \times \frac{P}{273 + t} \times \frac{44.90^{7}}{\text{Sample wt (mg)}}$$

$$V_c = (V \pm \text{calib. corr.}) - (V \times 0.011) - (\text{blank correction.}^{8})$$

Blank correction.—Analyze standard pure compound by above procedure and calculate correction as follows:

$$V_1 = \%\ \text{N} \times \frac{273 + t}{P} \times \frac{\text{Sample wt (mg)}}{44.90}$$

$$V_2 = (V \pm \text{calib. corr.}) - (V \times 0.011)$$

$$V_2 - V_1 = \text{blank correction}$$

V, P, and t are observed values in control analysis. % N is theoretical value for standard compound.

[5] If sample is liquid, weigh in capillary containing $KClO_3$ plug in closed end.

[6] Amounts of coarse and fine CuO used should not vary markedly because they affect the blank correction.

[7] $44.90 = \frac{273}{760} \times \frac{28,016}{22,414} \times 100.$

[8] From control analysis.

RESULTS

Twenty-one collaborators used the 1953 tentative method either as described or with only slight modification. They reported 103 values for sample 1, nicotinic acid. Twenty collaborators also analyzed sample 2, acetone-2,4-dinitrophenyl hydrazone, and reported 94 values for this material.

Each collaborator was asked to report all the data he obtained unless some known error was made in a determination and all values reported have been used to obtain the statistical data in this report.

Table 1 shows the tabulated results for both samples. In this table, n is the number of values reported, $\bar{x}$ the average, s the standard deviation and $s_{\bar{x}}$ the standard deviation of the $\bar{x}$ values.

TABLE 1.—*Summary of results obtained by tentative Dumas method*

COLLABORATOR NO.	NICOTINIC ACID (11.38% N)			ACETONE-2,4-DINITROPHENYL HYDRAZONE (23.52% N)		
	n	$\bar{x}$	s	n	$\bar{x}$	s
0	3	11.38	0.20	3	23.40	0.18
2	4	11.55	0.17	4	23.79	0.18
8	5	11.33	0.05	4	23.50	0.12
14	8	11.39	0.11	8	23.55	0.08
15	8	11.03	0.07	8	23.44	0.07
23	5	11.28	0.46	6	23.32	0.33
30	6	11.39	0.08	6	23.44	0.03
31	4	11.40	0.10	4	23.41	0.10
35	6	11.35	0.11	4	23.92	0.12
39	8	11.52	0.10			
44	5	11.06	0.09	5	23.61	0.27
45	4	11.31	0.07	4	23.46	0.13
59	3	11.45	0.07	3	23.40	0.06
71	6	11.68	0.23	6	23.81	0.14
72	4	11.40	0.17	4	23.50	0.19
74	4	11.38	0.07	4	23.90	0.13
75	4	11.17	0.04	4	22.84	0.05
76	8	11.49	0.28	8	23.34	0.10
77	4	11.43	0.07	4	23.47	0.07
79	5	11.33	0.03	5	23.54	0.05
80	5	11.40	0.06	4	23.21	0.14
Total No.	103	21		94	20	
Over-all Mean		11.37	0.13		23.45	0.13
$s_{\bar{x}}$		0.15			0.31	

The over-all means of 11.37 and 23.45 per cent N are only 0.01 and 0.07 per cent lower than the theoretical values, respectively. Neither of these deviations is significant by the t test (3). The average of the standard

deviations is 0.13 for both samples, a reasonably low value. Contrasted with this are the $s_{\bar{x}}$ values of 0.15 and 0.31 for samples 1 and 2, respectively. The value of 0.15 is acceptable but the poor precision between analysts shown by the $s_{\bar{x}}$ of 0.31 for acetone-2,4-dinitrophenyl hydrazone indicates a need for refinement of the tentative method tested or the search for a new and better method.

Each analyst supplied the following information about the procedures used when he reported his results.

(1) Whether a needle valve or stopcock was used to control the gas flow.
(2) Whether ground glass joint or rubber connections were used.
(3) Whether the sample burner was mechanically or manually operated.
(4) Whether the sample burner was electric or gas fired.

The effect of these variables permitted in the 1953 procedure was examined statistically in the same manner used to evaluate the variables in the 1952 study. The data were divided into groups according to the variable used and F and t tests were applied to each of the four pairs of groups. None of the variables could be shown to be consistently more precise or accurate than its alternate; that is, in no case did the F or t values for both samples exceed the critical F and t values.

Those collaborators who normally use the Shelberg or the Zimmermann procedure were asked to analyze the two samples by either of these, rather than by the tentative method. Table 2 shows the data obtained this year by the Shelberg and Zimmermann procedures and the data obtained last year for the same two materials by these same two procedures.

The over-all mean and standard deviation of the means were calculated for both the data obtained this year and for the combined 1952–1953 data. Even though the combined data include two sets of values by three laboratories, the fact that the sets were obtained one year apart was considered to allow for sufficient variation to treat the two sets as if they had come from different laboratories. This was done to increase the number of values so that a more reliable comparison could be made between these two methods and the tentative method.

The comparison in Table 2 of data obtained by the Shelberg and the Zimmermann methods shows that the Zimmermann procedure produced the more precise interlaboratory results for sample 2, while the results for sample 1 were not significantly different. This is shown by comparing the calculated F's with the critical $F_{.05}$ value; only for sample 2 does the calculated value exceed the critical value.

In addition to using the tentative method, collaborator 14 analyzed the two samples by the Dumas procedure described by Steyermark (4) with the following results: for sample 1, $n=9$, $\bar{x}=11.33$, $s=0.09$; for sample 2, $n=8$, $\bar{x}=23.37$, $s=0.10$. This collaborator's $\bar{x}$ values by the tentative method were higher and closer to the theoretical value, but his precision by the two methods was similar (see Table 1).

TABLE 2.—*Summary of results obtained by Shelberg and Zimmermann methods*

COLLAB. NO.[a]	1953			COLLAB. NO.[a]	1952		
	n	$\bar{x}$	s		n	$\bar{x}$	s
Nicotinic acid (11.38% N)							
22s	4	11.36	0.07				
27z	5	11.50	0.12				
29z	4	11.52	0.05	29z	4	11.36	0.16
37s	4	11.37	0.14	37s	6	11.35	0.15
63z	4	11.40	0.07	63z	4	11.44	0.05
65s	4	11.63	0.04	67s	4	11.39	0.09
78s	4	11.34	0.04				
	1953				1952–1953		
Total No.	29	7			47	11	
Over-all Mean		11.45	(0.08)			11.42	(0.09)
$s_{\bar{x}}$		0.11				0.09	
	n	$\bar{x}$	s	F	$F_{0.5}$		
Shelberg	6	11.41	0.11	2.47	6.26		
Zimmermann	5	11.44	0.07				
Acetone-2,4-Dinitrophenyl Hydrazone (23.52% N)							
22s	5	23.31	0.13				
27z	5	23.57	0.12				
29z	4	23.64	0.09	29z	3	23.41	0.11
37s	4	23.71	0.16	37s	7	23.63	0.10
63z	4	23.55	0.19	63z	6	23.45	0.09
65s	7	23.62	0.16	67s	4	23.34	0.12
78s	10	23.04	0.30				
	1953				1952–1953		
Total No.	39	7			59	11	
Over-all Mean		23.49	(0.16)			23.48	(0.14)
$s_{\bar{x}}$		0.24				0.19	
	n	$\bar{x}$	s	F	$F_{0.5}$		
Shelberg	6	23.44	0.26	8.34	6.26		
Zimmermann	5	23.52	0.09				

[a] The letters s and z after the collaborator number refer to the Shelberg and Zimmermann procedures, respectively.

Collaborator 49 was not able to obtain satisfactory micro bubbles using the high temperatures specified in the tentative method.

Collaborator 9 used his own procedure, which differed from the 1953 tentative method only in the method of correcting the observed volume of nitrogen. He determined a blank correction by burning dextrose, then used the data from different weight samples of a standard material to construct a calibration or correction curve. This was done by plotting the difference between the theoretical volume and the blank corrected, observed volume (STP) against the blank corrected, observed volume. His results were: for sample 1, $n=6$, $\bar{x}=11.39$, $s=0.04$; for sample 2, $n=4$, $\bar{x}=23.45$, $s=0.09$. Whether these values are better than the average values by the tentative method because of the method of obtaining the corrected volume of gas, because of slight changes in procedure, or for some other reason cannot be ascertained from the data available.

The results obtained during the last two years by the Dumas method are summarized in Table 3. In this table, n is the number of analyst's means or $\bar{x}$'s, $\overline{\bar{x}}$ is the over-all mean of $\bar{x}$'s, $s_{\bar{x}}$ the standard deviation of the $\bar{x}$'s, F the calculated F value, and $F_{.05}$ the critical value from the 5 per cent table. Although $F_{.05}$ is shown, calculated F's which exceed this critical value show differences significant at only the 10 per cent level because the larger variance, $(s_{\bar{x}})^2$, is arbitrarily placed in the numerator of the equation:

$$F = \frac{(s_{\bar{x}})_a^2}{(s_{\bar{x}})_b^2}.$$

TABLE 3.—*Summary of data obtained in 1952 and 1953*

METHOD	YEAR	n	$\bar{\bar{x}}$	$s_{\bar{x}}$	F	$F_{.05}$
Nicotinic Acid						
Shelberg–Zimmermann	1952–1953	11	11.42	0.09		
					2.78	2.77
Tentative	1953	21	11.37	0.15		
					2.25	2.07
Collaborator's[a]	1952	23	11.40	0.10		
Acetone-2,4-Dinitrophenyl Hydrazone						
Shelberg-Zimmermann	1952–1953	11	23.48	0.19		
					2.66	2.78
Tentative	1953	20	23.45	0.31		
					3.32	2.10
Collaborator's[a]	1952	22	23.47	0.17		

[a] Data obtained when each collaborator used the Dumas method normally employed in his own laboratory.

The interlaboratory precision using the tentative method tested this year was significantly lower than with the Shelberg–Zimmermann method and lower than that obtained when the collaborators used their own procedures. The differences were significant at the 10 per cent level for three of the four comparisons. Only for the comparison of the Shelberg–Zimmermann *vs* the tentative method for sample 2 was the calculated F less than the critical value and even here the difference between the two F values was slight. Student's t test (3) was applied to these same data to determine if there were any significant differences between means. None were found so the data in hand do not indicate any of the methods to be more accurate than the other two.

SUMMARY

The tentative micro Dumas method tested this year did not give results with as good interlaboratory precision as was obtained when the collaborators used the procedures they normally employed. The interlaboratory precision obtained by the Shelberg and Zimmermann methods was also better than that obtained by the tentative method and equal to that obtained by the more conventional Dumas procedures normally used. The over-all means by all methods agreed well with the theoretical values for the two samples analyzed, nicotinic acid and acetone-2,4-dinitrophenyl hydrazone.

It is recommended* that the 1953 tentative method should be revised before further collaborative work is done and that a rapid method similar to that of Shelberg or Zimmermann should also be tested collaboratively.

LIST OF COLLABORATORS

V. A. Aluise, Hercules Powder Company; C. J. Bain, Picatinny Arsenal; L. M. Brancone, Lederle Laboratories; L. E. Brown, Southern Utilization Research Branch; W. L. Brown, Eli Lilly & Company; B. L. Browning, Institute of Paper Chemistry; A. W. Dearing, Hunter College; T. DeVries, Purdue University; L. Dorfman, Ciba Pharmaceutical Company; K. K. Fleischer, Sterling-Winthrop Research Institute; E. E. Gansel, Ansco; J. Grodsky, Ortho Research Foundation; G. M. Gustin, Celanese Corporation of America; E. W. D. Huffman, Huffman Microanalytical Laboratory; G. A. Jones, E. I. duPont de Nemours & Company; D. F. Ketchum, Eastman Kodak Company; J. A. Kuck, American Cyanamid Company; J. A. Means, Chas. Pfizer & Company, Inc.; C. W. Nash, Rohm and Haas Company; P. B. Olson, Minnesota Mining & Mfg. Company; J. K. Owens, E. I. du Pont de Nemours & Company; P. Rothemund, Charles F. Kettering Foundation; E. T. Scafe, Socony-Vacuum Oil Company; S. A. Shrader, Dow Chemical Company; J. Sorensen, General Electric Company; A. Steyermark, Hoffman-LaRoche, Inc.; S. J. Tassinarri, National Dairy Research Laboratory, Inc.; W. H. Throckmorton, Tennessee Eastman Corporation; C. H. Van Etten, Northern Utilization Research Branch; C. L. Ogg, Eastern Utilization Research Branch.

* For report of Subcommittee A and action of this Association, see *This Journal*, 37, 63 (1954).

REFERENCES

(1) Ogg, C. L., *This Journal*, **36**, 344 (1953).
(2) Shelberg, Elmer F., *Anal. Chem.*, **23**, 1492 (1951).
(3) Snedecor, George W., *Statistical Methods*, 4th Ed., Iowa State College Press, Ames, Iowa, 1946.
(4) Steyermark, A., *Quantitative Organic Microanalysis*, Blakiston Company, New York, 1951.
(5) Willits, C. O., and Ogg, C. L., *This Journal*, **32**, 561 (1949).
(6) Zimmermann, W., *Mikrochemie ver. Mikrochim. Acta*, **31**, 42 (1943).
(7) "Report on Recommended Specifications for Microchemical Apparatus," *Anal. Chem.*, **21**, 1555 (1949).

REPORT ON STANDARD SOLUTIONS

SODIUM THIOSULFATE AND HYDROCHLORIC ACID

By H. G. Underwood (Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D. C.), *Referee*

Sodium thiosulfate solutions.—This subject was reopened following a comment by V. A. Stenger, *Anal. Chem.*, **23**, 1543 (1951), that thiosulfate solutions standardized by A.O.A.C. **39.35, 39.36** were found to give results deviating by ± 0.2 per cent in analyses of a standard iodate solution by various analysts. He suggested it would be desirable to have a thorough comparison of dichromate and iodate as standards for thiosulfate under various conditions. The preliminary study of the Associate Referee indicates that the present A.O.A.C. method gives easily reproducible results which compare favorably with those obtained by using iodine and potassium iodate. The Associate Referee recommends* that the study be continued to obtain collaborative results and comment and the Referee concurs.

Constant boiling hydrochloric acid.—Last year the Associate Referee modified the directions for the preparation of constant boiling hydrochloric acid **(39.11)** to make the method as specific as possible. The collaborative results supported the conclusion that the error of the method was insignificant as compared with the variation introduced by the standardization procedure. The proposed modification was adopted, first action. After the study had been reported to the Association, H. G. Pfeiffer of the General Electric Research Laboratory, Schenectady, N. Y., suggested that the data used by King, *This Journal*, **25**, 653 (1942), in deriving his equation for G (air wt of constant boiling HCl required to give one equivalent wt of HCl) be reviewed. He pointed out that no one

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1954).

straight line equation fitted all the data satisfactorily. A critical review of all available data by the Associate Referee and William Weiss during the past year resulted in the conclusion that the equations of two lines is preferable to the single equation of King. They recommend that the two equations be substituted for King's equation in the proposed modification of section **39.11**, *This Journal*, **36**, 96 (1953). The Referee concurs and recommends* that the method as further modified be adopted, first action.

REPORT ON STANDARDIZATION OF SODIUM THIOSULFATE SOLUTIONS

By W. H. MUNDAY (U. S. Food and Drug Administration, Department of Health, Education, and Welfare, Kansas City, Mo.), *Associate Referee*

The work this year on sodium thiosulfate solutions has been done because of the question raised by Stenger (1) as to the accuracy of the method of standardization described in *Official Methods of Analysis*, seventh edition.

Numerous reagents are used for the standardization of sodium thiosulfate. Some of the more common ones are potassium dichromate, potassium iodate, iodine, oxalic acid, iodine cyanide, and copper. It was decided to limit the present work to potassium dichromate (the reagent used in the A.O.A.C. *Methods of Analysis*), potassium iodate, and iodine.

Pure iodine would seem to be the ultimate standard by which the strength of sodium thiosulfate should be measured, since thiosulfate solutions are used for the titration of iodine. In most investigations of other substances proposed as standards for the standardization of thiosulfate, a comparison is made with the results obtained with pure iodine (2–4). Purified elemental iodine is not readily available as a primary standard and therefore must be purified before use. Since iodine has an appreciable vapor pressure, difficulties are presented in weighing it accurately. Reagent grade iodine purified by the method of Faulk and Morris (2), and weighed by the method described by Treadwell and Hall (5) was used in this study.

Potassium iodate is one of the more popular standards for sodium thiosulfate. According to Kolthoff (7), the salt may be obtained perfectly pure by several recrystallizations from water and drying at 180°C. It is not particularly hygroscopic and may be stored for some time. The principal objections to potassium iodate as a standard are its small equivalent

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1954).

weight and the fact that it is not readily obtainable in the purity required for a primary standard without recrystallization.

Potassium dichromate is one of the older standards. It is obtainable from the National Bureau of Standards as a primary standard and its equivalent weight is high enough for accurate weighing. Vosburgh (6) made a careful investigation of the effects of various factors, such as *p*H, concentration, and presence of oxygen upon dichromate, iodine, and thiosulfate; his findings agree with most of the other workers on the standardization of thiosulfate (6–10).

A solution of sodium thiosulfate was prepared as directed in **39.35,** Seventh Edition. This solution was standardized with potassium dichromate as directed in **39.36,** by the potassium iodate method as described by Willard-Furman (11), and by iodine as directed by Treadwell and Hall and Johnson (12).

In Table 1 are the results as obtained by the three methods:

TABLE 1.—*Standardization of thiosulfate*[a]

	IODINE	POTASSIUM IODATE	POTASSIUM DICHROMATE
	Normality	*Normality*	*Normality*
	0.1060	0.1060	0.1059
	0.1060	0.1059	0.1059
	0.1060	0.1059	0.1059
	0.1060	0.1059	0.1059
	0.1061	0.1059	0.1059
	0.1060	0.1059	0.1059
Av.	0.1060	0.1059	0.1059

[a] All values corrected to 20°C.

Roe's report "Standardization and Stability of 0.1 *N* Sodium Thiosulfate in Hot Weather" (13) indicates that the normality of thiosulfate increases with the temperature. The following experiments (Table 2) did not verify this.

TABLE 2.—*Effect of temperature*[a]

	20°C.	30°C.	40°C.
	Normality	*Normality*	*Normality*
	0.1059	0.1059	0.1059
	0.1059	0.1059	0.1059
	0.1059	0.1059	0.1059
Av.	0.1059	0.1059	0.1059

[a] All values corrected to 20°C.

Distilled water which contained a small amount of dissolved free chlorine was found to interfere in the iodometric titrations.

Using the reagents for the standardization of thiosulfate (except for the omission of the dichromate), and using distilled water containing 0.5 p.p.m. of free chlorine, a positive test for free iodine was obtained in the resulting solution. When titrated with 0.01 *N* thiosulfate this solution gave results as reported in Table 3.

TABLE 3.—*Effect of free chlorine*

TIME	*At once*	*10 min.*	*15 min.*
Ml of 0.01 *N* Thiosulfate	0.32	0.35	0.44
	0.38	0.35	0.44
	0.42	0.50	0.45

Table 4 shows a comparison of the observed normalities of a thiosulfate solution, when the water used contained free chlorine, with those obtained using chlorine-free water.

TABLE 4.—*Effect of chlorine*[a]

10 min.		*15 min.*		*20 min.*	
Cl FREE	CONT. Cl	Cl FREE	CONT. Cl	Cl FREE	CONT. Cl
.1059	.1058	.1059	.1057	.1059	.1057
.1059	.1058	.1059	.1057	.1059	.1057
.1059	.1058	.1059	.1057	.1059	.1056

[a] All values corrected to 20°C.

It was found that water from a normally operated laboratory still was contaminated with free chlorine. When the distilled water was boiled until free of chlorine by the ortho-tolidine test (14), there was no evidence of liberated iodine in potassium iodide solutions, even when the solution was allowed to stand overnight. This was not true when using distilled water containing free chlorine. The end point of the titrations was not stable and the blue color of the starch-iodine mixture would reappear after allowing the mixture to stand for a short time.

SUMMARY

In this preliminary study to evaluate the method for the standardization of sodium thiosulfate, it appears that the present A.O.A.C. method gives easily reproducible results which compare favorably to those obtained by using iodine and potassium iodate.

Distilled water which contains as much as 0.5 p.p.m. free chlorine interferes with iodometric titrations.

It is recommended* that the study of the method for standardization of sodium thiosulfate be continued to obtain collaborative results and comment.

ACKNOWLEDGMENT

The writer desires to acknowledge his gratitude to F. E. Yarnall for the use of his unpublished paper "Ultimate Standards in Iodometry," and to A. M. Allison for his guidance and helpful criticism.

REFERENCES

(1) STENGER, V. A., *Anal. Chem.*, **23**, 1543 (1951).
(2) FAULK, C. W., and MORRIS, S., *J. Am. Chem. Soc.*, **44**, 221 (1922).
(3) BAXTER, G. P., *ibid.*, **26**, 1579 (1904).
(4) RICHARD, THEO. W., and WILLARD, H. H., *ibid.*, **46**, 2204 (1924).
(5) TREADWELL and HALL, *Analytical Chemistry*, Vol. II, 8th Ed., John Wiley & Sons, Inc., Chapman & Hall, Limited, London.
(6) VOSBURGH, W. C., *J. Am. Chem. Soc.*, **44**, 2126 (1922).
(7) KOLTHOFF, I. M., *Pharm. Weekblad*, **59**, 66 (1922).
(8) BRAY, WM. C., and MILLER, H. E., *J. Am. Chem. Soc.*, **46**, 2204 (1924).
(9) WAGNER, J., *Z. Anorg. Chem.*, **19**, 427 (1899).
(10) KOLTHOFF, I. M., *Z. Anal. Chem.*, **59**, 40 (1920).
(11) WILLARD, H. H., and FURMAN, N. H., *Elementary Quantitative Analysis*, 2nd Ed., D. Van Nostrand Co., Inc., New York.
(12) JOHNSON, G. M., *This Journal*, **25**, 659 (1942).
(13) ROE, S. O., *Ind. Eng. Chem., Anal. Ed.*, **14**, 802 (1942).
(14) *Standard Methods for the Examination of Water and Sewage*, 8th Ed., Am. Public Health Association, New York, 1936.

REPORT ON CONSTANT BOILING HYDROCHLORIC ACID AS AN ACIDIMETRIC STANDARD

By SIDNEY WILLIAMS (Food and Drug Administration, Department of Health, Education, and Welfare, Boston, Mass.), *Associate Referee*, and WILLIAM WEISS (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

After last year's report on this subject (9) had been submitted, a communication was received from Dr. Pfeiffer calling attention to inaccuracies in the data used by King (8) in deriving his equation for G (air weight of constant boiling acid containing one equivalent weight of HCl). Dr. Pfeiffer also pointed out that no one straight line equation fitted all the data satisfactorily. This year's work has therefore consisted of reviewing

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1953).

the data in the literature and attempting to derive a better fitting equation for G.

The G values in the literature were plotted against pressure. Two straight line equations, one calculated from the data between 680–780 mm Hg, and another from the data between 540–660 mm Hg fit the observed points so that the variation of the points around the calculated lines is apparently random. The equations of the two lines are:

$$\text{for 540–660 mm:} \quad G = 162.255 + .02415P_0$$
$$\text{for 680–780 mm:} \quad G = 164.673 + .02039P_0.$$

Table 1 lists the values for G as derived from the data in the literature, as calculated from King's equation, and as calculated from the above two equations. It is readily seen that the present equations give G values closer to the literature values than does King's equation.

TABLE 1.—*Comparison of G values*

P_0 (mm Hg)	G (from literature)	REFERENCE	$G_{(calculated)}$	$G_{(calc)}-G_{(lit)}$ (diff.×1000)	$G_{calc}=\frac{P_0+7680}{46.8386}$	$G_{calc}-G_{lit}$ (diff.×1000)
			$G=162.255+0.02415\ Po$			
540	175.390	2	175.296	−94	175.496	106
600	176.558	2	176.745	187	176.777	219
620	177.224	3	177.228	4	177.204	−20
630	177.463	3	177.470	7	177.418	−45
640	177.703	3	177.711	8	177.631	−72
650	177.987	3	177.953	−34	177.845	−142
660	178.268	3	178.194	−74	178.058	−210
			$G=164.673+0.02039\ Po$			
680	178.486	1	178.538	52	178.485	−1
700	178.947	2	178.946	−1	179.192	245
700	179.026	3	178.946	−80	179.192	166
740	179.750	1	179.762	12	179.766	16
750	179.952	4	179.966	14	179.980	28
760	180.154	1, 2, 7	180.169	15	180.193	39
770	180.376	8	180.373	−3	180.407	31
780	180.589	8	180.577	−12	180.620	31

The collaborative results in last year's report (9) have been recalculated using the present equations for G. The resulting changes in the calculated normalities are very small with the greatest change being 3 parts per 10,000. The average of the calculated normalities changes less than one part in 10,000. These changes are shown in Table 2.

Using the revised calculated normalities, an analysis of variance was made for the borax and the NaOH data. This revision did not affect the

TABLE 2.—*Comparison of calculated normalities*

LABORATORY	P_s	NORMALITY: (AV. OF ALL TITRATIONS)	CALCULATED NORMALITY	
			$G=162.255+.02415\ P_s$ OR $G=164.673+.02039\ P_0$	$G=\frac{P_s+7680}{46.8386}$
Boston	752.2	.10062	.10061	.10061
	752.4	.10055	.10052	.10052
Chicago	740[a]	.09999	.10000	.10000
	740.3	.10006	.10002	.10002
Denver	633.9	.09989	.09980	.09983
	630.6	.100605	.10058	.10061
Kansas City	734.3	.10008	.10000	.10000
Washington	762.4	.10005	.10002	.10000
	763.2	.10025	.10027	.10026
Average		.10023	.10020	.10020

[a] Calculated from data submitted with A.O.A.C. results.

error due to standardization, which was calculated to be, for an average of 3 determinations, 1.4 parts per 1000 when standardizing against borax, and 2.8 parts per 1000 when standardizing against NaOH. The error of the method, again, is not significantly larger than the calculated error of standardization. As before, there is a significant positive bias, on the average, of 0.4 part per 1000 when the 0.1 N HCl solutions are standardized against borax.

RECOMMENDATIONS

It is recommended*—

(1) That the first action method for the standardization of hydrochloric acid by the constant boiling method, *This Journal*, **36**, 96 (1953) be revised as follows and adopted as first action:

Change the first four lines of the second paragraph (including the formula) to read:

Calc. air wt in grams (G) of this constant boiling HCl required to give one equivalent wt of HCl from one of the following equations:

For $P_0=540$–669 mm Hg: $G=162.255+0.02415\ P_0$

For $P_0=670$–780 mm Hg: $G=164.673+0.02039\ P_0$

(2) It is further recommended that this subject be closed.

* For report of Subcommittee A and action of the Association, see *This Journal*, **37**, 63 (1954).

REFERENCES

(1) BONNER, W. D., and WALLACE, R. E., *J. Am. Chem. Soc.*, **52**, 1747 (1930).
(2) ———, and TITUS, A. C., *ibid.*, **52**, 633 (1930).
(3) ———, and BRANTING, B. F., *ibid.*, **48**, 3093 (1926).
(4) FOULK, C. W., and HOLLINGSWORTH, M., *ibid.*, **45**, 1220 (1923).
(5) HENDRIXSON, W., *ibid.*, **37**, 2352 (1915).
(6) MOREY, G. W., *ibid.*, **34**, 1027 (1912).
(7) HULETT, G., and BONNER, W. D., *ibid.*, **31**, 390 (1909).
(8) KING, W. H., *This Journal*, **25**, 653 (1942).
(9) WILLIAMS, S., and WEISS, W., *ibid.*, **36**, 354 (1953).

ANNOUNCEMENT

SUGARS AND SUGAR PRODUCTS:

Dr. Robert J. Dimler, Northern Utilization Research Branch, Agricultural Research Service, Peoria, Illinois, has been appointed Associate Referee on Micro Sugar Methods.

CONTRIBUTED PAPERS

SELECTIVE ADSORPTION METHOD FOR DETERMINATION OF THE SUGARS OF HONEY*

By JONATHAN W. WHITE, JR., and JEANNE MAHER (Eastern Utilization Research Branch,† Philadelphia 18, Pa.)

Methods recommended (1) for the carbohydrate analysis of honey are little changed from those used 50 years ago. They are highly empirical and based upon insufficient knowledge of the sugars present in honey. In a recent study (2) of the determination of glucose and fructose, the authors examined five procedures and concluded that variance due to methods was as great as that due to differences among various floral types of honey. Thus, little confidence can be placed in comparison of results of analyses of honey by different existing methods. Täufel and Reiss (3) have concluded from a paper-chromatographic study of honey that the customary methods of analysis are inadequate.

This paper describes the use of carbon column chromatography as a pre-treatment for analysis, and presents analytical methods for determination of glucose, fructose, sucrose, reducing disaccharides, and higher sugars in the eluates. By separating honey into monosaccharide, disaccharide, and higher saccharide fractions before analysis, results are obtained that are more nearly related to the actual composition of the mixture. Because of the complexity of honey, however, some degree of empiricism still remains in the analytical procedure.

The procedure developed by Whistler and Durso (4) for separation of sugar mixtures into mono-, di-, tri-, and higher saccharides has been widely used for preparative work. In this method, the sugar mixture is adsorbed on a charcoal-celite column and successively eluted with water, 5 per cent ethanol, 15 per cent ethanol, etc., to separate the saccharides of increasing degree of complexity.

The quantitative aspects of this separation have not been neglected. McDonald and Perry (5) used carbon column adsorption to separate corn sirup into glucose, maltose, and dextrin fractions for analysis. They can separate a sample into these fractions in about one and one-half hours, using up to 50 pounds of pressure on the column. Eluate fractions are concentrated from 900 to 100 ml for analysis, which is done by conventional methods. They reported average recoveries of 96.5 per cent for glucose and 97.1 per cent for maltose from known mixtures.

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Alm (6) has recently described the use of carbon as the adsorbent in his gradient elution analyses of Schardinger dextrin hydrolyzates. The procedure as outlined does not appear suited for routine use on a number of samples, and if it were scaled up to provide sufficient material for fructose-glucose analysis of the monosaccharide fraction, excessive volumes and time would be required.

We have adjusted the column size, sample size, and eluant composition so that the time required for separation is reasonable, and little evaporation of fractions is required before analysis. This has required a study of the elution curves of honey for selection of the optimum eluant and adaptation of analytical methods to direct analysis of the effluent fractions.

The procedure described here has been applied to some 19 floral types of honey. Results show that considerably less glucose is present than heretofore realized, and reducing disaccharides are general components of all samples analyzed, as suggested by van Voorst (7) and Hurd, *et al.* (8). These results will appear in another publication.[1]

EXPERIMENTAL

Selection and preparation of column.—Since honey is largely a monosaccharide sirup, adjustment of sample size to weight of adsorbent was based largely on the disaccharide content. The adsorbent weight must be adequate to hold all disaccharide and higher material (estimated at below 100 mg for 1 g of average honey) whereas the preponderant monosaccharides pass through the column essentially unadsorbed. This adjustment to the disaccharide content permits the use of smaller columns (and hence more rapid flow and smaller eluant volumes) than if the column size were adjusted to the amount of monosaccharide likely to be present.

The columns were made from 22 mm (O.D.) Pyrex tubing, packed dry by the procedure of McDonald and Perry (5). After a glass wool plug is inserted and wetted, sufficient 1:1 mixture (by weight) of Darco G-60[2] and Celite 545 is added without tapping or suction to fill the column to a depth of 23.5 cm (about 20 g is required). Then suction is applied to the column outlet while the column is tapped gently with a cork ring. The depth of the packed portion should then be about 17 cm. After the upper part of the column is cleaned of carbon, a layer of filter aid is added, about 7–10 mm thick after gentle packing. The column is washed with 750 ml of water and next with 250 ml of 50% ethanol; and then is allowed to stand overnight under 50% ethanol before use. Washing with 250 ml water suffices to remove alcohol from the columns. The columns should not be permitted to run dry. Applying air pressure of 10 pounds should give flow rates with water of 6.3–7.7 ml per minute.

To find eluants suitable for quantitative recovery of honey sugars as mono-, di-, and higher saccharides in volumes convenient for analysis, three 1 g samples of clover honey were adsorbed, and then eluted with water, 1% ethanol, and 2% ethanol, respectively. With water, at least 350 ml was required for complete recovery, but with 1% ethanol, recovery of monosaccharides was complete with less than 250 ml. When 2% ethanol was used, disaccharide elution began before mono-

[1] See p. 478.

[2] Mention of trade names in this paper does not imply endorsement or recommendation by the United States Department of Agriculture over similar products not mentioned.

saccharide elution was complete. Hence, 1% ethanol was selected, with 250 ml as the required volume for elution.

For disaccharide elution, three 1 g samples were adsorbed as above and washed with 400 ml of water, then with 5, 7, and 8% ethanol, respectively. Collection of 250 ml of 7% ethanol was selected for quantitative recovery, although 200 ml of 8% ethanol would have served as well. For elution of remaining carbohydrate material remaining on the column, 100 ml of 50% ethanol was used without further investigation.

Variability of carbon.—During this work, several lots of carbon (Darco G-60) were used. Considerable differences were noted in flow rate and in adsorbing capacity. Maltose saturation (determined by carrying 0.5 g maltose through the procedure and determining the maltose in the 7% ethanol fraction) was 180 and 301 mg for two samples. The former column showed poor disaccharide recoveries after 8 uses. When possible, selection should be made for high flow rate (6–8 ml/mm) and high maltose saturation. One lot of the carbon–filter aid mixture had a rate of 3.5 ml/min., which lengthened the procedure unduly.

During the routine analyses of honey samples, it was noted that, though occasional recovery experiments with pure disaccharides indicated that the columns were functioning satisfactorily, runs with half-size samples of honey would give higher results for disaccharides. There appears to be a progressive deterioration in the column performance when honey samples are analyzed. The cause for this has not been determined. It has been satisfactory to limit the number of uses of a column to about 8 samples and replace it at that time.

Adsorption procedure.—The column is washed with 230–250 ml of water to remove the 50% alcohol under which it is stored. Then 20 ml of 1% ethanol is passed through. Air pressure is disconnected, and the sample, dissolved in 10 ml of 1% ethanol, is quantitatively transferred to the column and washed successively as described below. Filtrates are collected in volumetric flasks. The eluate collected during the introduction of the sample is included in the 250 ml of the "*A*" fraction. After the "*C*" fraction is collected, the column may be stored under the 50% ethanol without further treatment. About 2 to 3 hours is required for the adsorption separation.

When the procedure outlined above was applied to honey (1.0 g samples), the eluates contained the following amounts of sugars:

(*A*) 1% ethanol: 300–500 mg fructose and 200–400 mg glucose in 250 ml.

(*B*) 7% ethanol: 3–60 mg sucrose and 40–100 mg reducing disaccharide in 250 ml.

(*C*) 50% ethanol: 5–50 mg reducing sugar, after hydrolysis, in 100 ml.

Determination of individual sugars in carbon column eluates.—Methods were modified when necessary to allow determination of these sugars without concentrating the solutions. In the work described here, the sugars used were as follows:

Glucose—Bureau of Standards, Standard Sample 41, lot 4006. Less than 0.05 per cent moisture.

Fructose—$[\alpha]_D^{25} = +92.8°$, moisture 0.31 per cent.

Sucrose—Commercial table sugar was used, containing less than 0.04 per cent moisture.

Maltose—Eastman maltose hydrate was recrystallized from aqueous

ethanol. Moisture on drying *in vacuo* by procedure of Cleland and Fetzer (9) = 6.74%; $[\alpha]_D^{25} = 131.6°$ on hydrate basis; 138.7° anhydrous.

FRACTION "A"

Determination of fructose.—The direct determination of fructose in mixture with glucose has several advantages over its calculation by difference between total reducing sugars and glucose (10). We used the procedure of Marshall and Norman (10), with slight modification. In this method, glucose is oxidized by hypoiodite, and residual fructose is determined by copper reduction. The amounts of sugar specified for their procedure (40–80 mg. in 20 ml) are higher than those occurring in the solutions from the carbon columns. For this reason and because of our use of a Shaffer–Somogyi reagent[3] different from that used by Marshall and Norman, we calibrated the procedure for fructose rather than use the Marshall–Norman equation.

Solutions containing fructose and glucose as shown in Table 1 were subjected to the following procedure. Results are shown in the table.

TABLE 1.—*Shaffer-Somogyi titrations of residual fructose after hypoiodite oxidation of mixtures of glucose and fructose at 18°C.*

SUGARS IN SOLUTION OXIDIZED		FRUCTOSE IN SOLUTION ANALYZED	0.005 *N* THIOSULFATE REQUIRED
GLUCOSE	FRUCTOSE		
mg	*mg*	*mg*	*ml*
8.00	8.10	0.202	1.01
19.9	20.2	0.504	3.59
18.1	21.5	0.537	3.85
28.8	30.5	0.762	6.00
40.0	40.4	1.010	8.23
37.6	41.0	1.025	8.32
55.7	64.1	1.602	13.72
80.0	80.8	2.020	17.56

The sugars were dissolved in 20.0 ml of 1% ethanol in a 200 ml volumetric flask. After the addition of 40.0 ml of 0.05 *N* iodine solution and the slow addition (with shaking) of 25.0 ml 0.10 *N* sodium hydroxide, the flask was immersed in the 18° (±0.1°) bath. After 10 minutes, the reaction was stopped by the addition of 5.0 ml *N* sulfuric acid, the flask was removed from the bath, and the excess iodine was reduced with fresh 1% sodium sulfite, with 2 drops of starch indicator. The solution was then neutralized to bromocresol green with *N* sodium hydroxide and made to volume with water. Reducing value was determined in duplicate on 5 ml aliquots with the Shaffer-Somogyi reagent noted previously.

A straight line was fitted to the data in Table 1 using the values from 3.59 to

[3] The Shaffer-Somogyi reagent 50 (11) with 5 g KI and 250 ml 0.10 *N* potassium iodate /l was used because of greater stability.

17.56 ml thiosulfate. The equation, calculated from the line, for the determination of fructose by this procedure was

$$a = 0.1090b + 0.113 \quad \text{(I)}$$

where a = mg fructose in 5 ml aliquot between 0.50 and 1.75 mg fructose;
b = ml 0.00500 N thiosulfate.
Average deviation of the experimental values from the line was 0.37%.

$$500\,a = \text{Mg fructose in 250 ml 1\% ethanol column eluate} \quad \text{(II)}$$

Determination of glucose.—A recent study (2) of the determination of glucose and fructose in honey demonstrated the value of the hypoiodite oxidation method for glucose in comparison with indirect determination by difference between total reducing sugars and fructose determined polarimetrically or by oxidation. Significantly greater precision was found for the Lothrop–Holmes (12) procedure for glucose than for other methods studied. Marshall and Norman (10) have modified this method slightly, principally by specifying oxidation at 16–18°C. rather than 20°. They also used direct determination of fructose after hypoiodite destruction of glucose, rather than determination by difference, as recommended by Lothrop and Holmes.

The factors previously cited set the glucose concentration in the carbon column filtrate at about 250–450 mg in 250 ml, or 20–36 mg of glucose in a 20 ml aliquot. This sugar concentration is considerably lower than that used by Lothrop and Holmes (60–80 mg) or Marshall and Norman (40–80 mg).

Preliminary glucose oxidations by the Lothrop–Holmes procedure were carried out in the 20° (±0.1°) bath. Table 2 shows the results. This apparent increasing over-oxidation with decreasing glucose concentration was noted by Marshall and Norman (10).

TABLE 2.—*Oxidation of glucose at 20°C. by hypoiodite*

GLUCOSE OXIDIZED	0.05 *N* THIOSULFATE REQUIRED	GLUCOSE[a] FOUND	RECOVERY	MG GLUCOSE PER ML THIOSULFATE
mg	*ml*	*mg*	*per cent*	
16.6	3.96	17.8	107.1	4.202
23.9	5.62	25.3	105.8	4.253
35.8	8.20	36.9	103.1	4.366
39.3	8.94	40.2	101.1	4.396

[a] Theoretical factor of 4.502 mg glucose/ml thiosulfate was used.

A series of oxidations was carried out in which volume, iodine and alkali concentration, and extent of acidification were varied, but this error was not reduced. Marshall and Norman reported that for 50 mg glucose, recovery of 101.3 per cent at 20.5° was reduced to 99.6 per cent by using an

18° oxidation bath.[4] Table 3 shows the effect of oxidation in an 18° (±0.1°) bath upon recoveries.

TABLE 3.—*Oxidation of glucose at 18°C. by hypoiodite*

GLUCOSE OXIDIZED	0.05 *N* THIOSULFATE REQUIRED	GLUCOSE[a] FOUND	RECOVERY	MG GLUCOSE PER ML THIOSULFATE
mg	*ml*	*mg*	*per cent*	
12.20	2.75	12.38	101.47	4.436
24.40	5.44	24.49	100.37	4.485
20.56	4.58	20.62	100.29	4.489
41.12	9.06	40.79	99.19	4.539
				Av. 4.487

[a] Ml×4.502.

The conversion factor in the last column of Table 3 shows the same trend as for the oxidation in the 20° bath, though the spread is less. The average factor found, 4.487, is 99.67 per cent of theoretical and compares with the 4.484 value used by Marshall and Norman. The latter will be used here.

When mixtures of glucose and fructose are subjected to hypoiodite oxidation, some oxidation of fructose takes place. Lothrop and Holmes corrected the calculated glucose value by subtracting 1.3 per cent of the fructose content, an experimentally derived correction for the oxidation of fructose in the presence of glucose by alkaline iodine solutions. Although Marshall and Norman concluded that a constant correction of this type is not satisfactory for all concentrations, they used a constant correction for their range (40–80 mg. glucose and 40–80 mg fructose).

Various amounts of glucose and fructose were oxidized under the same conditions as in Table 3 (20 ml sugar solution in a cork-stoppered 250 ml Erlenmeyer flask, 20 ml 0.05 *N* iodine, 25 ml 0.1 *N* NaOH added over 30 seconds, placed in water bath at temp. 18±.1°; 5 ml 2 *N* H_2SO_4 added after 10.0 minutes and titrated with 0.05 *N* thiosulfate). Ratios of fructose to glucose were 1 and 1.5, the normal limits found in honey. Table 4 shows the results.

The factor from the last column in Table 4 was plotted against weight of fructose. The line

$$c = 0.0219 - 0.0002F \qquad \text{(III)}$$

was found to fit adequately between the limits 10 and 70 mg fructose where

c = ml 0.05 *N* thiosulfate per ml fructose and

F = mg fructose in the 20 ml.

[4] Analyses of the cooling curves of samples (initial temperature 26.5°) in the 20° and 18° baths for 10 minutes showed that the average temperature was 22.5° and 20.1°, respectively. Lothrop and Holmes specified oxidation "at 20°" (12) without stating whether this referred to the bath temperature, air temperature, or temperature of the solution.

TABLE 4.—*Oxidation of glucose at 18°C. by hypoiodite in the presence of fructose*

GLUCOSE	FRUCTOSE	TITER, 0.05 *N*	MG GLUCOSE ÷ 4.484	COL. 3–COL. 4	ML 0.05 *N* THIO PER MG FRUCTOSE
mg	*mg*	*ml*		*ml*	
14.6	15.3	3.54	3.26	0.28	0.018
25.4	23.3	6.08	5.66	0.42	0.018
31.7	31.8	7.53	7.07	0.46	0.015
41.1	39.2	9.61	9.16	0.45	0.012
14.0	22.8	3.53	3.12	0.41	0.018
24.3	37.4	6.01	5.42	0.59	0.016
31.5	47.6	7.68	7.02	0.66	0.014
39.6	60.9	9.54	8.83	0.71	0.012
40.9	44.0	9.61	9.12	0.49	0.011
39.8	59.6	9.61	8.88	0.73	0.012
20.3	31.1	5.07	4.53	0.54	0.017
21.4	20.9	5.16	4.77	0.39	0.019

From these data, the following equation for the iodometric determination of glucose in the presence of fructose under these conditions was derived:

G = factor (ml 0.05 N thiosulfate − correction for fructose oxidation), or

$$G = 4.484\left(\text{ml 0.05 } N \text{ thiosulfate} - F \times \frac{\text{ml thiosulfate}}{\text{mg fructose}}\right). \qquad \text{(IV)}$$

Since

$$\frac{\text{ml 0.05 } N \text{ thiosulfate}}{\text{mg fructose}} = 0.0219 - 0.0002F,$$

$$G = 4.484\,[\text{ml 0.05 } N \text{ thiosulfate} - F\,(0.0219 - 0.0002F)], \text{ or}$$

$$G = 4.484\,(\text{ml 0.05 } N \text{ thiosulfate} - 0.0219F + 0.0002F^2), \qquad \text{(V)}$$

where

G = mg glucose in the 20 ml oxidized, between the limits 10–50 mg glucose and 10–65 mg fructose.

12.5 G = mg glucose in the 250 ml 1% ethanol eluate (VI)

For substitution in equation (V), F may be found from equation I by multiplying a by 40.

Table 5 shows glucose recovery for the mixtures in Table 4, calculated by this equation. From the data in the last column, a variance of 0.11 mg and a standard deviation of 0.33 mg may be calculated.

In applying this procedure to the analyses of column eluates, it was found that the 1 per cent alcohol caused erratic results due to the iodoform reaction. Since correction by blank was not successful, it was necessary to remove the alcohol by evaporation before analysis. Therefore, 20 ml aliquots of the "*A*" fraction (1 per cent alcohol) in the 250 ml Erlenmeyer flasks used for the determination were evaporated to dryness on the steam bath in a current of air. Twenty ml of water was added before analysis.

Table 5.—Recovery of glucose in mixture with fructose by hypoiodite oxidation

GLUCOSE	FRUCTOSE	TITER, 0.05 *N*	GLUCOSE[a] FOUND	RECOVERY	ERROR
mg	*mg*	*ml*	*mg*	*per cent*	*mg*
14.6	15.3	3.54	14.6	100.0	0.0
25.4	23.3	6.08	25.5	100.4	+0.1
31.7	31.8	7.53	31.6	99.7	−0.1
41.1	39.2	9.61	40.6	98.8	−0.5
14.0	22.8	3.53	14.0	100.0	0.0
24.3	37.4	6.01	24.5	100.8	+0.2
31.5	47.6	7.68	31.8	100.9	+0.3
39.6	60.9	9.54	40.1	101.3	+0.5
40.9	44.0	9.61	40.5	99.0	−0.4
39.8	59.6	9.61	40.4	101.5	+0.6
20.3	31.1	5.07	20.5	101.0	+0.2
21.4	20.9	5.16	21.5	100.5	+0.1

[a] By equation V.

FRACTION "B"

Determination of reducing disaccharides as maltose.—Erratic values for maltose calibration in preliminary work indicated that a longer period of heating in the Shaffer–Somogyi oxidation might be necessary. It was found that thirty minutes in the boiling water bath was necessary to reach a constant titer in this determination.[5] Solutions (5 ml) of maltose in 7 per cent ethanol containing the amounts of anhydrous maltose shown in Table 6 were treated with the Shaffer–Somogyi reagent (as used for fructose) for thirty minutes in the boiling water bath. The titers are shown in Table 6.

TABLE 6.—*Determination of maltose in 7% ethanol with Shaffer-Somogyi reagent*[a]

ANHYDROUS MALTOSE PER 5 ML	0.005 *N* THIOSULFATE REQUIRED
mg	*ml*
0.183	0.57
0.456	1.61
0.913	3.56
1.460	6.15
1.826	7.67
3.652	15.81

[a] SS 50, 5 g KI and 250 ml 0.1 *N* iodate/l; 30 min. heating.

[5] During the course of the work it was demonstrated statistically that blank determinations using water, 1% or 7% alcohol, and 15 or 30 min. heating times all give the same titration value in the Shaffer-Somogyi determination; hence special blanks need not be run.

From these data the following relationship was obtained:

$$M = 0.2264e + 0.075 \quad \text{(VII)}$$

where

M = mg anhydrous maltose in 5 ml

e = ml 0.005 N thiosulfate.

50 M = milligrams anhydrous maltose in 250 ml 7% ethanol eluate. (VIII)

Determination of sucrose.—The 7 per cent ethanol eluate from the carbon column contains sucrose in addition to a considerable proportion of reducing disaccharide. To determine the sucrose, the change in total reducing value after mild acid hydrolysis was used. The procedure devised is outlined below. The hydrolysis is essentially that of the A.O.A.C. (1) for sucrose.

To a 5.00 ml sample in 7% ethanol in a 10 ml volumetric flask is added 1.00 ml dilute HCl (sp. gr. 1.1029) and 1 ml water. It is immersed in a water bath at 60° ±1°C. for 12 minutes and cooled to room temperature. The solution is made just alkaline to bromocresol green with 5 N NaOH and immediately brought to the acid side with 2 N H_2SO_4. It is then made to volume, and the reducing value of 5.00 ml is determined by the Shaffer-Somogyi procedure; 15 minute heating is used. Table 7 shows a calibration of the procedure with the weight of sucrose shown.

TABLE 7.—*Shaffer-Somogyi titration of hydrolyzed sucrose solutions*

SUCROSE IN 5 ML HYDROLYZED	0.005 N THIOSULFATE REQUIRED
mg	*ml*
0.510	1.75
1.004	3.95
2.008	8.72
2.510	11.28

To determine sucrose in the carbon column eluate, 5 ml aliquots are subjected to the procedure described above, and the sucrose equivalent is read from a curve constructed from the data in Table 7. From this value is subtracted the sucrose equivalent (from the curve) of the free reducing sugars in the solution. This may be obtained from the maltose titer. To avoid an extra determination of free reducing value with a 15 minute heating period, the maltose titer (30 min. heating) is multiplied by 0.92 (determined experimentally) and then used. The difference is then the milligrams of sucrose in 5 ml of the column eluate.

$$50(S_1 - S_2) = \text{mg sucrose in 250 ml 7\% ethanol eluate} \quad \text{(IX)}$$

where

S_1 = mg sucrose equivalent to sucrose titer

S_2 = mg sucrose equivalent to 0.92 × maltose titer (e in equation VII)

FRACTION "C"

Determination of higher sugars.—The 50 per cent ethanol eluate from the carbon column should contain all carbohydrate material from trisac-

charide to at least the heptasaccharide (13). To obtain an estimate of this fraction, it was hydrolyzed by the procedure which von Fellenberg (14) applied to honey dextrin—hydrolysis at 100°C. in 1 *N* sulfuric acid for 45 minutes.

To 25 ml of the 50% ethanol eluate in a 50 ml volumetric flask were added 5 ml of 6 *N* HCl and 5 ml of water. After heating by immersion in a boiling water bath for 45 minutes, the flask was cooled, neutralized to bromocresol green with 5 *N* sodium hydroxide, and made to volume; the reducing power was determined on 5 ml by the Shaffer–Somogyi reagent. Glucose equivalent may be obtained from published values (11).

$$\text{Milligrams of higher sugars in 100 ml 50\% ethanol eluate} = 40 \times \text{mg glucose found.} \quad (X)$$

ANALYSIS OF KNOWN MIXTURES

Mixtures of glucose, fructose, sucrose, and maltose in the approximate proportions found in honey were dissolved in 10 ml 1 per cent ethanol, placed on the carbon columns, and analyzed by the procedures outlined above. Table 8 shows the results. Average recovery of maltose and sucrose

TABLE 8.—*Analysis of known sugar mixtures*

MIXTURE NO.	GLUCOSE			FRUCTOSE			MALTOSE			SUCROSE		
	PRESENT	FOUND		PRESENT	FOUND		PRESENT	FOUND		PRESENT	FOUND	
	mg	*mg*	*per cent*	*mg*	*mg*	*per cent*	*mg*	*mg*	*per cent*	*mg*	*mg*	*per cent*
1	301.0	302.6	100.54	325.0	320.5	98.62	68.6	68.6	100.0	52.8	50.8	96.5
2	299.6	304.5	101.63	330.7	327.5	99.03	63.9	63.5	99.4	52.8	52.4	99.3
3	320.0	317.0	99.06	364.5	360.0	98.77	95.1	92.6	97.4	55.2	53.3	96.6
4	319.1	320.4	100.41	386.0	384.5	99.61	82.8	81.8	98.8	62.5	59.6	95.4
5	306.0	310.1	101.35	416.1	414.0	99.49	75.3	73.6	97.7	50.4	47.5	94.3
6	324.0	323.2	99.77	385.6	382.0	99.77	75.4	73.4	97.2	52.0	49.7	95.6
Av.			100.46			99.21			98.4			96.3

was of the same order as that found by McDonald and Perry (5). As expected, average recovery of monosaccharides was better and within the limits of the analytical methods, since adsorption is not so much of a factor under these conditions.

To determine whether materials present in honey have any effect upon the retention of the disaccharides on the carbon columns, weighed amounts of various sugars were added to four 0.8 g samples of a clover honey. These were adsorbed, eluted, and analyzed as already described. Table 9 shows the results.

In the table are shown three analytical values for each sugar as percentage of the honey; the value for the sample to which the sugar in question was added is shown in milligrams only. The average of these three values

TABLE 9.—*Analyses of mixtures of honey and added sugars*

HONEY	SUGAR ADDED		GLUCOSE		FRUCTOSE		SUCROSE		MALTOSE		HIGHER SUGARS	
grams	*Kind*	*mg*	*mg*	*per cent*	*mg*	*per cent*	*mg*	*per cent*	*mg*	*per cent*	*mg*	*per cent*
0.7644	Glucose	105.3	361.1	——	287.5	37.61	10.50	1.37	47.1	6.16	6.56	0.86
0.7867	Fructose	100.2	265.5	33.75	396.0	——	12.10	1.54	48.9	6.22	6.84	0.87
0.8085	Maltose	75.0	268.2	33.17	304.0	37.60	12.85	1.59	123.3	——	10.60	1.31
0.7735	Sucrose	60.5	259.7	33.57	296.5	38.33	68.15	——	48.7	6.30	7.00	0.90
Av.				33.50		37.85		1.50		6.23		

for each sugar was used to calculate the original amount of each sugar in the honey samples to which that sugar was added (Table 10). The difference between the values (sugar calculated in honey+sugar added) −(sugar found) is shown in Table 10. Here again, recoveries of the disaccharides are of the same order of magnitude as those in Table 8 for the pure sugar mixtures. Recovery calculated on the total amount of each sugar present is also shown in Table 10.

TABLE 10.—*Analytical recovery of sugars in honey-sugar mixtures*

	GLUCOSE	FRUCTOSE	SUCROSE	MALTOSE
Wt honey sample, g	0.7644	0.7867	0.7735	0.8085
Sugar in honey (Table 9), %	33.50	37.85	1.50	6.23
Wt sugar added, mg	105.3	100.2	60.5	75.0
Total sugar in sample, mg	361.4	398.0	72.1	125.3
Total sugar found, mg	361.1	396.0	68.2	123.3
Difference, mg	0.3	2.0	3.9	2.0
Recovery (% total sugar)	99.92	99.50	94.4	98.40

SUMMARY

A carbon column adsorption procedure was used for separation of the sugars of honey into monosaccharides, disaccharides, and higher sugars. Integrated with suitable analytical methods, the procedure permitted determination of the sugars of honey with greater accuracy than previously attained. A more realistic picture of the carbohydrate composition of honey was thus obtained.

Recoveries of glucose, fructose, maltose, and sucrose from mixtures averaged 100.46, 99.21, 98.42, and 96.28 per cent, respectively; recoveries of these sugars when added to honey were 99.92, 99.50, 98.40, and 94.40 per cent of the total individual sugar present.

REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D.C., 1950, pp. 518–521.
(2) WHITE, J. W., JR., RICCIUTI, C., and MAHER, J., *This Journal*, **35**, 859 (1952).
(3) TÄUFEL, K., and REISS, R., *Z. Lebensm.-Untersuch. u.-Forsch.*, **94**, 1 (1952).
(4) WHISTLER, R. L., and DURSO, D. F., *J. Am. Chem. Soc.*, **72**, 677 (1950).
(5) MCDONALD, E. J., and PERRY, R. E., JR., *J. Research Natl. Bur. Standards*, **47**, 363 (1951).
(6) ALM, R. S., *Acta Chem. Scand.*, **6**, 1186 (1952).
(7) VOORST, F. T., v. *Chem. Weekblad*, **38**, 538 (1941).
(8) HURD, C. D., ENGLIS, D. T., BONNER, W. A., and ROGERS, M. A., *J. Am. Chem. Soc.*, **66**, 2015 (1944).
(9) CLELAND, J. E., and FETZER, W. R., *Ind. Eng. Chem., Anal. Ed.*, **14**, 27 (1942).

(10) MARSHALL, C. R., and NORMAN, A. G., *Analyst*, **63**, 315 (1938).
(11) SHAFFER, P. A., and SOMOGYI, M., *J. Biol. Chem.*, **100**, 695 (1933).
(12) LOTHROP, R. E., and HOLMES, R. L., *Ind. Eng. Chem., Anal. Ed.*, **3**, 334 (1931).
(13) WHELAN, W. J., BAILEY, J. M., and ROBERTS, P. J. P., *J. Chem. Soc.*, (1953), 1293.
(14) FELLENBERG, T. v., and RUFFY, J., *Mitt. Gebiete Lebensm. Hyg.*, **24**, 367 (1933).

SUGAR ANALYSES OF HONEY BY A SELECTIVE ADSORPTION METHOD

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The authors have recently described a method (1) for the determination of the sugars of honey in which the sample is first subjected to carbon column chromatography to separate the sugars into monosaccharide, disaccharide, and higher sugar fractions. In the present paper are given results of the application of this method to 21 honey samples, representing 19 floral types of honey.

Many polarimetric and reduction methods have been used for the analysis of honey. These methods have not lacked in precision (2), but no assurance of accuracy can be obtained from an examination of the literature. For routine analytical purposes, honey has been regarded as a mixture of glucose and fructose with small amounts of sucrose, and of ill-defined carbohydrate materials collectively analyzed as honey "dextrin." Maltose, identified as the osazone (3), has been reported to occur in honey. van Voorst has described the application of his differential fermentation to 41 honey samples and stated that all contained maltose in amounts ranging from 2 to 7 per cent (4). Hurd, Englis, Bonner, and Rogers later applied a distillation method of sugar analysis to several honey samples. They found "maltose or some other reducing disaccharide" (5) to be present in all five samples examined. Neither of these methods is particularly well adapted to routine analysis, although that of van Voorst is somewhat better than that of Hurd, *et al.*

Paper chromatography shows promise of being most valuable in application to the problem of the identity of the sugars of honey. It has been used for this purpose by Täufel and Reiss (6), Malyoth (7), Vavruch (8), and at this laboratory. In general, no sugars have unequivocally been added to the list occurring in honey, but the complexity of the mixture in indicated. Täufel and Reiss have reported a total of 9 sugars, with 5 unidentified.

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METHODS OF ANALYSIS

The detailed method of analysis is described in another paper (1). In principle, the honey sample (0.8–1.0 g.) is subjected to adsorption on a column of carbon–celite under controlled conditions. Three fractions are obtained by successive elution:

(*A*) Monosaccharides in 1 per cent ethanol.
(*B*) Disaccharides in 7 per cent ethanol.
(*C*) Higher sugars in 50 per cent ethanol.

Glucose and fructose are determined in the *A* fraction by modification (1) of the Marshall–Norman (9) procedure. Sucrose in the *B* fraction is estimated by increase in reducing power following mild acid hydrolysis. Reducing disaccharides in the *B* fraction are determined by copper reduction calibrated against maltose. The higher sugars in the *C* fraction are determined by the reducing power after hydrolysis and are reported as glucose.

Replication.—For each honey type, a single sample was subjected to adsorption. For fructose, duplicate reducing-sugar determinations were done on aliquots from a single hypoiodite oxidation. For glucose, duplicate hypoiodite oxidations were done. For sucrose, duplicate aliquots from the 7 per cent ethanol eluate were hydrolyzed and one reducing sugar determination was done on each. For maltose, duplicate reducing sugar determinations were done on aliquots of the *B* fraction. For the *C* fraction, a single strong acid hydrolysis was done and duplicate reducing sugar values were obtained. In general, Shaffer–Somogyi titers agreed within ± 0.07 ml of .005 *N* thiosulfate; dextrose titers within ± 0.05 ml. of .05 *N* thiosulfate. The sucrose values are those found analytically, corrected for the 94.4 per cent recovery previously determined for the carbon column (1). Maltose values are likewise corrected for the 98.4 per cent recovery found for the columns. Other values are as found.

Paper chromatography of fractions.—As a routine check on the completeness of the carbon column separations, each fraction from the columns for each honey sample was examined by paper chromatography. The chromogenic reagent used (benzidine–citric acid (10)) is sensitive to 1 microgram of sugar on the paper. No appreciable contamination by components of other fractions was found. Traces of sugars from adjoining fractions were shown in the *B* and *C* fractions, but the authors believe that these are analytically insignificant. McDonald and Perry, using ammoniacal silver nitrate in a similar check of their carbon-column analysis (11) of corn sirups, state that no overlapping of the fractions was found. This may be due to the lower sensitivity of the latter reagent.

Figure 1 shows a typical papergram of the three fractions. It is obvious that the *B* fraction (disaccharides) is not limited to maltose and sucrose and that the *C* fraction (trisaccharides and higher sugars) is also a complex mixture. The monosaccharide fractions were all found to contain glucose

and fructose alone with no contamination by other sugars. This indicates that inaccuracy in the determination of these two sugars in honey arising from the presence of other sugars is eliminated and that the glucose and fructose values obtained by this procedure are closer to the actual composition of the honey than those obtained by other methods.

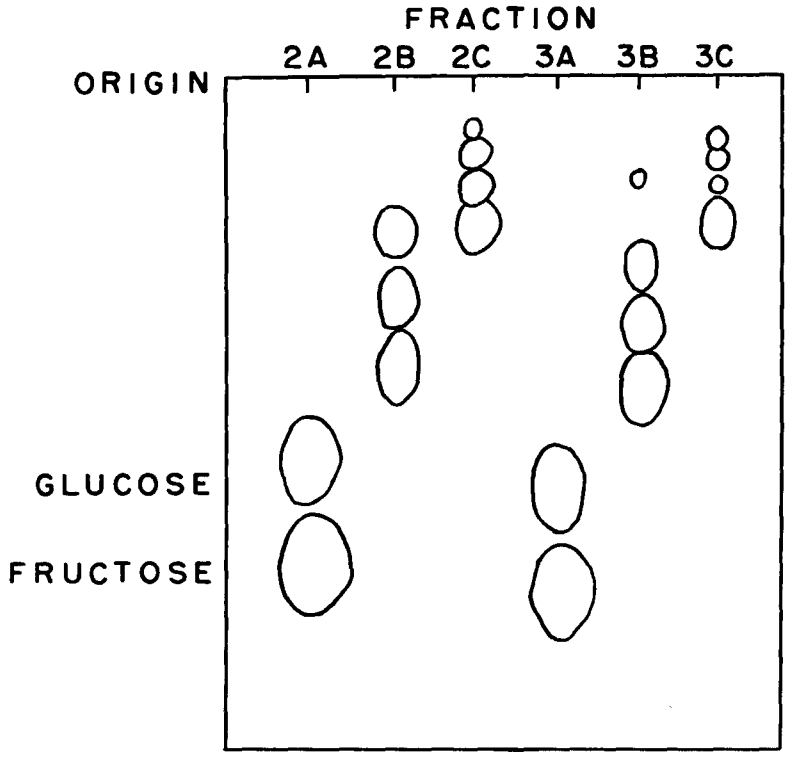

FIG. 1.—Tracing of paper chromatogram of carbon column fractions for samples 2 and 3. Downward irrigation with *n*-butanol–pyridine–water, 3:1:1.5; color spray benzidine–citric acid. The spots of identical R_F in fractions 3B and 3C have beer differentiated by other color reagents.

No claim is made that the reducing disaccharides determined in honey are in fact pure maltose, but for convenience they are so calculated. Figure 1 shows several sugars, three of which are reducing.

The *C*, or higher sugar fraction, probably contains the components determined by conventional honey analyses as "dextrins." It is apparent from a comparison of Figure 1 with the photograph of Buchan and Savage's papergrams of starch conversion products (12) that they are

not true dextrins. The authors believe that the term "higher sugars" is more appropriate for the carbohydrates of fraction *C* from honey.

RESULTS AND DISCUSSION

In Table 1 are shown the results of the application of this analytical procedure to 22 samples (21 honey and 1 honeydew) from 20 different

TABLE 1.—*Carbohydrate composition of honey as determined by selective adsorption method*

NO.	FLORAL SOURCE	CROP YEAR	H_2O[a]	GLUCOSE	FRUCTOSE	MALTOSE	SUCROSE	HIGHER SUGARS	TOTAL SUGARS
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Spanish Needle	1949	17.80	23.52	38.38	14.02	1.11	1.43	78.46
2	Calif. Sage	1948	14.00	25.78	41.97	11.66	1.49	2.54	83.44
3	Tupelo	1951	15.88	24.16	42.42	10.17	1.42	2.50	80.67
4	Basswood	1951	18.04	33.04	37.06	6.84	1.51	2.13	80.58
5	Heartsease	1951	19.08	28.04	38.84	9.16	0.33	0.78	77.15
6	White Thistle	1951	15.84	31.60	36.26	7.58	3.57	3.30	82.31
7	Fallflower	1951	18.00	33.82	37.86	6.16	1.15	0.80	79.79
8	Mesquite	1952	17.64	36.83	38.49	4.34	1.24	0.16	81.06
9	Alfalfa (Calif.)	1951	16.12	34.50	37.29	7.28	4.27	0.68	84.02
10	Eucalyptus	1951	16.60	32.98	39.81	7.80	1.04	0.74	82.37
11	Sweet Clover	1951	16.20	34.36	38.33	6.04	2.57	0.87	82.17
12	Alfalfa (Ariz.)	1951	16.20	33.76	39.13	6.46	1.19	0.65	81.19
13	Calif. Buckwheat	1951	13.40	32.20	41.42	8.15	0.46	0.67	82.90
14	Cotton	1952	19.36	33.66	37.80	6.00	1.11	0.46	79.03
15	Calif. Orange	1952	16.40	33.86	38.88	6.26	3.03	1.16	83.19
16	White Clover	1951	18.12	33.50	37.85	6.33	1.59	0.98	80.25
17	Buckwheat	1951	17.64	34.36	38.67	5.79	0.73	0.80	80.35
18	Pine Tree Forest[b]	1950	14.96	22.18	32.62	18.13	0.96	6.96	80.85
19	Wild Thyme	1950	14.60	24.87	42.42	10.17	1.50	2.50	81.46
20	Dandelion	1952	16.48	40.95	35.53	4.48	1.06	0.47	82.49
21	Tupelo	1953	16.60	26.54	43.95	6.38	0.52	1.74	79.13
22	Gallberry	1953	15.32	30.78	40.52	7.45	1.50	1.18	81.43
	Average		16.72	32.29	39.28	7.11	1.62	1.03	81.31

[a] By refractometer.
[b] Honeydew not included in average.

sources. Table 2 shows the analyses of the first 19 of these samples by other analytical methods. Samples 2, 18, and 19 were analyzed by the A.O.A.C. polarimetric method (13); the others by the Lothrop–Holmes (14) method. Sucrose was determined in all samples by the A.O.A.C. acid hydrolysis procedure (13). These analyses were carried out about six months previous to those in Table 1. In general, the new method gives lower glucose values than previous ones and shows the presence in all samples of reducing disaccharides, which were calculated as maltose. This confirms the observations of van Voorst; good agreement is also shown with the values reported by Hurd, *et al.*, in which the sugars were also separated into mono-, di-, and higher saccharides by distillation of the propionates.

TABLE 2.—*Carbohydrate analysis of honey (per cent) by conventional methods*[a]

NO.	H_2O[b]	GLUCOSE	FRUCTOSE	SUCROSE[c]
1	18.12	32.3	41.5	3.6
2	14.68	31.2	45.3	2.5
3	15.88	28.2	43.8	2.4
4	18.12	35.9	37.0	2.3
5	19.40	33.2	37.2	1.2
6	15.88	33.7	39.6	5.0
7	18.00	37.5	37.4	1.6
8	17.80	39.3	38.9	1.8
9	15.60	37.5	39.0	4.7
10	16.60	36.2	41.6	1.5
11	16.16	37.0	41.0	3.0
12	16.16	37.0	41.8	1.5
13	13.40	36.3	42.3	0.9
14	19.36	37.0	38.8	1.6
15	16.56	35.9	39.6	4.0
16	18.64	36.5	38.1	2.6
17	17.48	38.8	39.0	1.7
18	16.52	28.0	34.0	8.15
19	15.32	31.2	45.4	1.98

[a] Samples 2, 18, 19, by A.O.A.C. polarimetric method; 1, 3–17, by Lothrop-Holmes method.
[b] By refractometer.
[c] Authors are indebted to Mrs. M. S. Gaspar for these sucrose analyses.

Table 3 shows a comparison of the values for selected samples from Table 1 with analyses reported by Hurd, *et al.* (5) for honey samples from the same floral sources. These latter were determined by conversion of the sugars to proprionate esters, followed by fractional distillation; the "tri- and higher saccharides" were calculated from the residue after distillation. Hurd's values as shown in Table 3 were recalculated to the same moisture contents as the samples in this laboratory to facilitate comparison. Agreement is reasonably good, especially in the disaccharide fractions.

The average analyses of honey as reported by several investigators are shown in Table 4. Also shown in Table 4 is the average of the 19 samples reported in Table 2, analyzed by conventional procedures, and the average of the same 19 samples analyzed by the new procedure.

Glucose.—The average content of this sugar was lower by 4 per cent (11.5 per cent less on the individual sugar basis) when determined by the new method than when the same samples were analyzed by other procedures. The average glucose content of all domestic samples in Table 1 is about 2 per cent lower than reported for the 198 samples of Browne and of Eckert and Allinger.

Fructose.—The average fructose content is somewhat lower (1.35 per cent, or 3.3 per cent on the fructose basis) when analyzed by the new method than was found by other methods on the same samples. It is 1.65

TABLE 3.—*Comparison of honey analyses by carbon column adsorption (A) with those reported for a distillation method (B)*

NO.	FLORAL SOURCE	METHOD	H_2O	MONO-SACCHARIDE	DI-SACCHARIDE	TRI- AND HIGHER SACCHARIDE
			per cent	*per cent*	*per cent*	*per cent*
11	Sweet Clover	A	16.2	72.7	8.6	0.9
		B	16.2[a]	74.8	6.3	2.5
5	Heartsease	A	19.1	66.9	9.5	0.8
		B	19.1[a]	66.4	10.1	3.8
15	Calif. Orange	A	16.4	72.7	9.3	1.2
		B	16.4[a]	70.1	10.8	2.7
17	Buckwheat	A	17.6	73.0	6.5	0.8
		B	17.6[a]	70.6	6.9	1.9
3	Tupelo	A	15.9	66.6	11.6	2.5
		B	15.9[a]	68.6	11.4	4.1

[a] All values shown for method B have been recalculated from the original data (Table 1 in ref. (5)) to the moisture contents shown for method A.

per cent lower than the average value for the 198 samples referred to above. This discrepancy may in part be brought about by the presence of reducing ketose (fructose) disaccharides and higher sugars determined as fructose in older methods. This may be confirmed by the determination of reducing ketose groups in the disaccharide fraction. In one honey reported in Table 1 (No. 16) such a determination gave a value of 0.9 per cent for reducing ketose disaccharide content, equivalent in reducing power to 0.5 per cent fructose.

Sucrose.—The average sucrose content by the adsorption method is also lower than was found for the same samples by conventional methods

TABLE 4.—*Average composition of honey as determined by different methods*

SAMPLES	H_2O	GLUCOSE	FRUCTOSE	SUCROSE	MALTOSE	DEXTRIN	METHODS
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
19 Domestic	16.75	32.20	38.80	1.59	7.47	1.24	Selective adsorption, this paper[a]
19 from Table 2	16.82	34.9	40.1	2.7			cf. Footnote *a*, Table 2
19 from Table 1	16.63	30.90	38.75	1.58	8.33	1.62	Selective adsorption, this paper[b]
92 Domestic	17.70	34.02	40.50	1.90		1.51	Polarimetric; reduction (18)
106 California	16.04	34.54	40.41	2.53		0.91	A.O.A.C.: polarimetric, reduction (19)
41 European		30.5	41.5		4.6	1.7	Reduction, differential fermentation (4)

[a] Table 1 with samples 18, 19, 21 excluded.
[b] Table 1, with samples 20, 21, 22 excluded, hence the same 19 samples reported in Table 2.

TABLE 5.—*Approximate melezitose content of honeys and honeydews*

NO.	SOURCE	MELEZITOSE	HIGHER SUGARS
		per cent	*per cent*
18	Pine-tree honeydew	4.33	6.96
19	Wild thyme	0.18	2.50
23	Honeydew mixture	3.51	7.50
24	Honeydew mixture	2.91	6.72
25	Honeydew mixture	3.96	6.31
6	White thistle	1.90	3.30

(Table 4). In both cases the acid hydrolysis procedure was used. It is probable that this difference is caused by sucrose-type linkages in tri- or higher saccharides in the *C* fraction. Such sugars as melezitose or maltosyl fructose (15) would simulate sucrose in the conventional analytical procedure. For example, sample 18 showed 8.15 per cent sucrose by the old procedure and only 0.96 by the new. As shown in Table 5, this honey contains 4.33 per cent of higher sugars containing labile linkages, calculated as melezitose. Sample 19, in which sucrose by both procedures is reasonably in agreement, showed (Table 5) only 0.18 per cent of such compounds.

Maltose.—The presence of reducing disaccharides in the honey samples reported in Table 2 is ignored in the analytical procedures used. As seen in Table 4, these same samples averaged 8.33 per cent of reducing disaccharides (calculated as maltose). This amount of disaccharide would simulate $180/342 \times 8.33 = 4.38$ per cent glucose if determined by A.O.A.C. methods. This value may be compared with the average 4.0 per cent lower glucose content for these samples found by the selective adsorption procedure.

Dextrin.—No comparative values are available for dextrin by conventional procedure and higher sugars by the new method on the same samples. However, the 1.30 per cent average for higher sugars for the 22 samples in Table 1 may be compared with the average of 1.19 per cent dextrin for the 198 samples analyzed by Browne and by Eckert and Al Allinger (Table 4). These 198 samples were analyzed for "dextrin" by alcoholic precipitation, while the new method determines higher sugars after isolation from monosaccharides and disaccharides, by copper reduction after hydrolysis.

Determination of melezitose.—The trisaccharide melezitose is a component of honeydew (16). The melezitose content of a sample can be approximated from the increase of reducing power brought about by the mild hydrolysis of the "*C*" fraction. According to von Fellenberg (17), the application of mild hydrolysis to melezitose produces reducing sugar equivalent to 67.9 per cent of its weight of glucose. The higher sugar fraction of several samples has been analyzed in this manner. Results are shown in Table 5.

Undetermined.—It may be seen from Table 1 that on the average 1.95 per cent of the 21 honey samples remains unaccounted for. This undetermined fraction varies from 4.27 per cent in sample 21 (tupelo) to zero (within the limits of error of the analysis) for samples 9 (California alfalfa) and 15 (California orange). Browne (18) reported 3.7 per cent undetermined for his 92 domestic samples; Eckert and Allinger (19) reported 4.7 per cent. For the 25 samples reported by Lynn, *et al.* (20), 4.1 per cent was not accounted for. The principal reason for this improvement over older procedures is the reducing disaccharide fraction, representing an average of 7.11 per cent. In the A.O.A.C. procedure, it is analyzed on the basis of its reducing value and simulates about half its weight of glucose. Thus there is a net gain in dry matter recovery.

A picture of the carbohydrate composition of honey may be deduced from these considerations. Although based upon only 21 samples of 19 different floral types, the following generalizations are proposed. Honey contains somewhat less dextrose and levulose than heretofore supposed, less sucrose, and appreciable amounts of reducing disaccharide material which is largely aldose, some reducing ketose disaccharide, and small amounts of trisaccharide and of higher sugars, some of which contain linkages of about the same ease of hydrolysis as the sucrose linkage.

SUMMARY

Twenty-one honey samples representing 19 floral sources have been analyzed for carbohydrate content by a new selective adsorption procedure. Average values found were: moisture, 16.72 per cent; glucose, 32.29 per cent; fructose, 39.28 per cent; sucrose, 1.62 per cent; maltose, 7.11 per cent; and higher sugars, 1.03 per cent. This represents a considerably lower glucose content, somewhat lower fructose and sucrose levels, and significant amounts of reducing disaccharides (as maltose) when compared with average results by previous methods. Previously postulated occurrence of reducing disaccharides in honey is confirmed.

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REFERENCES

(1) WHITE, J. W., JR., and MAHER, J., *This Journal*, **37**, 466 (1954).
(2) WHITE, J. W., JR., RICCIUTI, C., and MAHER, J., *ibid.*, **35**, 859 (1952).
(3) ELSER, E., *Mitt. Gebiete Lebensm. Hyg.*, **15**, 92 (1924).
(4) VOORST, F. T. v., *Z. Untersuch. Lebensm.*, **83**, 414 (1942).

(5) HURD, C. D., ENGLIS, D. T., BONNER, W. A., and ROGERS, M. A., *J. Am. Chem. Soc.*, **66**, 2015 (1944).
(6) TÄUFEL, K., and REISS, R., *Z. Lebensm.-Untersuch. u. -Forsch.*, **94**, 1, 1952.
(7) MALYOTH, E., *Naturwiss.*, **38**, 478 (1951).
(8) VAVRUCH, I., *Chem. Listy*, **46**, 116 (1952); through *Chem. Abst.*, **46**, 11497h (1952).
(9) MARSHALL, C. R., and NORMAN, A. G., *Analyst*, **63**, 315 (1938).
(10) WHITE, J. W., JR., and MAHER, J., *Arch. Biochem. Biophys.*, **42**, 360 (1953).
(11) MCDONALD, E. J., and PERRY, R. E., JR., *J. Research, Natl. Bur. Standards*, **47**, 363 (1951).
(12) BUCHAN, J. L., and SAVAGE, R. I., *Analyst*, **77**, 401 (1952).
(13) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950.
(14) LOTHROP, R. E., and HOLMES, R. L., *Ind. Eng. Chem., Anal. Ed.*, **3**, 334 (1931).
(15) WHITE, J. W., JR., and MAHER, J., *J. Am. Chem. Soc.*, **75**, 1259 (1953).
(16) HUDSON, C. S., and SHERWOOD, S. F., *ibid.*, **42**, 116 (1920).
(17) FELLENBERG, T. v., and RUFFY, J., *Mitt. Gebiete Lebensm. Hyg.*, **24**, 367 (1933).
(18) BROWNE, C. A., *Bull. 110, Bur. Chem.*, U. S. Department of Agriculture, 1908.
(19) ECKERT, J. E., and ALLINGER, H. W., *Bull. 631, Agric. Expt. Sta.*, University of California (1939).
(20) LYNN, G. E., MILUM, V. G., and ENGLIS, D. T., *33rd and 34th Ann. Rept., Ill. State Beekeeper's Assoc.*, 54 (1934).

A POLAROGRAPHIC METHOD FOR THE QUANTITATIVE DETERMINATION OF SACCHARIN IN FOODS*

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Saccharin in pure solution can be determined polarographically (2); however, when attempts are made to measure it quantitatively in some foods, methods suitable for pure solutions fail because of interfering substances. In a preliminary investigation to this study, it was determined that the inability to obtain polarographic waves of saccharin in food was due to small amounts of the oxalate ion and somewhat larger amounts of the acetate ion, which interfered with the polarographic wave. Since a large number of foods contain one or both of these substances, it was necessary that acetate and oxalate be removed from the food before the polarographic procedure could be applied.

CALIBRATION CURVES

A series of 25 linear regression calibration curves were made, consisting of seven dilutions each. Each curve was set up with sodium saccharin in

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concentrations of 2.00, 1.60, 1.40, 1.20, 0.80, 0.40, and 0.20 mg per ml. The supporting electrolytes consisted of: (*a*) 5.0 ml of 0.1 *N* HCl and 5.0 ml of 0.1 *N* H_3PO_4; and (*b*) 5.0 ml of 0.1 *N* HCl and 5.0 ml of 0.1 *N* KCl. In all cases 5.0 ml aliquots of the various standard saccharin solutions were used for the determinations.

METHOD

A sample of food, to which a known amount of sodium saccharin had been added, was mixed with water in a Waring blendor to bring the mixture to a workable consistency. An excess of five per cent lead acetate solution was added and the mixture was shaken vigorously. The resulting lead precipitates were removed by filtration and the filtrate was strongly acidified with HCl. The saccharin was extracted by washing each sample three times with 80 ml portions of ethyl ether (1). The extractions were carried out by shaking the mixtures and the ether for five minutes in a separatory funnel. Foreign material was removed from the solvent by washing the solution with about ten ml of distilled water, and the extracts were evaporated to dryness. The residues were washed into 50 ml volumetric flasks and brought to volume with 0.1% Na_2CO_3. A five ml aliquot of the carbonate–saccharin solution was placed in an electrolysis cell with the supporting electrolyte. The applied E. M. F. for all determinations was $-1.4V$.

RESULTS AND DISCUSSION

Calibration curves.—The mean error for the 25 calibration curves was 0.92 per cent, calculated by the square of the correlation coefficient, *r*. The mean diffusion coefficient was 1.82×10^{-7} cm^2 sec^{-1}; the use of statistical confidence limits (0.05 probability level) demonstrated that the true diffusion coefficient was between 1.66×10^{-7} and 1.98×10^{-7} cm^2 sec^{-1}. The half-wave potentials ($E_{\frac{1}{2}}$) ranged from -1.14 to -1.19 *V;* with a mean $E_{\frac{1}{2}}$ value of -1.16 *V*. The regression equation for the composite calibration curve was $i_d = -0.732 + 0.467\ X$ microamperes (*X* equals the concentration in millimoles/liter). Since none of the individual calibration curves varied significantly from the composite curve, the results reported are based on the composite curve.

A summation of the results of polarographic runs may be found in Table 1. More consistent results were obtained using the HCl–H_3PO_4 supporting electrolyte than the HCl–KCl supporting electrolyte. The latter was therefore discontinued in the second part of this study. Only results obtained with the HCl–H_3PO_4 supporting electrolyte are reported. Difficulties were encountered in extracting the saccharin from Russian type dressing, candy mints, ketchup, and ice cream; a semi-gel was

TABLE 1.—*The per cent recovery of saccharin and the half-wave potentials of polarographic determinations in 11 representative foods*

FOODS	NO. OF SAMPLES	pH[a]	$E_{\frac{1}{2}}$	PERCENTAGE RECOVERY	
				RANGE	MEAN
Watermelon rind preserves	5	1.86	−1.17	90.2– 95.2	92.5
Dill pickles	9	1.87	−1.15	91.2– 96.8	94.4
Wine	5	1.81	−1.15	92.8– 94.1	93.5
Russian type dressing	5	1.82	−1.14	82.0– 91.8	86.0
Fruit preserves	5	1.95	−1.14	88.1– 92.1	89.6
Carbonated cola type beverage	11	1.84	−1.16	96.1–101.2	99.3
Ice cream	5	1.67	−1.16	93.2– 99.1	95.0
Ketchup	5	1.75	−1.16	95.0–104.4	103.3
Candy mints	5	1.85	−1.15	81.9– 86.2	83.3
Non-carbonated fruit drink	4	1.84	−1.15	96.1–100.1	97.2
Liqueur	4	1.79	−1.16	94.2– 98.6	96.8

[a] The pH values given are for the samples in HCl-H_3PO_4 supporting electrolyte after completion of the determination.

formed upon the addition of ether to these substances. The gelation did not significantly affect the results but made the extraction more difficult. The extraction was facilitated in the carbonated cola type of beverage and in the dill pickles by making the food alkaline with sodium hydroxide before extraction.

SUMMARY

(1) Acetate and oxalate ions were shown to interfere with the quantitative polarographic determination of saccharin in foods, using HCl–H_3PO_4 and HCl–KCl supporting electrolytes.

(2) A procedure is presented for the quantitative polarographic estimation of soluble saccharin in foods. The method is considerably more rapid than the present official objective method (1). The amount of saccharin recovered from 11 representive foods by the method reported herein ranged from 81.9 per cent to 104.4 per cent. The mean recovery of the saccharin was 93.7 per cent.

REFERENCES

(1) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C., 1950.

(2) KOLTHOFF, I. M., and LINGANE, J. J., *Polarography*, Vol. 2, 2nd Ed., Interscience Publishers, Inc., New York, 1952.

COLOR-MOISTURE RELATIONS OF YELLOW SWEET CORN*

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Methods for the sorting of fruits and vegetables based on color are being developed at the Western Regional Research Laboratory as an aid in processing and utilization (1, 2). An experimental lemon sorter was recently developed by and successfully operated at the Davis Agricultural Experiment Station of the University of California (6). The machine was capable of dividing the lemons into five grades or stages of maturity, at a rate of from 5 to 10 lemons per second. The starting point for this development was provided by basic color data, in the form of spectrophotometric reflectance curves, obtained from lemons in various stages of ripening.

This paper deals with methods of obtaining the color reflectance data of yellow sweet corn as a function of its moisture content. The moisture content is one of the criteria for maturity used in the processing of sweet corn.

COLOR DATA

Spectrophotometric data for three samples of Golden Cross T strain yellow sweet corn are reproduced in Fig. 1. The curves show their reflectance in per cent of that of a vitrolite working standard for narrow wavelength bands in the visible region. These traces were obtained from the undisturbed exposed surfaces of the kernels on fresh cob corn. The same kernels were removed and their moisture content determined by a vacuum oven method.[1] The specifications of color were obtained through tristimulus integration (7) of the spectrophotometric curves. These color specifications are composed of numbers representing the three attributes by which any color may be described.

The data, shown in Fig. 1, illustrate that as the moisture content of the corn kernels decreases, an increase is observed in the two attributes of the quality or kind of color. First, the attribute of excitation purity, which corresponds to saturation, richness, or Munsell chroma, refers to the location of the sample color between a neutral gray and the corresponding pure spectrum color. In this instance there is a change from 46.0 per cent to 56.3 per cent on the scale where gray is 0 and the pure spectrum color is 100 per cent. This increase is exhibited in the spectrophotometric curves by the increased difference between the upper and

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† Bureau of Agricultural and Industrial Chemistry, Agricultural Research Service, U. S. Department of Agriculture.

[1] The samples of corn were blended with an equal weight of water and aliquots of the mixture predried in a forced draft oven for 16 hours at 60°C. The samples were then dried in a vacuum oven for 40 hours at 70°C.

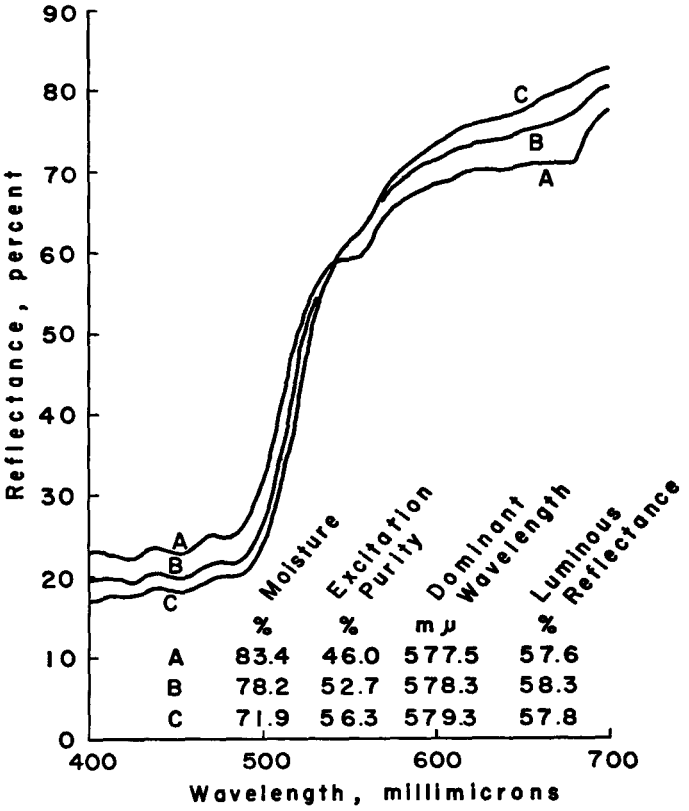

FIG. 1.—Spectrophotometric curves and color data for three samples of Golden Cross T strain yellow sweet corn of different moisture content.

lower portion of each curve. Second, the attribute of dominant wavelength, which corresponds to hue, that is, red, yellow, green, blue, etc., refers to the wavelength of the pure spectrum color which most nearly matches the sample. The small shift in dominant wavelength, from 577.5 to 579.3 millimicrons, is difficult to see from an examination of the spectrophotometric curves, but it may be illustrated here by noting the relative positions of the more nearly vertical portions of the traces. No definite trend is illustrated here by the values of the third attribute of color, namely, luminous reflectance. This attribute corresponds to lightness or Munsell value, and is the proportion of total light reflected relative to the amount incident upon the sample. The values given refer to a scale where 0 is black and 100 per cent is white. In the present case, the vitrolite glass reference standard of reflectance is assumed to have a luminous reflectance of 100 per cent.

Fig. 2 shows a plot of color data obtained on fifty samples of yellow sweet corn. The first two attributes of color quality, discussed above in connection with corn samples A, B, and C of Fig. 1, were obtained from this portion of the chromaticity diagram, or Maxwell triangle, as employed by the International Commission on Illumination (I.C.I.) color system (4, 7). The plotted points representing the data obtained from curves A, B, and C are encircled and labeled. The encircled but unlabeled

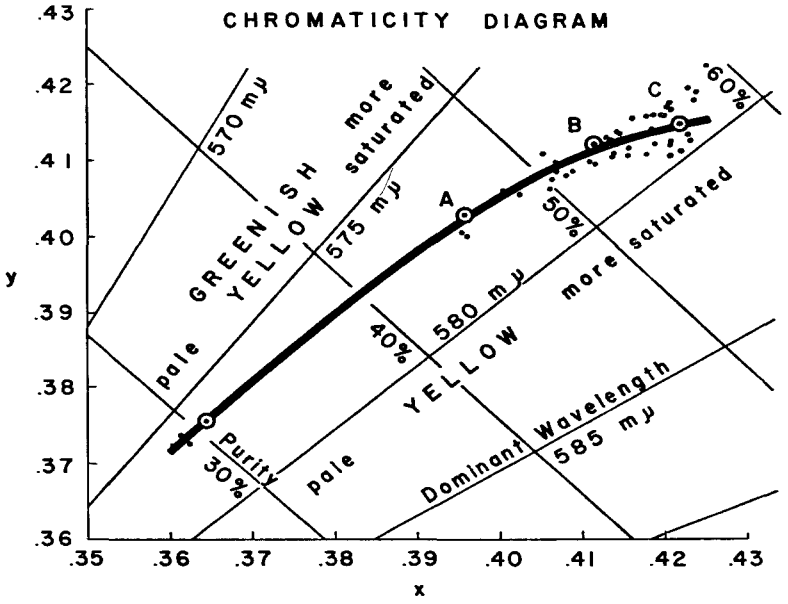

FIG. 2.—Variations of dominant wavelength and purity for samples of Golden Cross T strain yellow sweet corn. A wide range of moisture content is represented; samples containing a very high percentage appear at lower left and those of lower percentage toward the upper right.

point at the extreme lower left corner of Fig. 2 was obtained from a very immature, pale yellowish-white sample of corn. Toward the upper right are the more mature samples of lower moisture content, higher purity, and more yellow dominant wavelength. The per cent moisture increments between adjacent encircled points are approximately equal, the values of per cent moisture decreasing from lower left to upper right by increments of 5.2, 5.2, and 6.3, respectively. The color-moisture relation, presented here, illustrates that the increase in purity is relatively greater than the increase in dominant wavelength. These data show also that the purity of the color does not continue to increase at a uniform rate with per cent moisture, but tapers off. On the other hand, the dominant wavelength

appears to increase in a more uniform manner with respect to changes in per cent moisture.

The next two figures present the relationships of per cent moisture with each of these two color quality attributes separately. First, in Fig. 3, is shown the non-linear relation obtained by plotting per cent moisture versus per cent purity. From left to right the color strength increases from a pale, nearly neutral shade, toward a more saturated shade containing a greater proportion of a pure spectrum color. Second, Fig. 4 presents a plot of per cent moisture versus dominant wavelength. In this figure the change from left to right corresponds to a change in hue from a greenish yellow to yellow in the direction toward orange and red.

Both Figs. 3 and 4 present relationships which are possible bases for development of automatic means of rapidly measuring the percentage of moisture contained in the kernels of individual ears of yellow sweet corn without altering or destroying the samples. The purity-moisture relation, while not as desirable from a linearity standpoint, does have the advantage that larger light energy differences are available which facilitate the detection of small moisture differences. The moisture-dominant wavelength relation, however, has the desirable feature of better linearity especially in the regions of lower moisture content.

INSTRUMENT DEVELOPMENT

The purpose of developing the instrument (5) shown in Figs. 5 and 6 is to test means of determining the moisture content of yellow sweet corn by measuring related color attributes. It is desirable that the methods be readily adaptable to equipment designed for rapidly and automatically sorting yellow sweet corn for processing. In using this laboratory photometer to measure the purity of the corn color, the sample under examination is placed in position on the integrating sphere at top center. A projection lamp is mounted in the lower right enclosure. Light from it is arranged to pass though a rotating disk composed of red and blue filter segments. The corn is illuminated alternately in this manner by red and blue lights corresponding to upper and lower portions of the spectrophotometric curves of Fig. 1. The reflected light falls on a bank of phototubes beneath the integrating sphere. The phototubes are arranged to control an electric current which then pulsates in accordance with the sample's difference in reflective properties for lights of these two colors. The magnitude of these pulsations is determined by a modified electronic voltmeter circuit. Readings obtained from this meter are plotted against purity in Fig. 7, which shows the degree of linearity obtained by this method and the agreement of the two sets of data for these samples.

Some results of measurements with the photometer on this set of fifty ears of Golden Cross T strain yellow sweet corn[2] are shown in Fig. 8.

[2] Supplied by the Agricultural Experiment Station of the University of California, Davis, Calif.

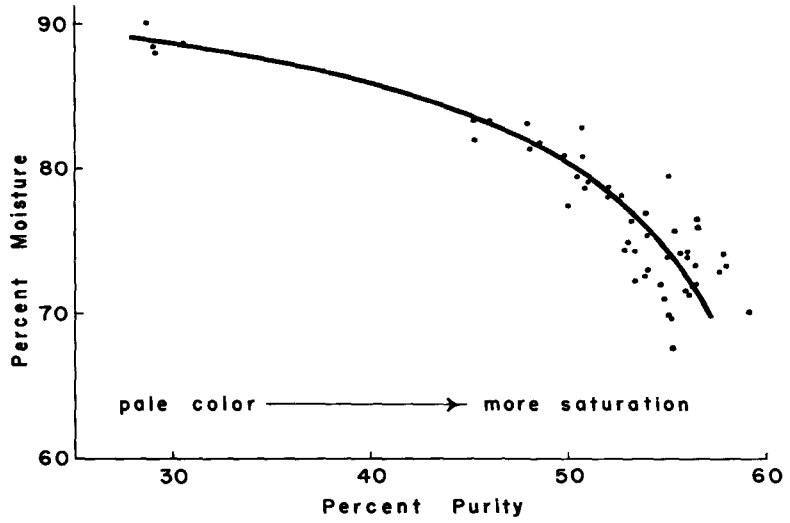

FIG. 3.—The color-moisture relation showing the development of a more saturated shade with decreasing moisture content.

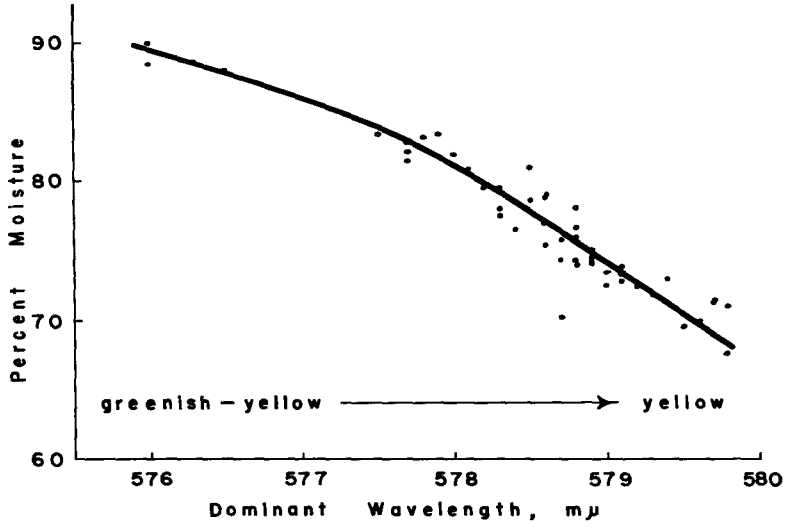

FIG. 4.—The color-moisture relation showing the development of a more yellow hue with decreasing moisture content.

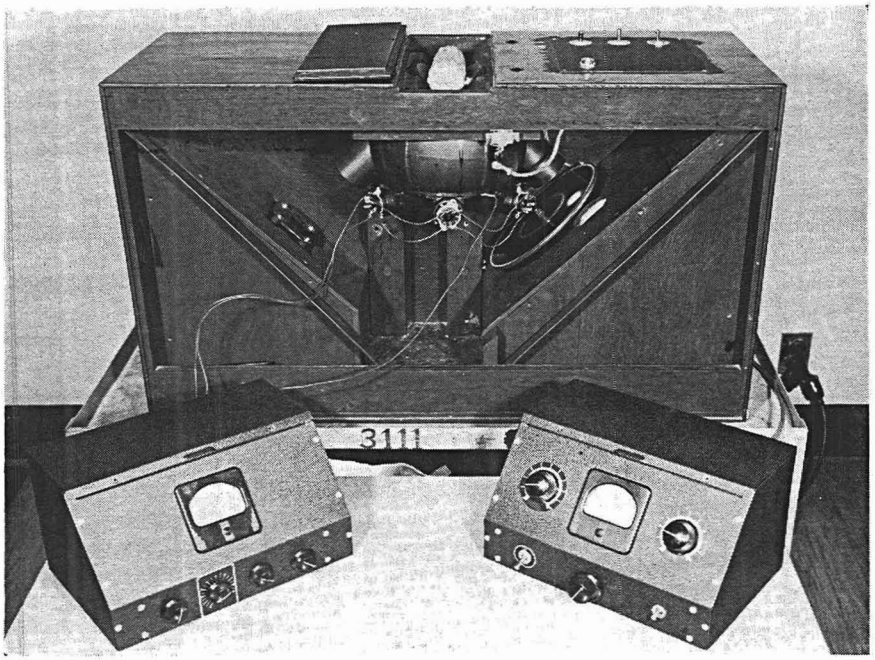

FIG. 5.—The experimental photoelectric photometer adapted to measure the excitation purity of the color of yellow sweet corn.

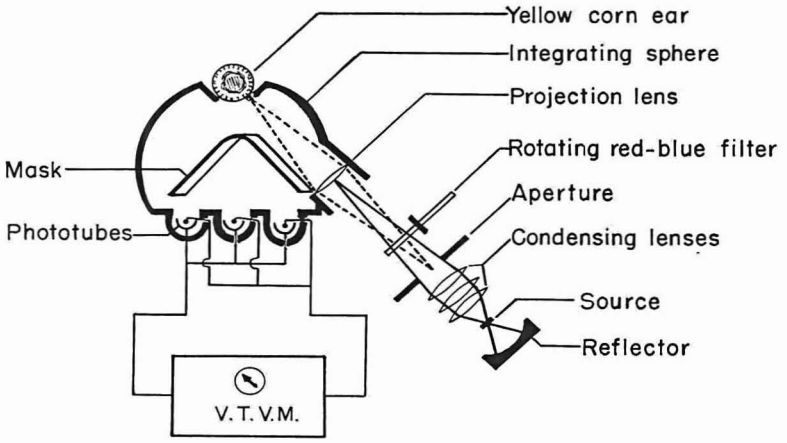

FIG. 6.—Schematic diagram of the optical system and rotating filter disk of the experimental photoelectric photometer adapted to measure the excitation purity of the color of yellow sweet corn.

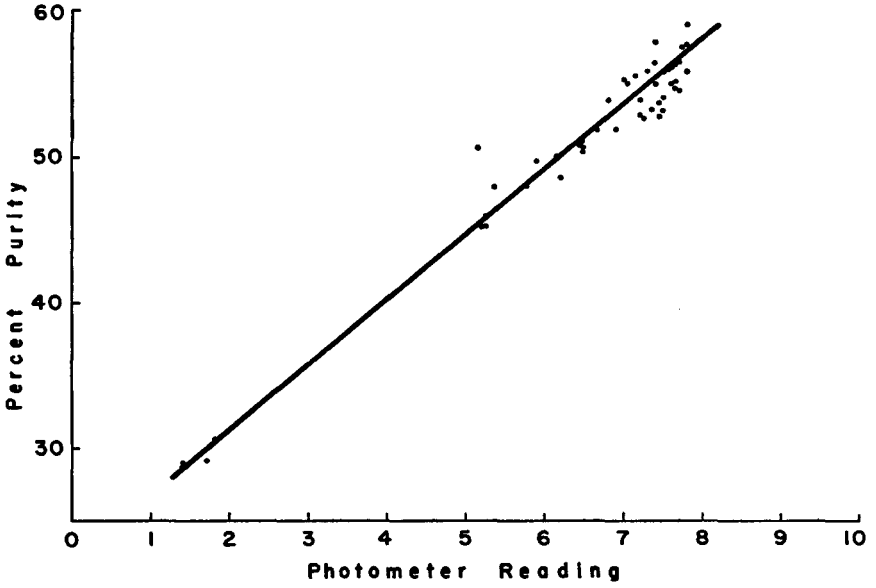

FIG. 7.—Agreement of the data obtained on the experimental photoelectric photometer with excitation purity specifications.

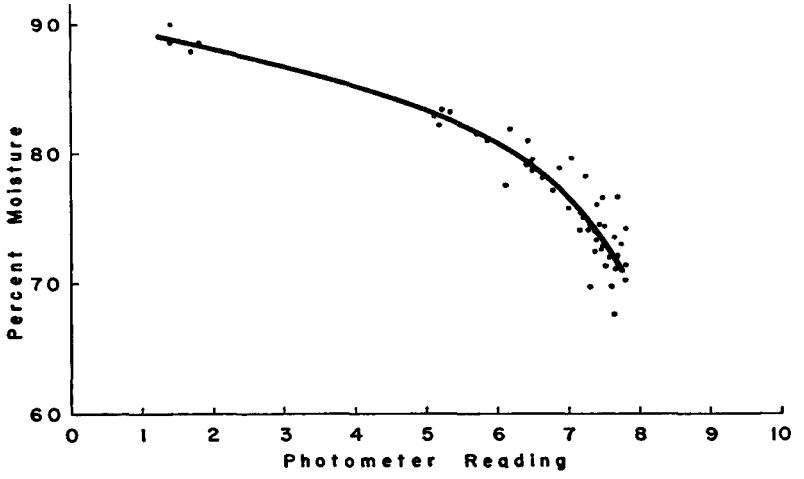

FIG. 8.—Relation of moisture content with experimental photoelectric photometer data on purity of the color of Golden Cross T strain yellow sweet corn.

These samples were harvested from one plot, iced in the husk within fifteen minutes and measured about five hours later. An attempt was made to include samples of a wide range of moisture content. The four high-moisture samples, represented at upper left, had developed only a very pale yellow color. All measurements were obtained from an area of the kernels visually selected for uniformity of color in an attempt to reduce most of the variations of nonuniform development. However, the data are considered as containing variations arising from natural phenomena as well as those due to the experimental difficulties encountered.

Some differences found in varieties of yellow sweet corn are illustrated in Fig. 9. Curves representing data on Golden Cross Bantam[3] and Iochief[3] (broken lines) are compared with the one from Fig. 8 on Golden Cross T strain (solid line). Variables of soil and climate as well as variety should receive consideration.

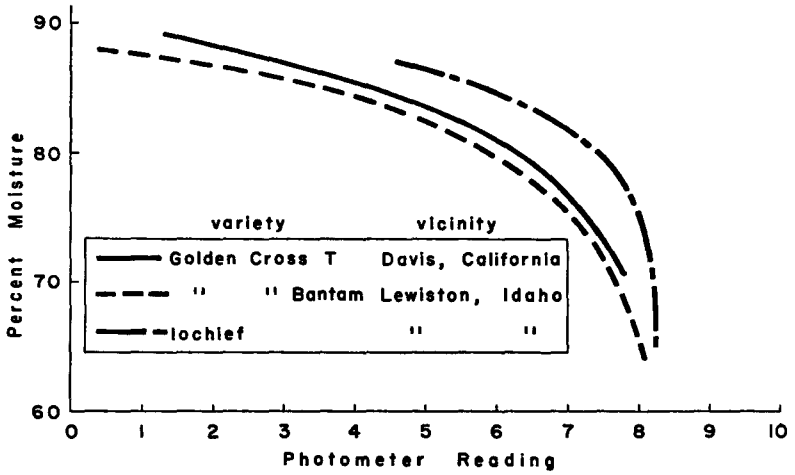

FIG. 9.—Comparison of the moisture-purity relations as determined by the experimental photoelectric photometer on different varieties of yellow sweet corn.

A variation of the other color-moisture relation, per cent moisture versus dominant wavelength (Fig. 4), might be found very useful as a basis for moisture determinations through color measurement. The more nearly linear relationship provided is shown in Fig. 10, where per cent moisture is plotted against the difference of two sums obtained by tristimulus integration of the spectrophotometric curves. The first sum, $S(x)$,[4] represents

[3] Supplied by the Agricultural Experiment Station of the University of Idaho, Moscow, Idaho, and grown at the Lewiston Field Station, Lewiston, Idaho.

[4] $S(x)$ and $S(y)$ are sums obtained through mechanical integration, according to the selected ordinate method (7), with a tristimulus integrator which was fitted with a scale marked with thirty ordinates for I.C.I. illuminant C.

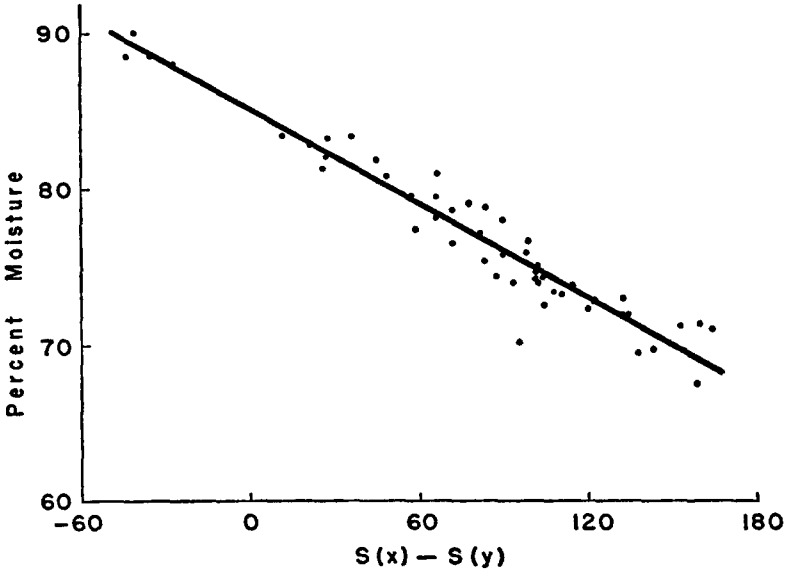

FIG. 10.—A practical variation of the color-moisture relation–per cent moisture versus dominant wavelength. ($S(x)$ and $S(y)$ are factors related to the reflectances of the sample for the corresponding I.C.I. primaries.)

the reflectance of the sample for red plus blue light. The other sum, $S(y)$, refers to that of the green region. While this combination appears to be a measure of the dominant wavelength for the present purpose, this conclusion must not be inferred for the general case.

The adaptation of the present laboratory photometer to measure the dominant wavelength of the corn color could be accomplished. The red segment of the rotating disk would be replaced by a suitable combination filter which transmits in both the amber and blue regions, approximating $S(x)$, and a suitable green segment for $S(y)$ would be substituted for the blue one. Then, with necessary minor changes, such as increasing the sensitivity of the electronic voltmeter circuit, one should be able to obtain data resembling those of Fig. 10. To illustrate this type of measurement, Fig. 11 presents data obtained with the Hunter Color-Difference Meter (3, 4) on the same fifty samples of Golden Cross T strain yellow sweet corn. Per cent moisture is plotted against readings obtained with the Hunter chromaticity scale[5] which embodies filters of similar nature. Hunter's selection and arrangement of filters, photocells and other components are such that the readings obtained with this particular chromaticity

[5] The a_R scale. This one of the Hunter chromaticity scales is defined (4) in terms of the I.C.I. tristimulus values, X and Y, of the color.

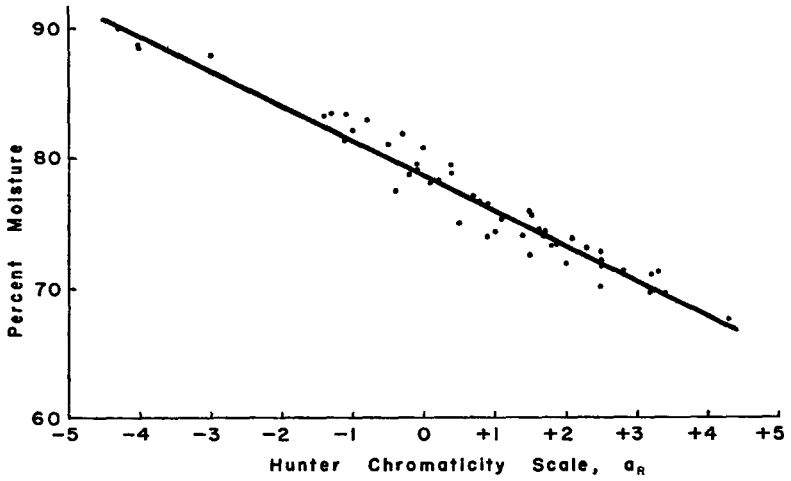

FIG. 11.—Application of the Hunter Color-Difference Meter to a method for approximating the dominant wavelength–per cent moisture relation of Golden Cross T strain yellow sweet corn.

scale are related to the differences between the two tristimulus integration sums, $S(x)$ and $S(y)$, which were employed in plotting Fig. 10. Results of this type lend credibility to the conclusions reached through this study.

CONCLUSIONS

Definite and reproducible relationships exist between the color of yellow sweet corn and its moisture content. These relationships should be of value in the detection of the stage of development of the sweet corn. The experimental results indicate that color changes due to variety and to agronomic or climatic conditions must be taken into consideration. A laboratory experimental model of a photoelectric photometer for evaluating the color of yellow sweet corn has been constructed and tested. This type of apparatus for evaluating color should be suitable for adaptation to automatic sorting for processing.

ACKNOWLEDGMENTS

The authors are indebted to Henry M. Wright and Marion C. Long of the Analytical Section of this Laboratory for the moisture determinations; to Prof. J. E. Knott of the Agricultural Experiment Station of the University of California, Davis, and to Prof. George W. Woodbury of the Agricultural Experiment Station of the University of Idaho, Moscow, for supplying the samples of yellow sweet corn for this work; and to

K. J. Palmer, W. C. Dietrich, and several other members of the staff of the Western Regional Research Laboratory for valuable assistance and suggestions during the course of this study.

REFERENCES

(1) EASTMOND, E. J.. PETERSON, J. E., and STUMPF, RUTH, *Food Technol.*, **5**, 121 (1951).
(2) EASTMOND, E. J., *Advances in Chemistry Series* No. **3**, 3 (1950).
(3) HUNTER, R. S., *J. Optical Soc. Am.*, **38**, 661 (1948).
(4) JUDD, D. B., *Color in Business, Science and Industry*, John Wiley & Sons, Inc., New York, 1952.
(5) LUKENS, H. C., MACKENZIE, R. P., and KUNSMAN, C. H., *J. Optical Soc. Am.*, **41**, 873 (1951).
(6) POWERS, J. B., GUNN, J. T., and JACOB, F. C., *Agricultural Engineering*, **34**, 149 (1953).
(7) Staff of Massachusetts Institute of Technology, Color Measurement Laboratory; *Handbook of Colorimetry*, The Technology Press, Cambridge, Mass., 1936.

SOURCES OF VARIATION IN THE DETERMINATION OF VITAMIN D BY THE LINE TEST

By C. I. BLISS,* C. WHITMAN,† F. G. MCDONALD,‡ and C. E. BILLS§

In the line test for Vitamin D, the experimenter scores in each rat the degree of calcification of the metaphysis in a leg bone and computes the relative potency of an unknown preparation from a series of such scores. Although he is aided by sample bones, photographs, or diagrams, his scores are estimates on an arbitrary scale with an imprecision that limits the reliability of an assayed potency. This, however, is only one of several sources of potential error in the vitamin D assay. The bone selected for scoring, the number of rats assigned to each dose, and their allotment in respect to litter, age, and body weight—all may influence the precision of the assay. These and other factors have been controlled or balanced in the U.S.P. assay (8).

A quantitative evaluation of their relative importance, as demonstrated in an experiment carried out for this purpose at Mead Johnson and Co., in 1942, is attempted in the present study. Since the potency of vitamin D continues to be determined biologically, our findings are still relevant to the design of efficient assays and are presented for their possible value in the drafting of the vitamin D assay for the U.S.P. XV.

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EXPERIMENTAL PROCEDURE

The experimental rats were of the inbred, pied, Wisconsin laboratory strain. At birth, litters were reduced to eight rats of which six were used later in the experiment. They were raised from birth (in February) on a stock diet consisting of 75 per cent of Mead's Pablum and 25 per cent of powdered whole milk. When they were twenty-one days old, they were transferred to the ricketogenic McCollum Diet No. 3143, which had been cooked under steam pressure and dried on a rotary drum drier. Its Ca:P ratio was 5:1 and it produced florid rickets in twenty days. During this depletion period half of the litters were caged together; the rats in the other half were caged individually. The difference had no detectable effect upon the assay results and has been disregarded in the analysis presented here. During the assay period all rats were caged individually. Twenty-eight litters were carried to the assay period, at which time four unsuitable ones were discarded.

The vitamin D supplement consisted of U.S.P. Reference Standard Cod-Liver Oil No. 2 diluted with corn oil. It was fed at six dosage levels, starting at the equivalent of 1.06 mg of the reference oil per day and increasing by a factor of $\sqrt{2}$ to the highest dose of 6.0 mg daily for six days. Hence the data provided a dosage–response curve with six dosage levels, A–F.

Rats were assigned to these doses in two series, each series consisting of two 6×6 Latin squares. Columns represented litters, and letters indicated different doses of vitamin D. The rows balanced two graded or quantitative variables within litters—body weight and age—although the effects of rows were studied by co-variance in the analysis. In the first series the rats of each litter were placed upon the test diet on the same day, so that all dosage levels had the same average age of depletion. Within litters, the rats were assigned to rows in order of weight. In the second series the rats in each litter were placed upon the test diet as each individual reached approximately the same weight as its heaviest ricketic litter mate. Here the rows of the design represented ranking by age within litters. One Latin square is shown below, the other three followed a similar pattern; each was randomized separately.

Ranking by weight (or age)	*Litter No.*					
	1	2	3	4	5	6
1	B	A	C	E	F	D
2	F	E	D	A	B	C
3	C	D	F	B	A	E
4	D	F	B	C	E	A
5	A	C	E	F	D	B
6	E	B	A	D	C	F

At the end of the assay period of seven days, the rats were killed and the radii and tibiae were removed from both the left and right legs of each rat. These bones were prepared for the line test in the usual manner (8) and labeled so as to conceal the dosage of vitamin D. Two observers read each bone independently, scoring the metaphyseal healing in grades from 0 to 4 by quarter units on the Bills scale (1). To simplify later calculations, each reading was multiplied by 4 to convert it to a whole number, but most of the computed results are reported in units on the original scale. Records were kept of the weight of each rat at depletion, of its food consumption, and of its gain or loss in weight during the seven-day assay period.

The error in reading the line test.—The error in reading the line test was examined by comparing scores assigned independently to the same bone by each of two observers. For this purpose the dose of vitamin D and the source of the bond were not relevant, provided the rat was neither completely ricketic nor completely healed. On these grounds, nineteen rats showing no healing in three or four bones were dropped from this part of the analysis and twenty-seven others were omitted due to mistakes or omissions in recording. There remained a total of ninety-eight individuals in which all four bones were scored by both observers.

Frequency distributions were then prepared from the difference for each bone between the score of observer A minus that of observer B. Judging from an analysis of variance of these differences, the means of the scores assigned by the two observers did not differ in any of the four bones, left and right radii and tibiae. Although the observers agreed in their average scores for each bone, they might not have agreed over different parts of the scoring scale. This possibility has been tested by tabulating the differences between the two scores for each bone against their sums and combining the results on all bones (with the exception of twenty bones having a total score of zero; Table 1).

From an analysis of variance, the column means in Table 1 differed more from one another than would be expected to occur by chance. The trend of the mean scores in the last row suggest that observer B may have tended to overscore poor healing and underscore good healing in comparison with observer A. Because of this difference, later calculations were based only upon the scores recorded by the more experienced observer A.

By segregating the variation between the columns in Table 1, the reading error in assigning line test scores could be computed. The standard deviation within columns (with 364 degrees of freedom) was divided by $4\sqrt{2}$ to obtain $s = 0.204 \pm 0.008$ as the standard deviation in scoring a single bone on the original scale from 0 to 4. Since this is less than the one-quarter unit in which the bones were graded, an experienced observer should be able to score the line test in even smaller graduations than were used here.

TABLE 1.—*Relation of the difference (Observer A–Observer B) to the sum of the scores (×4) assigned independently by the two observers*[a]

DIFFERENCE BETWEEN SCORES (×4) FOR EACH BONE	SUM OF TWO SCORES (×4) FOR EACH BONE AND NUMBER OF BONES IN EACH INTERVAL								TOTAL
	1–4	5–8	9–12	13–16	17–20	21–24	25–28	29–32	
4	——	——	——	——	——	——	2	——	2
3	——	1	——	1	——	2	——	——	4
2	4	3	1	1	6	10	2	1	28
1	4	3	2	2	27	18	1	4	61
0	14	8	3	63	53	21	2	4	168
−1	4	4	5	6	37	10	4	3	73
−2	1	4	5	3	8	6	——	——	27
−3	——	——	1	——	3	1	——	——	5
−4	——	——	2	——	2	——	——	——	4
Total:	27	23	19	76	136	68	11	12	372
Mean difference	0.22	0	−1.16	−0.07	−0.23	0.28	0.82	0.25	−0.06

[a] Including both left and right radii and tibiae.

Comparison of bones.—In the present experiment, four bones were removed and scored from each rat although only a single bone ordinarily is used. If different bones from the same rat were to vary sufficiently, the scoring of more than one from each individual might increase the precision of the assay, since their average should represent the rat more adequately than a single bone.

The first comparison was that of the difference in the scores assigned by observer A to the left and right bones for 96 paired tibiae and 98 paired radii, omitting all cases where both bones were scored 0 for no healing. On the original scale of 0 to 4, the mean difference of right–minus–left for tibiae was 0.042 ± 0.036; and for radii, -0.028 ± 0.032. There was obviously no difference here in the healing of left and right bones. In units of a single bone, the standard deviation between left and right bones from the same rat was $s = 0.239 \pm 0.012$, a value but little larger than the scoring error.

A similar comparison of paired tibiae and radii for 102 left pairs and 101 right pairs respectively, again showed no significant difference; the mean differences for the left side was -0.046 ± 0.075, and that for the right side, 0.020 ± 0.069. However, the variability between tibiae and radii was more than twice that determined for the same bone on opposite sides, giving a standard deviation of $s = 0.510 \pm 0.025$ in units of a single bone on a scale of 0 to 4.

Although the tibia and radius gave equivalent mean scores, one might be more sensitive than the other to differences in the dosage of vitamin D. To test this possibility, a dosage–response curve was determined for

each bone from the five log-doses in the linear range with litters 1 to 6 and 9 to 12; the only suitable ones were represented equally by tibiae and radii. When the standard deviation in the response (s) was divided by the slope of the log-dose response curve (b) to convert it into units of log-dose ($\lambda = s/b$), the tibia ($\lambda = 0.620/2.494 = 0.249$) proved here a more sensitive indicator than the radius ($\lambda = 0.741/2.284 = 0.324$). From these estimates, 70 per cent more rats would be needed in an assay based upon the radius if it were to equal the precision of a similar assay scored on the tibia (2).

Although the tibia was more sensitive than the radius, the possibility remained that a combined score based upon both bones might be more sensitive than either alone. The relative weights of each bone in such a combination could be determined by discriminant analysis, which would allow for the correlation ($r = 0.76$) between their scores within each rat. For maximal sensitivity, the appropriate weights for tibiae and for radii were 270 and 1 respectively. An analysis of variance showed, however, that if the tibia was scored, no pertinent information was added by the radius, but that if the radius was scored, the information remaining in the tibia could increase the precision of an assay.[1] Because of this finding, the results of observer A were further restricted to her scores on tibiae in later calculations.

Previous experience of the experimenters who evaluated the healing dealt mostly with the tibia, but a familiarity with radius readings was such that noted differences could not have been the result of bias. The reason for the superiority of the tibia is not hard to explain: In splitting the bones with a scalpel, it is almost impossible to avoid some mutilation of the fine structure. The softer the bone, i. e., the more ricketic, the more likely this is to occur. A given amount of mutilation is less, percentage-wise, in a large bone than in a small bone. Hence the advantage of the tibia over the radius. Of less importance, but probably still a factor, is the fact that the larger tibia is easier to manipulate, and hence to cut with minimum damage.

Variables affecting the assay.—Apart from differences between litters, four variables have been assumed in the United States Pharmacopoeia (8) to modify the precision of a vitamin D assay: (1) the age of the depleted rat at the start of the assay period; (2) Its body weight on depletion; (3) Its food consumption while on assay; and (4) Loss in weight during the assay period. Individual rats must not exceed specified limits for each criterion, and for an official assay, the average values of the first two criteria in rats receiving different preparations or doses must agree more closely. If these controls could be incorporated in the design, or proved unnecessary and omitted, the assayer's task would be easier.

[1] For readers interested in the observed dosage–response curves, the mean scores for these ten litters on the Bills scale of 1:4 for the six successive dosage levels were the following: Tibia—1.51, 1.30, 1.54, 1.88, 2.14, 2.88; radius—1.82, 1.24, 1.48, 1.99, 2.19, 2.60. The lowest dose, clearly below the linear range of the dosage–response curve in this experiment (see last section), was omitted from the calculation of slope.

In the first series, the rats in each of twelve litters comprising two Latin squares were started on the assay diet on the same day, so that their average age at the end of depletion was the same for all six doses. This method of confounding differences in age at depletion with litters proved quite manageable and, in fact, is currently used in a number of assay laboratories. Variations in weight on depletion were balanced among doses by assigning rats to the doses in each column of the first two Latin squares in order of increasing weight. In the second series of two Latin squares, a similar attempt was made to equalize the body weight at the end of depletion by assigning rats to doses within each column in the order in which they grew to the approximate weight of the heaviest litter-mate to reach depletion first. This led to an average range of nineteen days in the age at which litter-mates were transferred to the assay diet, and it was evident as the experiment proceeded that the method led to overdepletion in many rats. In both series the third and fourth variables were measured for all rats. They confirmed the unsuitability of the second series, which will not be considered further in the analysis.

In the first series, one entire litter was eliminated in which all rats showed nearly maximal healing. Three individual animals were also omitted—one which showed little or no healing at the next to the highest dosage level, and two with a high degree of healing at one of the two smallest doses. Several of these omissions were not unlike those specified in the U.S.P. One other rat was lost from these eleven litters during the experiment.

The restricted data were then analyzed by the method for randomized blocks, considering each litter as a block. The importance of differences in body weight, which were balanced between the rows of the original Latin squares, was tested by co-variance. In addition, two other quantitative factors were examined in the same analysis of co-variance—gain (or loss) in weight, and food consumption during the assay period. For computational purposes the four missing test scores and their corresponding initial or concomitant measurements were replaced by the missing plot technic for randomized blocks. Whether the observed variation in the three concomitant measures increased the error of the assay could be judged from the partial regression coefficients computed from the error row.

Weight on depletion had no effect upon the degree of healing. Hence the present results agree with those of Coward and Kassner (5), where inequalities in body weight did not change the error significantly ($P = 0.08$) when differences between litters had been segregated. However, in a line test measured planimetrically, Gridgeman (6) found that the degree of healing increased significantly with weight when tested by co-variance ($P = 0.008$). The absolute effect of this factor was small. Any potential bias could be avoided by balancing differences in body weight with a

Latin square design similar to that used in this series, which would also provide some randomization in distributing rats to doses. For comparisons of particular importance, the effect of differences in depleted body weight could be tested by co-variance and adjusted if necessary.

Variation in food consumption during the assay period similarly had no significant effect. The U.S.P. XIV requires that each rat must consume at least 28 g of basal ration during the assay period. In this series twelve individuals apparently consumed less than this amount. Experimental data on food consumption are not very reliable and any information to be gained from such a record is probably supplied more effectively by the gain in body weight during the assay period. The two measurements were correlated significantly in the present series with $r=0.42$ ($P<0.01$).

Gain in weight during the assay period is also a requirement in the U.S.P. assay. Any rat which loses weight is supposed to be discarded as being in danger of showing false healing, a dictum that was not followed

TABLE 2.—*Combined analysis of variance for both series and estimated components of variance*

VARIANCE	D.F.	MEAN SQUARE	VARIANCE COMPONENT
Litters	10	$4.1105=\hat{\sigma}_1^2+\hat{\sigma}_2^2+2\hat{\sigma}_3^2+12\hat{\sigma}_4^2$	$0.2640=\hat{\sigma}_4^2$
Rats within litters	46	$0.9421=\hat{\sigma}_1^2+\hat{\sigma}_2^2+2\hat{\sigma}_3^2$	$0.4424=\hat{\sigma}_3^2$
Bones within rats	194	$0.0572=\hat{\sigma}_1^2+\hat{\sigma}_2^2$	$0.0157=\hat{\sigma}_2^2$
Scoring error	364	$0.0415=\hat{\sigma}_1^2$	$0.0415=\hat{\sigma}_1^2$

here. In the present series, 20 rats lost from 1 to 5 g during the assay period, although the entire series of 62 rats averaged a gain of 1.2 g. The partial regression coefficient measuring the effect of a change in weight upon the score in the line test differed insignificantly from zero. These data suggest that the loss of only a few grams may be negligible and in critical cases it could be adjusted by co-variance.

The litter effect.—Litter mates have long been known (1, 3, 4) to react more nearly alike to vitamin D than individuals from different litters, so that mean differences between litters have been segregated here both from the error and from the treatment effects. The mean square attributable to differences between litters was highly significant in the present experiment, as expected. It has been converted to Bills units for one reading on a single bone to obtain the values in Table 2. The mean square for litters tends to be too large because of the missing values which have been replaced, but probably by not more than 5 per cent.

The last column in Table 2 shows the component of variance, in units of one reading on a single bone, attributable to litter differences, to the variation among individual rats, to the contrast between bones within rats, and to the observer's error in scoring the line tests. The variance of

a single reading on one rat would be the sum of these four components if no litter restrictions were placed upon the assay and one bone were read once from each rat. By designing the assay so as to segregate differences between litters, the first component, representing 35 per cent of the variance, would not affect the comparison of doses, which was the case here. If, instead, the rats had been assigned to doses completely at random, the number of rats would have had to be increased by more than 50 per cent to obtain the same precision. Litter effects of about the same magnitude have been reported by other observers (2, 5–7), all confirming the necessity of segregating litter differences in a well designed assay.

The smallest variance component, computed from the contrast between right versus left bones, in effect measured the extent to which a given rat could be represented by a single bone. If one bone were scored once, its scoring variance would be $\hat{\sigma}_1^2+\hat{\sigma}_2^2=0.0572$. If read twice independently, its scoring variance would be $(\hat{\sigma}_1^2/2)+\hat{\sigma}_2^2=0.0365$. If both the right and left tibiae of the same rat were each read once instead, the scoring error would be reduced to $(\hat{\sigma}_1^2+\hat{\sigma}_2^2)/2=0.0286$. However, when these are compared with the component for rats within litters, $\hat{\sigma}_3^2=0.4424$, the gain in assay precision would seldom justify the added labor of removing both tibiae from each rat and handling twice as many bones.

Relation between dosage and response.—The precision of an assay technic depends not only on the error variance of the response, but also on the slope of its dosage–response curve. The mean response observed at each of the six dosage levels in eleven rats has been plotted in Fig. 1 against the logarithm of the dose of cod liver oil in mg per day. The means defined a curve which tended to flatten at its lower end, as if it were approaching a floor asymptotically. The dosages in a bioassay are restricted by choice to the approximately linear central part of the total dosage–response curve.

The apparent curvature in the six points plotted in Fig. 1 has been tested by fitting a parabola and determining by an analysis of variance whether the quadratic term, representing simple curvature, was significant statistically. From the quadratic term in the left side of Table 3,

TABLE 3.—*Alternative analysis of variance of the six dosage levels in the first series*[a]

TERM	D.F.	*F*	TERM	D.F.	F
Doses 1 to 6: Linear	1	38.80[b]	Doses 2 to 6: Linear	1	38.00[b]
Quadratic	1	4.30[c]	Quadratic	1	0.26
Scatter	3	0.51	Scatter	2	0.31
			Dose 1 *vs.* doses 2–6	1	5.74[c]

[a] Each has the same mean square for litters×doses as the error.
[b] $P<0.001$.
[c] $P<0.05$.

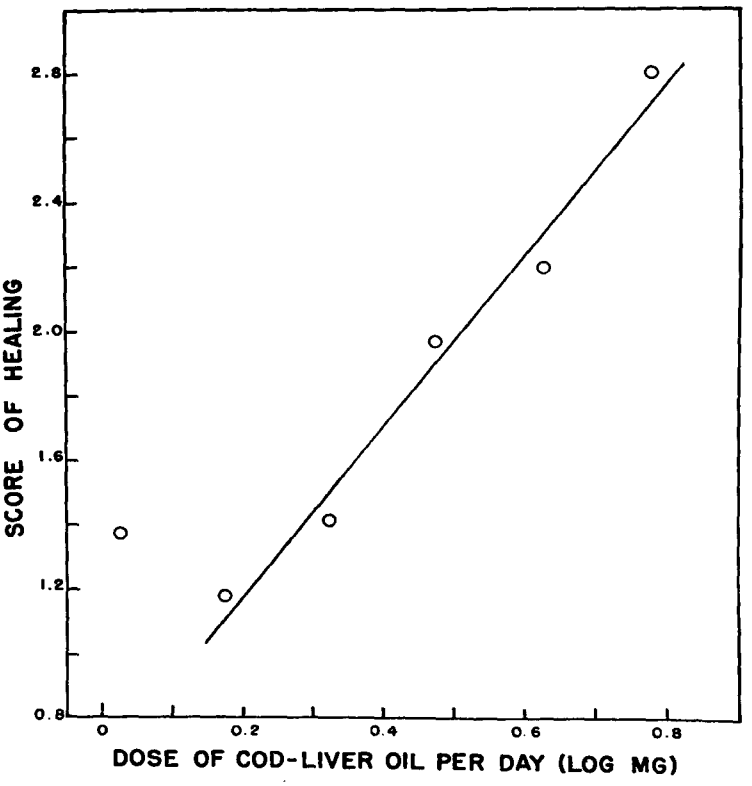

FIG. 1.—Dosage response curve for 5 larger doses.

the apparent curvature would be considered real. When the smallest dose was omitted, however, the remaining observations exhibited a satisfactory linearity, which is evident from the alternative analysis in the right hand side of Table 3. Accordingly a straight line has been fitted to the five larger doses with a slope of $b = 2.68 \pm 0.43$ for the response in Bills units of 0 to 4, plotted against the log-dose of cod liver oil per day. Since the standard deviation of the response about the line was $s = 0.686$, the standard deviation in log-dose units inferred from the linear part of the dosage–response curve (above a daily dose of 1.5 mg of cod liver oil) was $\lambda = 0.256 \pm 0.051$. This is of the same order of precision as the assays reported by Coward (4) as $\lambda = 0.68/3.1 = 0.22$, from a long series of data of the British Pharmaceutical Society. The fact that the smallest dose gave here a larger mean response than the next succeeding dose (Fig. 1) suggests that the chance of remaining in the linear range of the dosage–response curve in future similar experiments could be improved by avoiding dosages as small as either of the two lowest levels.

SUMMARY

Some of the quantitative characteristics of the line test have been examined from a dosage–response curve determined with six-rat litters and six dilutions of the U.S.P. Reference Cod Liver Oil No. 2. Each bone was scored in quarter units on the Bills scale from 0 for no healing to 4 for full healing. On this scale, paired readings by two independent observers of 272 radii and tibiae showed a standard deviation of 0.204 unit. With a scoring error of this magnitude, scoring to a one-quarter scale unit is fully justified. In a comparison of their relative effectiveness as indicators for vitamin D tibiae proved more sensitive than radii from the same rats.

When differences between litters were segregated and all rats in a litter were started on the test diet on the same day, weight on depletion and food consumption during the assay period had no effect upon the score as tested by multiple co-variance. A change in weight during the assay period had a negligible effect upon the error of the assay even when one-third of the rats lost from 1 to 5 grams.

The variability between litters was significantly greater than that within litters, and of a magnitude such that the neglect of litter-mate control would require an increase in the number of rats of more than 50 per cent to obtain a given precision.

At a daily dose of cod liver oil of 1.5 to 6 mg per day and a seven-day assay period, the dosage–response curve could be fitted by a straight line when the mean score was plotted against the log dose. At this level or below, the curve flattened out. The inherent precision of the assay in terms of the standard deviation of the log dose was $\lambda = 0.256 \pm 0.051$, a value of the same magnitude as those determined by previous workers.

REFERENCES

(1) BILLS, C. E., *Biol. Symposia*, **12**, 409 (1947).
(2) BLISS, C. I., *The Statistics of Bioassay with Special Reference to the Vitamins*, Academic Press, Inc., New York, 1952.
(3) ———, and GYORGY, P., in *Vitamin Methods*, Vol. II, P. GYORGY, Editor, Academic Press, Inc., New York, 1951, p. 41.
(4) COWARD, K. H., *The Biological Standardisation of the Vitamins*, Balliere, Tindall and Cox, London, 1947.
(5) ———, and KASSNER, E. W., *Biochem. J.*, **35**, 979 (1941).
(6) GRIDGEMAN, N. T., *Quart. J. Pharm. Pharmacol.*, **18**, 15 (1945).
(7) JONES, J. I. M., *ibid.*, **18**, 92 (1945).
(8) *United States Pharmacopeia*, Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950.

EVALUATION OF TWO METHODS FOR THE DETERMINATION OF CAROTENE

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The present A.O.A.C. method for carotene was adopted as official in 1948 (1). Because a number of potential sources of error existed in the procedure as outlined, a proposed modification of the method was reported by this laboratory in 1951 to increase the precision (2). In the same year, the Associate Referee asked the collaborators to compare the A.O.A.C. method with the Western Regional Research Laboratory (W.R.R.L.) modification. The A.O.A.C. method gave lower carotene values in almost all laboratories (3). Based on this comparative study, the Associate Referee recommended that the W.R.R.L. modification should not be adopted until the reasons for the variation between the two methods could be ascertained. Since the Associate Referee reports that uncontrolled factors are still influencing the results to a considerable degree (4), this laboratory initiated further work to evaluate both the A.O.A.C. and its own method. This paper presents the results of this investigation which established that variations in the A.O.A.C. method were caused by a combination of factors. The W.R.R.L. modification reduces or eliminates many of them and thus leads to greater precision.

EXTRACTION OF CAROTENE

At least three factors may be expected to cause variations in extraction of carotene. These are: possibility of isomerization of the carotene, oxidation during extraction, and incomplete extraction of the carotene.

Possibility of isomerization during hot extraction.—The A.O.A.C. procedure offers as alternatives either cold (overnight) or hot (1 hour) extraction. Carotene in extracts of alfalfa will isomerize rapidly when exposed to heat (2) or light (5). The extent and degree of isomerization will depend on the relative amounts of stereoisomers initially present in the meal and upon the degree of illumination of the sample during the hot extraction. It has been shown previously that during normal A.O.A.C. hot extraction of a typical dehydrated meal, sufficient isomerization can occur to increase the total apparent absorption about 6 per cent when determined by a filter-type instrument (2). Little isomerization occurs during cold extraction for 16 hours in the dark (2). These observations would favor the cold extraction procedure.

Possibility of oxidation during extraction.—It is difficult to evaluate the

amount of oxidation that may occur during hot extraction, because the simultaneous isomerization, with its consequent enhancement of color intensity, tends to mask the decrease in color intensity caused by oxidation unless stereoisomer analysis is undertaken. That oxidation must be considerable can be deduced, however, from the results of last year's "Carotene Workshop"[1] wherein collaborators were asked to compare cold (overnight) with hot (1 hour) extraction on identical samples when all other operations were comparable. The results showed an apparent 2 to 3 per cent lower carotene content for the hot extraction than for the cold. Zscheile and Whitmore (6) also found about a 3 per cent loss through heat decomposition or oxidation during hot extraction on samples containing 200 to 300 p.p.m. of carotene. They further stated that these losses are undoubtedly higher on a percentage basis for samples very low in carotene. Pepkowitz (7) showed that carotene in petroleum ether is subject to photochemical destruction in the presence of chlorophyll and acetone. Little, if any, oxidative loss occurs in the cold (overnight) extraction procedure.

Possibility of incomplete extraction.—Zscheile and Whitmore (6) employed a re-extraction technique on various samples of meal during the development of their analytical method, and were able to show that by refluxing the residues for a period of 2.5 hours with an acetone–hexane mixture (30–70), they were able to obtain 2 per cent additional carotene. Cooley and Koehn (8) recently confirmed this observation, and demonstrated that by employing a more efficient solvent mixture they were able to extract from 6.5 to 15 per cent of additional carotene from various samples of dehydrated alfalfa meal. Pumpelly, *et al.* (9), demonstrated that cold (overnight) extraction will remove 2.5–4.5 per cent more carotene than the hot extraction. However, even a 16 hour extraction in the cold does not always extract all of the carotene (Table 1). Extraction techniques which employ a preliminary rehydration of the meal permit more quantitative extraction of the pigments (10).

CHROMATOGRAPHIC PURIFICATION OF THE EXTRACTED CAROTENE

Incomplete elution of carotene.—The lower values obtained by the collaborators with the A.O.A.C. procedure were probably caused by a combination of factors. Primarily, it is evident that many of the laboratories were not eluting all of the carotene from the column when the specified directions were followed. Thus, of the 46 laboratories that reported results by both methods in the comparative study (3), 10 reported values from 12 to 20 per cent lower by A.O.A.C., 8 reported values from 20 to 35 per cent lower, and one reported an even lower value.

[1] A collaborative study held at Purdue University, April 23–24, 1953. See ref. (4).

TABLE 1.—*Extraction of carotene by A.O.A.C. overnight extraction*

SAMPLE	A.O.A.C. CAROTENE MG/LB	ADDITIONAL CAROTENE IN RESIDUE[a]	
		MG/LB	PER CENT
Dehydrated alfalfa meal A	37	2.0	5.4
	38	1.4	3.7
Dehydrated alfalfa meal B	58	4.4	7.6
	60	3.6	6.0
Dehydrated alfalfa meal C	74	0.8	1.1
	75	1.0	1.3
Dehydrated alfalfa meal D	94	9.3	9.9
	89	7.2	8.1
Dehydrated alfalfa meal E	123	5.3	4.3
	118	5.2	4.4

[a] Residue from 30% acetone extraction re-extracted by rehydration and acetone extraction (10).

To determine the minimum amount of eluant required to elute the carotene under the conditions employed in this laboratory, a solution of carotene was prepared by dissolving purified crystalline carotene in a 1+9 mixture of acetone–hexane. One hundred ml of this solution was added to an A.O.A.C. column. At the point when all of the added carotene solution had just passed into the column, 30 per cent of carotene had already been eluted from the column (Table 2). The column was then developed with a series of 10 ml portions of eluant until 120 ml had been collected. Al-

TABLE 2.—*Volume of eluant required to elute carotene quantitatively from A.O.A.C. column*

VOLUME OF ELUANT ADDED	PER CENT OF ADDED CAROTENE ELUTED	
(*ml*)	*Trial 1*	*Trial 2*
0	29.8	30.1
10	37.2	39.9
20	46.6	49.9
30	57.9	60.9
40	69.4	70.4
50	86.6	80.3
60	90.7	89.6
70	96.4	95.1
80	98.4	97.7
90	99.4	98.9
100	99.8	99.5
110	99.9	99.8
120	100.0	99.9

though about 95 per cent of the added carotene was eluted with 75 ml of the 1+9 acetone–hexane, an additional 35 ml was required to elute the remaining 5 per cent.

To ascertain the amount of carotene that might be expected to be retained on the column when the A.O.A.C. procedure is followed as directed, collaborative sample No. 48 was analyzed independently by 8 chemists at our laboratory, 5 of whom had had carotene assay experience. Cold overnight extraction was employed. During the chromatographic purification step, an operator visually ascertained when all the carotene had apparently been eluted, noted the amount of eluant required, then developed the column with an additional 50 ml of eluant and collected this portion separately. The carotene solutions were given to one analyst who completed the colorimetric determinations. The analyses on all samples were completed during one morning. Each chemist performed triplicate assays of the meal by both the A.O.A.C. and W.R.R.L. methods. The average carotene value obtained by the A.O.A.C. procedure was 5 per cent lower than by the W.R.R.L. method but the values were the same when the second eluate was included (Table 3). Pumpelly, *et al.* (9), also showed that the lower results obtained in the A.O.A.C. method were caused mainly by incomplete elution of the carotene from the adsorbent.

Inclusion of impurities with carotene.—Under the conditions employed in the A.O.A.C. method for chromatography of the carotene fraction (very retentive adsorbent; strong eluting solution), several non-carotene pigments having very similar chromatographic characteristics on magnesia to that of beta-carotene will follow behind so closely as to form overlapping bands with the carotene. It is impossible visually to separate these pigments from carotene under the elution conditions employed in the A.O.A.C. method. However, these pigments are present in the tailings from the carotene zone and are ordinarily left behind along with some carotene in the A.O.A.C. procedure. The amount of carotene left behind on the column varies with each operator and even with the same operator under ordinary operating conditions (Table 3).

In the W.R.R.L. procedure, which employs a smaller column and a larger ratio of eluant to pigment, certain of these pigments are carried along with the carotene through the column. Re-chromatography of the combined eluates from the W.R.R.L. columns on hydrated lime showed that less than 1 per cent of non-carotene pigments were present. Re-chromatography of the combined second eluates from all the A.O.A.C. columns indicated also that at least 88 per cent of this material was beta-carotene. The remainder of the material consisted of several different pigments.

To obtain better characterization of these non-carotene pigments, a cold extraction of 200 grams of alfalfa meal from collaborative sample

TABLE 3.—*Comparison of A.O.A.C. and W.R.R.L. chromatographic methods for carotene*

ANALYST	A.O.A.C. METHOD			W.R.R.L. METHOD
	1ST ELUATE		1ST+2ND ELUATE	MICROGRAMS
	VOLUME	MICROGRAMS	MICROGRAMS	
J.G.	68	69.1	72.0	72.4
	76	68.0	71.6	73.7
	79	69.1	71.7	73.2
C.R.T.	80	64.5	69.7	72.7
	74	59.3	69.0	72.1
	84	66.4	70.8	71.8
G.F.B.	112	68.1	70.8	69.6
	57	71.8	71.8	71.2
	77	69.1	70.0	72.4
I.V.F.	78	66.3	71.0	70.6
	78	70.3	72.8	72.4
	74	67.6	72.3	72.4
E.D.W.	94	69.1	69.3	72.4
	82	72.3	72.3	72.4
	78	70.6	71.2	71.2
R.F.E.	70	72.3	72.3	70.6
	74	69.2	71.6	71.0
	88	70.6	71.5	73.7
A.L.L.	90	58.7	67.7	70.4
	96	70.6	71.8	72.6
	94	69.1	71.1	71.0
E.M.B.	110	55.9	66.7	72.4
	76	64.5	67.4	70.2
	78	66.4	69.9	71.8
	74	65.0	69.8	70.2
Mean		68.4	71.6	71.8
Std. Dev.		2.6	1.3	0.96

No. 48 was made at this laboratory with 3 liters of a 3+7 mixture of acetone–hexane, and after removal of the acetone by washing with water, the entire fraction was added to a 75 mm diameter Zechmeister type column packed to a height of 30 cm with hydrated lime. Development of the column with 1.5 per cent *p*-cresyl methyl ether in hexane (11) resulted in the separation of the carotene stereoisomers from one another and from

the non-carotene pigments which occurred above the carotene and below the cryptoxanthin on the column. These non-carotene pigments were termed "impurity A" by Kemmerer and Fraps (12), and were present in what they called their "crude carotene" fraction.

In the present work, these non-carotene pigments were eluted from the column as a group and re-chromatographed to separate them from one another. Eleven pigments were so isolated. They represented about 8 per cent of the total "crude carotene" fraction (Table 4). The spectrophotometric properties of these pigments demonstrated that they were not carotenes.

TABLE 4.—*Relative amounts of non-carotene pigments distributed between carotene and cryptoxanthin on the chromatographic columns and their interference with WRRL carotene assay*

FRACTION	APPARENT CAROTENE	PURIFIED CAROTENE	RELATIVE AMOUNT OF W.R.R.L. CAROTENE ELUATE
	micrograms	*(per cent)*	*(per cent)*
Carotene	2103	100	98.85
Impurity A-Band 1	12	0.5	0.50
Impurity A-Band 2	20	1.0	0.35
Impurity A-Band 3	4	0.2	0.10
Impurity A-Band 4	1	0.05	0.03
Impurity A-Band 5	8	0.4	0.10
Impurity A-Band 6	3	0.1	0.02
Impurity A-Band 7	13	0.6	0.05
Impurity A-Band 8	33	1.5	None
Impurity A-Band 9	47	2.2	None
Impurity A-Band 10	33	1.5	None
Impurity A-Band 11	5	0.2	None
Cryptoxanthin	—	—	None

Adding the isolated pigments either alone or together with an extract from dehydrated alfalfa as obtained in the cold (overnight) extraction procedure to a W.R.R.L. column and developing with 25 ml of a 1+9 mixture of acetone–hexane showed that most of these pigments were retained by the column under these conditions, and that less than 1.25 per cent of the "total carotene" eluate would consist of these pigments (Table 4). A similar study was made on a poor quality meal which had been stored until its carotene content had been reduced from about 100 mg to about 50 mg per pound. Two hundred grams of this meal were extracted and processed as described for collaborative sample No. 48. Comparable data on the relative amounts of non-carotene impurities and their spectrophotometric and chromatographic properties were obtained.

Variation in activity of adsorbent.—When collaborative studies were

begun in 1947, the Associate Referee mailed to the various collaborators a sample of the magnesium oxide then being employed in his laboratory, with the instructions that it be used for the collaborative assay. When the specified procedure was followed with the magnesia in use in this laboratory, 50 ml was insufficient eluant to completely remove the carotene, although it was sufficient when the magnesia supplied by the Associate Referee for comparative purposes was used. The official procedure has been modified recently to read " . . . use 50 ml or slightly more if necessary of acetone–hexane (1+9) to develop the chromatogram and wash the visible carotene through the adsorbent" (3). One of the main sources of variability between laboratories is caused by this step, since it is extremely difficult to determine visually the exact point at which all of the carotene has come through the column. A recently proposed technique for standarization of the magnesia can be used to reduce or elimi-

TABLE 5.—*Carotene recovery after successive passage through four magnesia columns*[a]

COLUMN	CAROTENE RECOVERED FROM COLUMN (MICROGRAMS)	
	A.O.A.C.	W.R.R.L.
1	60.3	29.7
2	59.9	29.7
3	61.3	30.0
4	60.1	29.8

[a] Columns were developed with 1+9 mixture of acetone-hexane.

nate variation (13). By increasing the ratio of eluant to sample placed on the column the W.R.R.L. modification practically eliminates the error caused by the variability of magnesia from one batch to the next.

Destruction of carotene on the chromatographic column.—It has been shown by Wiseman, *et al.*, that celite destroys carotene by contact with solutions of the pigment in hexane (14). They state that destruction or alteration of pigments may be negligible when time of filtration is short, although in longer filtrations the tendency of celite to isomerize and oxidize carotene cannot be ignored.

Table 5 presents the results of passing a solution of beta-carotene successively through four A.O.A.C. columns. The solutions were prepared by dissolving purified crystalline beta-carotene in a 1+9 mixture of acetone–hexane and the columns were developed with 120 ml of 1+9 acetone–hexane. No loss of carotene occurred under these conditions. Similarly, no loss occurred with the W.R.R.L. column.

MEASUREMENT OF THE CAROTENE CONTENT OF THE PURIFIED EXTRACT

Composition of the purified extract.—Since the A.O.A.C. method as originally outlined involved hot extraction of the meal with the conse-

quent change of ratio of carotene isomers, only "total carotene" was determined and no attempt was made to measure the individual stereoisomers. However, the Associate Referee stated in his report for 1948 (1) that "further efforts should be made to work out analytical procedures which will not produce isomerization." As has been pointed out above, the alternate cold (overnight) extraction procedure does not cause isomerization of the carotene. Since as much as 60 per cent of the carotene in certain samples of alfalfa meal may exist in partially cis forms (11), and since these cis isomers have only a fraction of the vitamin A activity of all-trans-beta-carotene (15–18), the calculated nutritional value may be as much as 30 per cent lower than found by a "total carotene" analysis.

Kemmerer *et al.* (19) recognized this problem a decade ago and suggested that in the case of certain forages, the vitamin A activity of the pigment be obtained by multiplying the crude carotene value by the factor 0.77. More recently, simplified procedures have been developed at this laboratory to permit the determination of the relative amounts of beta-carotene isomers present in the "total carotene" fraction by either chromatographic (11, 20) or spectrophotometric (21) techniques.

Calibration of instruments.—While a spectrophotometer, suitably calibrated and operated, is inherently capable of the most accurate results (22–25), a properly calibrated colorimeter can also yield satisfactory analytical data. The spectrophotometer wavelength scale may be easily checked against the numerous mercury and cadmium emission lines in the visible and ultraviolet spectra and the photometric accuracy compared by means of alkaline potassium dichromate solutions (26).

The use of potassium dichromate solutions for calibration of colorimeters is of doubtful reliability because the dichromate absorption spectrum is so different from that of beta-carotene and its stereoisomers that small differences in wavelength of the pass band of the colorimeter filter may introduce significant differences in calibration between different instruments. Thus, a group of 440 mμ colorimeter filters of the same manufacture gave a range greater than 5 per cent in the relative ranking of a dichromate and a carotene solution when examined in a particular photoelectric colorimeter of that manufacture (Table 6).

TABLE 6.—*Comparison of calibration of 440 mμ filters in a photoelectric colorimeter*

440 FILTER NUMBER	ABSORBANCE OF CAROTENE SOLUTION / ABSORBANCE OF DICHROMATE SOLUTION
1	1.029
2	1.059
3	1.006
4	1.012
5	1.026

Crystalline beta-carotene may be the ideal material for colorimetric calibration, except for the inconvenience of preparation of authentic material of high purity in most control laboratories (27) and the necessity of verifying the purity of the commercially available material (28). The authors consider that in the absence of concern for the different nutritive values of the several beta-carotene stereoisomers, the indirect calibration of the colorimeter proposed by Comar (28) by means of chromatographed carotene extracts whose absorbance has been determined at 451 mμ (29) on a spectrophotometer has great superiority for both convenience and reliability in inter-laboratory comparisons such as those now being conducted on the A.O.A.C. carotene method (4).

SUMMARY

Possible sources of error that may be present in the A.O.A.C. method for carotene have been indicated and evaluated. Errors may arise during the extraction of the meal because of the possibility of re-isomerization, oxidation, or incomplete extraction of the carotene. Errors may also arise during the preparation of the purified carotene fraction because of the variations in activity of the chromatographic adsorbent, incomplete elution of carotene, contamination of carotene eluate with impurities, or destruction of carotene on the column. The advantages of indirect calibration of the colorimeter by means of the spectrophotometer are indicated.

The proposed modification of the A.O.A.C. method now in use at the Western Regional Research Laboratory has been compared with the A.O.A.C. method. Carotene values obtained by the W.R.R.L. modification are at least 5 per cent higher than those obtained by the A.O.A.C. method. This may be due to a combination of factors, perhaps the most important being that the A.O.A.C. method does not always permit quantitative elution of all the carotene from the column unless extreme precautions are taken. Because of increased simplicity the W.R.R.L. modification lends itself better to the routine determination of large numbers of samples.

ACKNOWLEDGMENT

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REFERENCES

(1) QUACKENBUSH, F. W., *This Journal*, **32**, 480 (1949); **32**, 48 (1949).
(2) THOMPSON, C. R., and BICKOFF, E. M., *ibid.*, **34**, 219 (1951).
(3) QUACKENBUSH, F. W., *ibid.*, **35**, 736 (1952).
(4) ———, *ibid.*, **37**, No. 3 (1954), (in press).

(5) THOMPSON, C. R., BICKOFF, E. M., and MACLAY, W. D., *Ind. Eng. Chem.*, **43**, 126 (1951).
(6) ZSCHEILE, F. P., and WHITMORE, R. A., *Anal. Chem.*, **19**, 170 (1947).
(7) PEPKOWITZ, L. P., *J. Biol. Chem.*, **149**, 465 (1943).
(8) COOLEY, M. L., and KOEHN, R. C., *Anal. Chem.*, **22**, 322 (1950).
(9) PUMPELLY, C. T., MITCHELL, H. L., and SILKER, RALPH E., *This Journal*, **34**, 821 (1951).
(10) BICKOFF, E. M., LIVINGSTON, A. L., and VAN ATTA, G. R., *ibid.*, **35**, 826 (1952).
(11) ———, and THOMPSON, C. R., *ibid.*, **32**, 775 (1949).
(12) KEMMERER, A. R., and FRAPS, G. S., *Ind. Eng. Chem., Anal. Ed.*, **15**, 714 (1943).
(13) WILKIE, J. B., and JONES, S. W., *Anal. Chem.*, **24**, 1409 (1952).
(14) WISEMAN, H. G., STONE, S. S., SAVAGE, H. L., and MOORE, L. A., *ibid.*, **24**, 681 (1952).
(15) DEUEL, H. J., JR., JOHNSTON, C., SUMNER, E., POLGAR, A., and ZECHMEISTER, L., *Arch. Biochem.*, **5**, 107 (1944).
(16) DEUEL, H. J., JR., JOHNSTON, C., MESERVE, E. R., POLGAR, A., and ZECHMEISTER, L., *ibid.*, **7**, 247 (1945).
(17) JOHNSON, R. M., SWICK, R. W., and BAUMANN, C. A., *ibid.*, **22**, 122 (1949).
(18) KEMMERER, A. R., and FRAPS, G. S., *J. Biol. Chem.*, **161**, 305 (1945).
(19) KEMMERER, A. R., FUDGE, J. F., and FRAPS, G. S., *J. Am. Soc. Agr.*, **36**, 683 (1944).
(20) BICKOFF, E. M., ATKINS, M. E., BAILEY, G. F., and STITT, F., *This Journal*, **32**, 766 (1949).
(21) STITT, F., BICKOFF, E. M., BAILEY, G. F., THOMPSON, C. R., and FRIEDLANDER, S., *ibid.*, **34**, 460 (1951).
(22) GOLDRING, L. S., HAWES, R. C., HARE, G. H., BECKMAN, A. O., and STICKNEY, M. E., *Anal. Chem.*, **25**, 869 (1953).
(23) HISKEY, C. F., *ibid.*, **21**, 1440 (1949).
(24) GIBSON, K. S., and BALCOM, M. J., *J. Res., Natl. Bureau Stand.*, **38**, 601 (1947).
(25) O'CONNOR, R. T., HEINZELMAN, D. C., and JEFFERSON, M. E., *Ind. Eng. Chem., Anal. Ed.*, **18**, 557 (1946).
(26) HAUPT, G. W., *J. Research, Natl. Bur. Stand.*, **48**, 414 (1952).
(27) FRAPS, G. S., and KEMMERER, A. R., *Chem. Eng. News*, **19**, 846 (1941).
(28) COMAR, C. L., *Ind. Eng. Chem., Anal. Ed.*, **18**, 626 (1946).
(29) BICKOFF, E. M., WHITE, L. M., BEVENUE, A., and WILLIAMS, K. T., *This Journal*, **31**, 633 (1948).

THE DETERMINATION OF 2-NITRO-1,4-DIAMINOBENZENE IN HAIR DYES*

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The use of 2-nitro-1,4-diaminobenzene in commercial hair dyes to obtain red shades makes it desirable to have an analytical procedure for its identification and estimation.

In this investigation a spectrophotometric method was developed utilizing the yellow and orange-red colors of acid and alkaline aqueous solutions of 2-nitro-1,4-diaminobenzene. In this procedure, fatty acids and resorcinol are first extracted from an acid solution of the hair dye. The solution is then made strongly alkaline and the nitrodiamine extracted with ether. The strongly alkaline solution holds back decomposition products of other amine compounds. The compound is re-extracted from the ether with dilute acid and determined spectrophotometrically in both acid and alkaline aqueous solutions.

METHOD

APPARATUS

A spectrophotometer capable of isolating a wave band of 10mμ, or less, in the region 400–700 mμ. (A Cary recording spectrophotometer Model 11 was used.)

REAGENTS

(a) *Sodium hydroxide solution, 10% (w/v).*

(b) *Sodium hydroxide solution, 50% (w/w).*

(c) *Standard soln of 2-nitro-1,4-diaminobenzene (30 mg/l in alcohol).*—Dissolve 60 mg of the compound in 200 ml of alcohol. (The 2-nitro-1,4-diaminobenzene used was in the form of black needles with a greenish lustre (m.p. 138–140°C.))

PROCEDURE

Add a sample of the hair dye contg 2–10 mg of 2-nitro-1,4-diaminobenzene to a separatory funnel, dil. to 18 ml with H_2O, acidify with HCl, and add 2 ml HCl in excess. Ext. with five 25 ml portions of water-washed ether. Reserve aq. soln. Combine ether extracts and wash with two 5 ml portions of ca 0.1 *N* HCl. Combine acid washings, ext. with 25 ml of ether, and then add acid washings to reserved aq. soln. Discard ether extracts. Cool aq. soln in ice bath, add 30 ml of an ice cold 50% NaOH soln contg 100 mg of Na_2SO_3. Cool the resulting mixt. to room temp. Ext. with four 35 ml portions of ether and filter the combined ether extracts thru a large cotton plug into a separatory funnel. Wash the plug with 25 ml of ether. Discard remaining aq. soln. Add 15 ml of H_2O to the ether ext., acidify with HCl, shake mixt. well, and draw off aq. layer into a 50 ml volumetric flask. Repeat this acid extn

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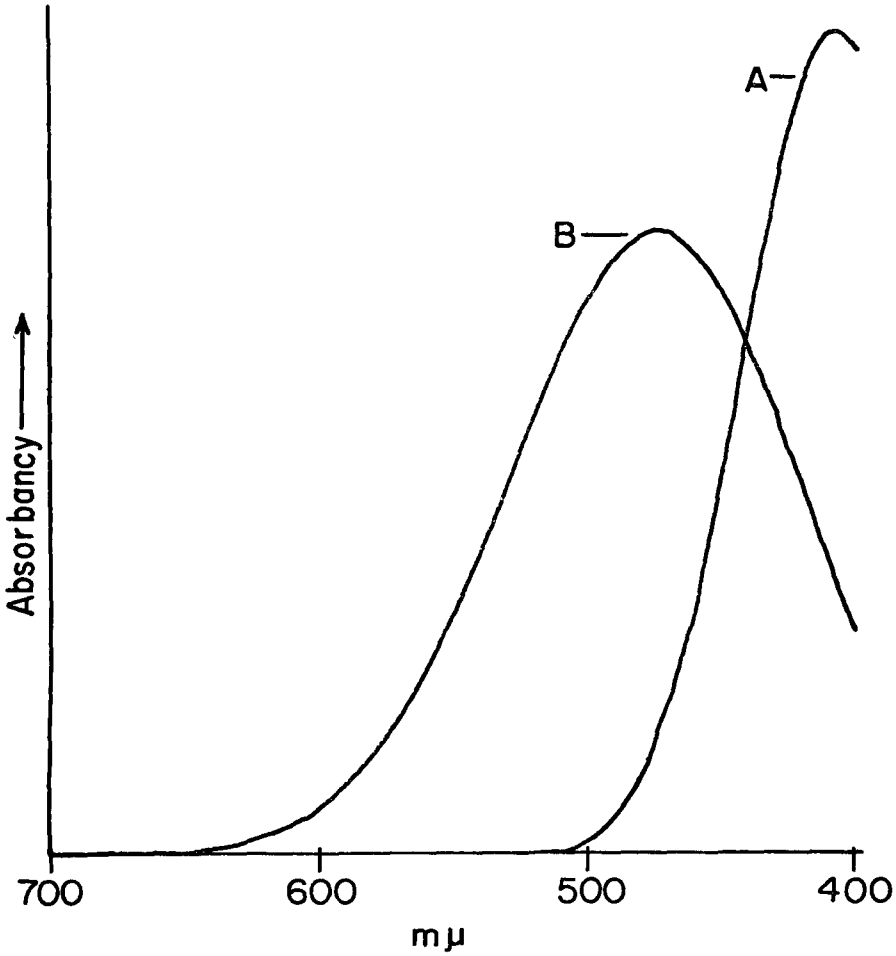

FIG. 1.—Absorbance curves of 2-nitro-1,4-diaminobenzene. A: 40 mg per liter in 0.1 *N* HCl containing 10% alcohol. B: 40 mg per liter in 0.1 *N* NaOH containing 10% alcohol and 1% Na_2SO_3.

twice, and transfer the extracts to the 50 ml volumetric flask. Fill the flask to the mark with H_2O and mix. Discard remaining ether soln.

SPECTROPHOTOMETRIC DETERMINATION

Prepare the following acidic solns:

(1) A 10% soln of alcohol made ca 0.1 *N* with HCl (1 ml concd HCl per 100 ml of soln).

(2) An aliquot of the soln obtained in the extraction procedure dild to a known

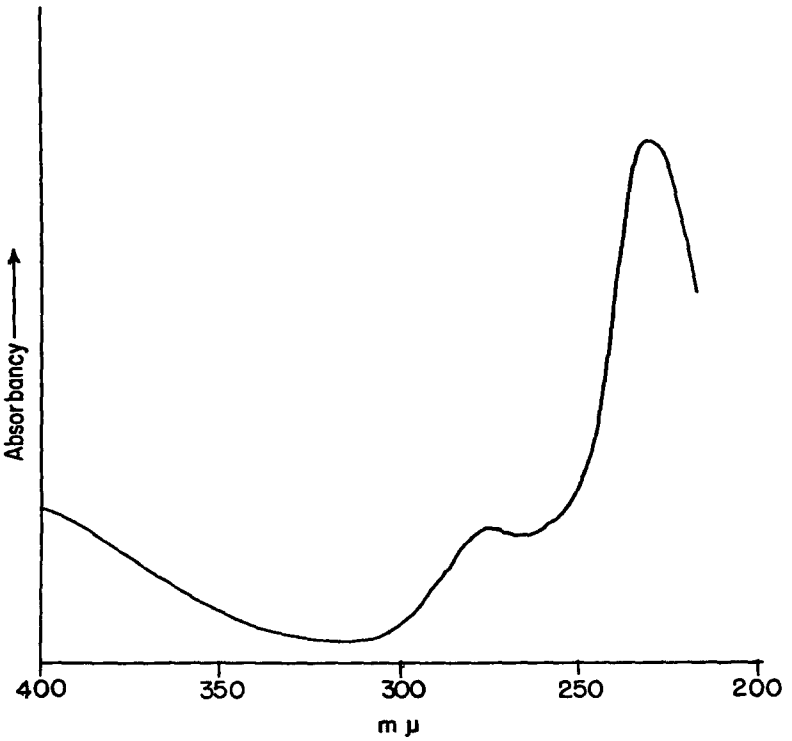

FIG. 2.—Ultraviolet absorbance curve of 2-nitro-1,4-diaminobenzene (10 mg per liter) in 0.1 *N* HCl containing 10% alcohol.

vol. with sufficient alcohol, HCl, and H_2O to yield a final soln contg 20–30 mg/l of the nitrodiamine in 10% alcohol, ca 0.1 *N* with HCl.

(3) A soln of 30 mg/l of 2-nitro-1,4-diaminobenzene in 10% alcohol, ca 0.1 *N* with HCl.

Det. the absorbance of these solns in 1 cm cells at 408 mμ, using H_2O in the reference cell.

Correct the observed absorbance of the sample and standard for the solvent blank, if any, and calc. the concn of 2-nitro-1,4-diaminobenzene in the sample.

Prepare the following alkaline solns:

(1) A 10% aqueous alcohol soln made about 0.1 *N* with NaOH (4 ml 10% NaOH soln per 100 ml).

(2) An aliquot of the soln obtained in the extraction procedure dild to vol. with sufficient alcohol, H_2O, and NaOH soln to yield a final soln contg 20–30 mg/l of the nitrodiamine in 10% alcohol, ca 0.1 *N* with NaOH.

(3) A soln of 30 mg/l of 2-nitro-1,4-diaminobenzene in 10% alcohol, ca 0.1 *N* with NaOH.

Det. the absorbance of these solns in 1 cm cells at 473 mμ, using H_2O as the blank.

Correct the observed absorbance of the sample and standard for the blank and calc. the concn of 2-nitro-1,4-diaminobenzene in the sample.

EXPERIMENTAL

Acidic solns of 2-nitro-1,4-diaminobenzene obey Beer's law to within ±1% at 408 mμ in the concn range 10–40 mg/l. Similar results were obtained with alkaline solns at 473 mμ. The absorbance curves are shown in Fig. 1.

Solns of 2-nitro-1,4-diaminobenzene in 0.1 *N* HCl contg 10% alcohol obey Beer's law to within ±1% at 230 mμ in the concn range 2–10 mg/l. The ultraviolet absorbance curve is shown in Fig. 2.

A hair dye of the following composition was prepared:

	per cent
Ethanol	10
Oleic acid	10
Ammonia	2
Sodium sulfite	0.2
p-Phenylenediamine	1
o-Aminophenol	1
p-Aminophenol	1
Resorcinol	1
Water	73.8

Known amounts of 2-nitro-1,4-diaminobenzene were added to 5 ml aliquots of the prepd hair dye and the resulting solns were analyzed by the proposed procedure. Typical results are presented in Table 1.

TABLE 1.—*Recoveries of 2-nitro-1,4-diaminobenzene from hair dye*

EXPT. NO.	NITRODIAMINE ADDED[a]	RECOVERY			
		IN ACID SOLUTION AT 408 mμ		IN ALKALINE SOLUTION AT 473 mμ	
	mg	*mg*	*per cent*	*mg*	*per cent*
1	51.2	48.8	95.3	48.8	95.3
2	10.04	9.86	98.2	9.7	96.6
3	2.51	2.51	100	2.50	99.6

[a] The 2-nitro-1,4-diaminobenzene was added to 5 ml of the prepared hair dye.

DISCUSSION

The recoveries of the 2-nitro-1,4-diaminobenzene in either acid or alkaline solution are 95 per cent or better.

Acid and alkaline solutions of the nitrodiamine were found to be spectrophotometrically stable for at least twenty-four hours.

The dual determination serves as a check on the possible presence of other absorbing materials. For more positive identification the complete absorbance curves for the spectral region 400–700 mμ should be obtained in both acid and alkaline solutions.

Although the lowest concentration of nitrodiamine determined in the

prepared hair dye was 0.05 per cent, it is believed that the procedure can be effectively used for concentrations as low as 0.01 per cent.

The nitrodiamine's strong ultraviolet absorbance in acid solution (Fig. 2) should make possible its determination in any preparation containing only small amounts of the other amines commonly used in hair dyes. In such cases, the absorbance measurement should be made on the acid solution remaining after the initial ether extraction.

SUMMARY

A sensitive spectrophotometric method has been developed for the identification and estimation of 2-nitro-1,4-diaminobenzene.

STUDIES ON COAL-TAR COLORS. XVIII. FD&C YELLOW NO. 1

By CHARLES STEIN and KENNETH A. FREEMAN (Division of Cosmetics, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

The disodium salt of 2,4-dinitro-1-naphthol-7-sulfonic acid (Naphthol Yellow S) is certifiable under the Coal-Tar Color Regulations (1) as FD&C Yellow No. 1. This paper describes the preparation of pure samples of the free color acid which were used to establish spectrophotometric standards and to check the accuracy of the titanium trichloride titration in the quantitative determination of the dye.

The dipotassium salt of 2,4-dinitro-1-naphthol-7-sulfonic acid is certifiable as FD&C Yellow No. 2. Although no samples of FD&C Yellow No. 2 have, as yet, been submitted for certification, the data presented in this paper should be applicable to this dye as well as to FD&C Yellow No. 1.

The color acid was prepared in two ways. In the first method, 1-naphthol-2,4,7-trisulfonic acid was prepared by the sulfonation of α-naphthol and the trisulfonic acid was converted to the desired nitro compound by treating with nitric acid. The procedure described by Gatterman (2) was followed.

In the second synthesis of FD&C Yellow No. 1, the starting material was 1-naphthol-2,4,7-trisulfonyl chloride. Gebauer–Fuelnegg, *et al.* (3), have prepared this compound and established its structure and melting point. The trisulfonyl chloride was hydrolyzed in concentrated sulfuric acid and the resulting monosulfonic acid was nitrated to obtain the dye.

The two samples of the dye showed identical spectrophotometric properties. Either method is suitable for the preparation of a spectro-

photometric standard. The procedure involving the direct sulfonation of α-naphthol is considerably more rapid than that using 1-naphthol-2,4,7-trisulfonyl chloride and may be preferred for this reason.

Knecht and Hibbert (4, 5) have shown that Naphthol Yellow S can be determined quantitatively by titration with titanium trichloride. They added an excess of standardized titanium trichloride and back titrated with a standardized solution of ferric iron. In this work the official A.O.A.C. method (6) was found to give satisfactory results, although the addition of alcohol appears to improve the accuracy of the titration.

Six representative samples of commercial dyes were analyzed both by titration with titanium trichloride and spectrophotometrically. Sodium chloride and volatile matter were also determined. The results are shown

TABLE 1.—*Total analysis of commercial samples of FD&C Yellow No. 1 dye by titration with titanium trichloride*

SAMPLE NO.	DYE BY TITRATION	VOLATILE MATTER	SODIUM CHLORIDE	TOTAL
	per cent	*per cent*	*per cent*	*per cent*
1	90.2	5.8	3.6	99.6
2	90.6	5.8	2.9	99.3
3	92.2	5.7	1.5	99.4
4	90.7	4.8	4.0	99.5
5	91.2	6.3	1.8	99.3
6	92.6	4.3	3.3	100.2
				Av. 99.6

in Table 1 (total analysis based on titration with titanium trichloride) and Table 2 (total analysis based on spectrophotometric analysis). Sulfates, water-insoluble matter, ether extracts, mixed oxides, and Martius Yellow were determined to complete the analysis. In each case, however, the sum of these constituents was negligible, and the results are not included in the tables. The titration and spectrophotometric analyses agree within 1 per cent.

EXPERIMENTAL

Preparation of FD&C Yellow No. 1 (color acid) from α-naphthol.—Gatterman's procedure (2) was followed. The dye was recrystallized four times from 15% HCl, and then dried, first over $CaCl_2$ and finally over H_2SO_4 and KOH. The material thus obtained corresponds quite closely to the dihydrate as shown by loss in weight when dried in an Abderhalden apparatus. The melting point of 151° reported by Gatterman could not be duplicated, probably because of slight variations in the water content. The melting point of the dihydrate does not appear to be a satisfactory criterion of purity. This dihydrate can be converted to the anhydrous material by drying in an Abderhalden apparatus over P_2O_5 at 135° at a pressure of 2 mm of Hg. Analytical determinations were made on the anhydrous color acid.

Calcd. for $C_{10}H_6O_8N_2S$: N = 8.9; S = 10.2. *Found:* N = 8.7, 8.7; S = 10.3, 10.2

TABLE 2.—*Total analysis of commercial samples of FD&C Yellow No. 1 dye by spectrophotometric analysis*

SAMPLE NO.	DYE BY SPECTRO-PHOTOMETER	VOLATILE MATTER	SODIUM CHLORIDE	TOTAL
	per cent	*per cent*	*per cent*	*per cent*
1	91.3	5.8	3.6	100.7
2	92.0	5.8	2.9	100.9
3	92.8	5.7	1.5	100.0
4	91.7	4.8	4.0	100.5
5	92.1	6.3	1.8	100.2
6	92.7	4.3	3.3	100.3
				Av. 100.4

Titration with titanium trichloride (6).—One gram of anhydrous color acid theoretically requires 381.9 ml of 0.1 *N* $TiCl_3$. One gram of the disodium salt requires 335.0 ml of 0.1 *N* $TiCl_3$. The dye is best titrated by preparing a stock solution and taking aliquots containing about 0.05 g of dye. Use 150 ml of water, 50 ml of alcohol, and 20 g of sodium bitartrate. Found: 99.3%, 99.3%.

Preparation of FD&C Yellow No. 1 (color acid) from 1-naphthol-2,4,7-trisulfonyl chloride.—The trisulfonyl chloride was prepared by the method of Gebauer-Fuelnegg (3) (m.p. 175–175.5°C.; reported (3), 174°C.). The trisulfonyl chloride (8 g) was mixed with 10 g of concd H_2SO_4 in a large test tube and the tube was heated in an oil bath at 125°C. After 2 hrs at 125°C., the original paste was converted to a dark colored solution and all evolution of gas had ceased. A sample of this solution did not give a precipitate when diluted with water. The solution was cooled and poured over 30 g of cracked ice. To the cold solution, 6 g of concd HNO_3 was added and the solution was heated at 50°C. for 30 mins. The solution was cooled and stored in a refrigerator overnight. The color acid was filtered off from the cold solvent on a fritted glass filter and recrystallized three times from small portions of 15% HCl. It was dried over KOH and H_2SO_4.

Titration of the anhydrous material with $TiCl_3$: 99.2%, 99.2%.

SPECTROPHOTOMETRIC DATA

Figure 1 shows the absorption spectra in the visible region of the purified color in 0.04 *N* ammonium acetate, 0.1 *N* NaOH, and 0.1 *N* HCl.

The absorption spectrum in acid solution shows no peak and is unsatisfactory for analytical purposes. The absorption spectra in ammonium acetate and NaOH solutions are very similar. Since commercial dyes are the disodium salt of the color acid, the calculations were based on the results obtained in NaOH solution. In 0.1 *N* NaOH, the absorbance of the color acid is 0.0555 per mg per liter. This is equivalent to an absorbance of 0.0487 per mg per liter for the disodium salt. Solutions of the dye in 0.1 *N* NaOH and ammonium acetate solutions obey Beer's law. These solutions are stable for at least twenty-four hours when stored in the dark.

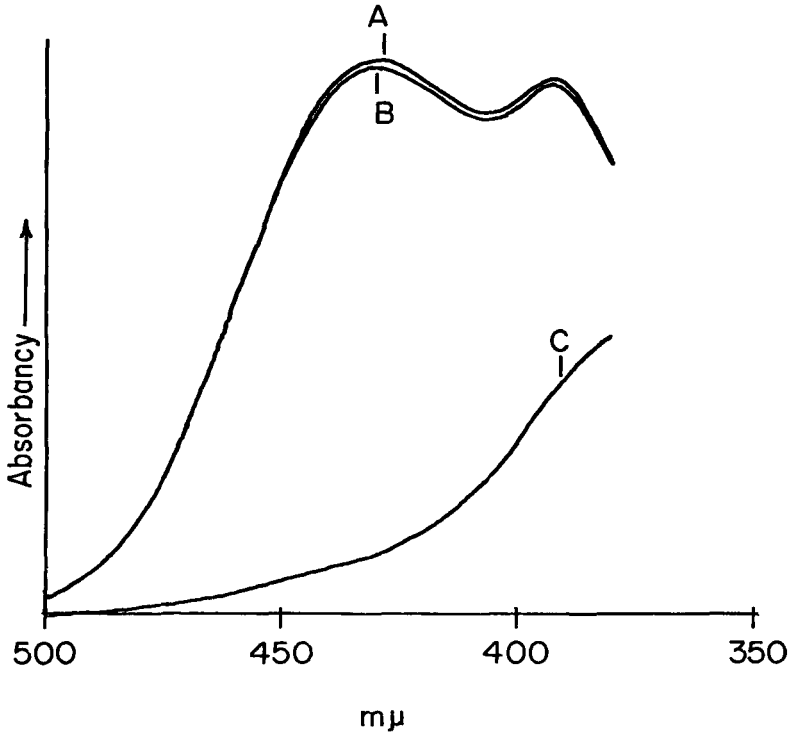

FIG. 1.—Absorbance curves of FD&C Yellow No. 1. Concn: 17.22 mg/l. Solvent: Curve A—0.04 *N* ammonium acetate. Curve B—0.1 *N* NaOH. Curve C—0.1 *N* HCl. Cells: 1 cm.

SUMMARY

Pure samples of FD&C Yellow No. 1 (color acid) have been prepared in two ways.

Titration with titanium trichloride and spectrophotometric analysis in 0.1 *N* NaOH are satisfactory methods for the quantitative determination of the dye.

Complete analyses of representative commercial samples show that the specifications in the Coal-Tar Color Regulations are accurate and practical.

REFERENCES

(1) Service and Regulatory Announcements, F.D.C. 3, Coal-Tar Color Regulations.
(2) GATTERMAN, L., and WIELAND, H., *Laboratory Methods of Organic Chemistry*, The MacMillan Co., New York, N. Y., 1932, p. 186.

(3) GEBAUER-FUELNEGG, E., and HAEMMERLE, E., *J. Am. Chem. Soc.*, **53**, 2648 (1931).
(4) KNECHT, E., and HIBBERT, E., *Ber.*, **36**, 1549 (1903).
(5) ———, *ibid.*, **37**, 3475 (1904).
(6) *Official Methods of Analysis*, 7th Ed., 1950, Association of Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Wash. 4, D. C., p. 671, **34.21(c)**.

THE SEPARATION OF AMARANTH AND TARTRAZINE

By G. G. McKEOWN (Food and Drug Laboratory, Department of National Health and Welfare, Ottawa, Canada)

The section of the A.O.A.C. *Methods of Analysis* (1) dealing with water-soluble food colors does not include a method of separating amaranth (FD&C Red No. 2) and tartrazine (FD&C Yellow No. 5). While indirect procedures of identification and estimation are provided for dealing with a mixture of these two colors, the development of a practical method of separation would be a distinct improvement.

In this paper, a chromatographic separation of amaranth and tartrazine is described. The procedure is rapid and convenient, and the separated colors are recovered almost quantitatively in an unaltered form.

PROCEDURE

APPARATUS

Alumina column.—Use a glass column 150 mm in length and 15 mm I.D. with absorbent cotton as a base plug. Run a slurry of alumina in water into the column to a height of 75 mm. Wash the column with distilled water until the percolate becomes clear. (The alumina is supplied by Fisher Scientific Company, Adsorption Alumina, 80–200 M M.)

REAGENTS

Hydrochloric acid soln.—(1:100). *Sodium hydroxide soln.*—0.4%. *Sodium acetate soln.*—3.0% (anhydrous salt).

METHOD

Activate the column by passing thru ca 100 ml of the HCl soln. Dissolve ten mg of the color mixture to be separated in 20 ml of the HCl soln, pass into the column, and follow by 100 ml of distd water to wash thru the acid. (The colors are fixed on the upper 5–6 mm of the column in the form of an alumina lake).

Pass sodium acetate soln thru the column at a rate of 5 ml per min. and collect the eluate in 100 ml fractions. (A yellow band of tartrazine soon separates from the amaranth on the column. Tartrazine begins to appear in the second 100 ml fraction of the eluate.) Continue the process until the eluate becomes nearly colorless or faintly pink. (At this point, all or almost all of the tartrazine has been removed from the column.) Then remove amaranth from the column with 100–200 ml of the NaOH soln.

Bring each fraction of the eluate to a *p*H of ca 6.0 with concd HCl and examine spectrophotometrically.

TABLE 1.—*Separation of mixtures containing 1 mg of tartrazine and 9 mg of amaranth*

FRACTION NO.	SOLVENT	RUN NO. 1		RUN NO. 2	
		TARTRAZINE	AMARANTH	TARTRAZINE	AMARANTH
		mg	*mg*	*mg*	*mg*
1	NaOAc soln	—	—	0.002	—
2	NaOAc	0.134	0.003	0.149	0.003
3	NaOAc	0.398	0.008	0.476	0.005
4	NaOAc	0.327	0.013	0.294	0.008
5	NaOAc	0.108	0.018	0.061	0.018
6	NaOAc	0.033	0.023	0.018	0.030
7	NaOH	—	8.94	—	8.94

RESULTS

The colors used were commercial samples supplied by the Dye and Chemical Company of Canada, Kingston, Ontario. All weights of color refer to pure color calculated on the basis of pure dye content.

A total of 10 mg of color was used in each separation. Duplicate separations were carried out using three ratios of tartrazine and amaranth. Tables 1, 2, and 3 show the amount of color found in each successive 100 ml fraction of the eluate. It will be seen from the tables that no color is lost during the separation. The sodium acetate fractions contain 99–100 per cent of the tartrazine plus 1–3 per cent of the amaranth. The sodium hydroxide fractions contain 97–99 per cent of the amaranth plus 0–1 per cent of the tartrazine.

DISCUSSION

The separation has been applied to food color work in this laboratory and was found to be quite satisfactory for both qualitative and quantitative studies.

TABLE 2.—*Separation of mixtures containing 5 mg of tartrazine and 5 mg of amaranth*

FRACTION NO.	SOLVENT	RUN NO. 1		RUN NO. 2	
		TARTRAZINE	AMARANTH	TARTRAZINE	AMARANTH
		mg	*mg*	*mg*	*mg*
1	NaOAc soln	0.007	—	0.016	—
2	NaOAc	0.328	—	0.468	—
3	NaOAc	1.308	—	1.313	—
4	NaOAc	2.300	0.003	2.010	0.003
5	NaOAc	1.017	0.028	1.119	0.021
6	NaOAc	0.040	0.018	0.074	0.028
7	NaOH	—	4.95	—	4.95

TABLE 3.—*Separation of mixtures containing 9 mg of tartrazine and 1 mg of amaranth*

FRACTION NO.	SOLVENT	RUN NO. 1		RUN NO. 2	
		TARTRAZINE	AMARANTH	TARTRAZINE	AMARANTH
		mg	*mg*	*mg*	*mg*
1	NaOAc soln	—	—	—	—
2	NaOAc	0.154	—	0.122	—
3	NaOAc	0.722	—	0.578	—
4	NaOAc	1.527	—	1.220	—
5	NaOAc	2.437	—	1.927	—
6	NaOAc	2.892	—	2.605	0.002
7	NaOAc	1.062	0.009	2.205	0.005
8	NaOAc	0.168	0.024	0.275	0.019
9	NaOH	0.038	0.967	0.068	0.974

The nature of the separation is of some interest. There have been many comprehensive investigations of the chromatography of water-soluble food colors on alumina columns (2–4). In each case, the separations were based on the differences of physical adsorption, and the well-known chemical phenomenon of lake formation was avoided as an undesirable side effect. In the foregoing study, however, it will be noted that the colors were intentionally fixed upon the column in the form of a lake.

Alumina lakes may be decomposed to yield the free color by washing with salts or bases of sodium, potassium, ammonia, etc. As each color has its own affinity towards lake formation, it was reasoned that preferential removal of colors from a column of alumina might be possible. It was found that strongly alkaline solutions removed colors from a column *en masse* and thus effected no separation. Dilute salt solutions, however, removed the colors very slowly and permitted an equilibrium to be established between the formation and decomposition of the lakes. In this particular case, amaranth has a much greater tendency towards lake formation than has tartrazine and thus the latter descends the column at a much faster rate. The process closely resembles that of an anion exchange.

ACKNOWLEDGMENT

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REFERENCES

(1) *Official Methods of Analysis, A.O.A.C.*, 7th Ed., 1950, pp. 656–657.
(2) RUGGLI, P., and JENSEN, P., *Helv. Chim. Acta.*, **18**, 624 (1935); **19**, 64 (1936).
(3) RUIZ, SAENZ LASCANO, I., *Ind. parfum.*, **1**, 187 (1946).
(4) ———, *Ann. fals. et fraudes.*, **41**, 211 (1948).

SEPARATION AND IDENTIFICATION OF CHLORINATED ORGANIC PESTICIDES BY PAPER CHROMATOGRAPHY. V. DDT (*ORTHO,PARA'* AND *PARA,PARA'* ISOMERS), RHOTHANE, AND METHOXYCHLOR

By LLOYD C. MITCHELL (Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

The investigation described in this paper deals with the separation and identification by paper chromatography of the *o,p'*[1] and *p,p'*[2] isomers of DDT, rhothane[3] (also known as DDD and TDE), and methoxychlor (MC).[4]

METHOD

APPARATUS

The chromatographic tank and accessories have been described in earlier publications (1–3).

REAGENTS

(a) *Immobile solvents.*—
  (1) Dimethylformamide (DMF):[5] Dil. 25 ml to 100 ml with ethyl ether, ACS grade.
  (2) Soybean oil: Dil. 1 ml refined oil to 100 ml with ethyl ether.

(b) *Mobile solvents.*—
  (1) Petroleum ether (PE): ACS grade, B. P. 30–60°C.
  (2) Acetone, ACS grade, and water, 75+25, v/v.

(c) *Chromogenic agents.*—
  (1) 0.05 *N* $AgNO_3$ in ethanol.
  (2) Formaldehyde soln (ca 37%).
  (3) 2 *N* KOH in methanol.
  (4) Concd HNO_3 and 30% H_2O_2, 1+1 v/v.

(d) *Standards.*—
  0.01 and 0.1 *M* solns of *o,p'*-DDT, *p,p'*-DDT, rhothane, and methoxychlor, and mixtures of all four components in 0.01 and 0.1 *M* concns, in ethyl acetate.

(e) *Filter paper.*—
  Whatman No. 1 in 8×8″ sheets. The particular batch of filter paper used in this work did not contain enough Ag reactants to interfere with the location of the developed spots. With other batches of papers, it may be desirable to take the precaution of washing (2) the filter paper prior to use with mobile solvents contg H_2O.

PROCEDURE

One 8×8″ paper sheet was spotted with 0.005 ml portions of the 0.1 *M* solns, and a second paper with 0.01 *M* solns, at equal intervals along base line; papers were sprayed with immobile solvent (reagent **a**-1 or **a**-2), and chromatograms were de-

[1] *o,p'*-DDT is 1,1,1-trichloro-2(*o*-chlorophenyl)-2(*p*-chlorophenyl) ethane.
[2] *p,p'*-DDT is 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl) ethane.
[3] Rhothane is 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl) ethane; obtained from Rohm & Haas Co., Philadelphia, Pa.
[4] Methoxychlor is 1,1,1-trichloro-2,2-*bis*(*p*-methoxyphenyl) ethane; obtained from E. I. du Pont de Nemours, Inc., Wilmington, Del.
[5] Obtained from Matheson Coleman and Bell, Inc.

veloped with mobile solvent (reagent b-1 or b-2), as previously described (3, 5). When mobile solvent approached top of sheets (about ¾ hr for PE and about 2¼ hrs for acetone), papers were removed from tank, solvent front was marked, and papers were hung from rod in hood until apparently dry (ca 5–10 mins.). Wearing rubber gloves, the operator sprayed papers with chromogenic agents (reagents c-1 to c-4) as previously described (4). A tank lined with blotting paper was used for PE (1; cf. 6, 7). About 50 ml PE was added to bottom of tank *just prior* to inserting papers into troughs therein.

DISCUSSION

Volatile solvents, such as petroleum ether (30–60°), are difficult to use in the ascending procedure of paper chromatography because they tend to evaporate too rapidly from the chromatogram to ascend the paper for any appreciable distance. Some provision must be taken to saturate the air within the container with solvent vapor other than that from the trough and chromatogram. One way to achieve this condition is to line the container with blotting paper (ordinary desk blotters) (1; cf. 6, 7), the lower edge of which dips into mobile solvent added to the bottom of the tank *just prior* to inserting the paper(s). The solvent front on the liner should move upward slightly ahead of the solvent front on the chromatogram. If it lags behind the chromatogram front, the solvent evaporates from the latter to such an extent that there is no distinctly visible front. This behavior contributes to excessive diffusion (visible only after application of the chromogenic agents) with the result that the developed spots of different components overlap and are indistinct. On the other hand, if the front on the liner moves too far ahead of that on the chromatogram, vapor tends to condense on the chromatogram to cause diffusion of the spots when the chromatogram front moves into the area of condensed vapor.

Although petroleum ether can be employed at temperatures of 26–27°, or even up to 30°, it performs better at 25° and below. Large differences may occur in R_F values, as illustrated in Table 1, where the values for 24° are compared with those for 27°.

Successful separations of the four substances (*o,p'*-DDT, *p,p'*-DDT, DDD, and MC) can be obtained with the solvent system, DMF (immobile) and PE (mobile), when the proper amount of DMF is added to the paper. With 10 per cent DMF in ethyl ether (v/v), the two DDT isomers separate from DDD and MC, but not from each other. With 25 per cent DMF, which is about the maximum amount this paper will hold, the isomers of DDT separate from each other and from DDD and MC, but the DDD and MC are so close together that the spots overlap. Other aliphatic hydrocarbons [hexane, isohexanes, heptane, mixed (or *iso*) octanes] or cyclohexanes did not satisfactorily separate the four substances.

Another peculiarity of this system is the tendency for mixtures of DDD

TABLE 1.—*R_F values of o,p'-DDT, p,p'-DDT, DDD, and methoxychlor*[a]

NO.	0.01 M CONCN.								NO.	0.1 M CONCN.							
	DDT *o,p'*	DDT *p,p'*	ALL FOUR SUBSTANCES				DDD	MC		DDT *o,p'*	DDT *p,p'*	ALL FOUR SUBSTANCES				DDD	MC
Temperature, 24°																	
A1	.69	.51	.67	.51	.22	.17	.20	.10	A2	.68	.60	.76	.59	.26	.21	.22	.14
B3	.69	.51	.63	.49	.21	.17	.19	.13	B4	.71	.56	.70	.53	.24	.19	.21	.18
C5	.69	.54	.71	.55	.26	.21	.22	.16	C6	.66	.56	.70	.55	.27	.21	.26	.18
D7	.72	.59	.72	.55	.27	.22	.24	.15	D8	.73	.60	.71	.56	.26	.21	.25	.19
A1	.65	.47	.63	.48	.24	.19	.26	.19	A2	.63	.53	.68	.50	.23	.18	.22	.17
B3	.64	.46	.63	.45	.23	.19	.21	.14	B4	.68	.59	.66	.51	.25	.20	.25	.19
C5	.57	.43	.57	.42	.20	.15	.20	.15	C6	.61	.52	.62	.46	.21	.18	.19	.13
D7	.67	.53	.69	.50	.24	.20	.23	.15	D8	.68	.54	.67	.53	.25	.21	.25	.19
Temperature 27°																	
A1	.94	.87	.96	.89	.55	.44	.52	.38	A2	.96	.94	.98	.91	.57	.47	.55	.43
B3	.91	.79	.91	.82	.48	.39	.47	.33	B4	.92	.85	.93	.83	.45	.38	.43	.32
C5	.92	.86	.94	.86	.49	.38	.49	.41	C6	.93	.90	.90	.86	.50	.41	.53	.43
D7	.83	.74	.83	.69	.41	.35	.39	.31	D8	.89	.80	.89	.79	.42	.33	.40	.30

[a] Solvent system: Immobile—Dimethylformamide in ethyl ether, 25+75, v/v. Mobile—petroleum ether.

TABLE 2.—*R_F values of o,p'-DDT, p,p'-DDT, DDD, and methoxychlor*[a]

NO.	0.01 M CONCN							NO.	0.1 M CONCN							
	DDT *o,p'*	DDT *p,p'*	ALL FOUR SUBSTANCES			DDD	MC		DDT *o,p'*	DDT *p,p'*	ALL FOUR SUBSTANCES			DDD	MC
A1	.48	.47	.48	.58	.72	.57	.72	A2	.48	.48	.47	.58	.70	.57	.69
B3	.51	.51	.51	.59	.73	.58	.72	B4	.50	.50	.50	.60	.75	.60	.73
C5	.51	.49	.50	.59	.74	.58	.74	C6	.49	.49	.48	.58	.73	.58	.71
D7	.48	.48	.49	.58	.73	.57	.73	D8	.48	.48	.48	.58	.72	.57	.70

[a] Solvent system: Immobile—refined soybean oil in ethyl ether, 1+99, v/v. Mobile—acetone and water, 75+25, v/v.

and MC to give R_F values closer together than the corresponding values for these materials applied to the paper separately. The reverse of this behavior applies to *o,p'*- and *p,p'*-DDT mixtures, which tend to have R_F values farther apart than those obtained from chromatograms of the individual isomers.

Where it is unnecessary to separate the two isomers of DDT from each other, but is desirable to separate DDT, DDD, and MC from one another, solvent systems consisting of oil[6] as the immobile solvent and aqueous solutions of acetic acid, acetone, ethanol, methyl cellosolve, pyridine, or any combination of the above mentioned solvents may be employed. Successful separations were obtained with water content in the mobile phase from 20 to 30 per cent, v/v. Of the above-named solvent systems, oil–aqueous acetone separated DDT, DDD, and MC distinctly.

Experiments were conducted in which the mobile phase was varied from 100 to 65 per cent acetone, v/v, in steps of 5 per cent. With 100 per cent acetone, the three substances separated in the upper quarter of the chromatogram, while with 65 per cent acetone, separation occurred in the lower quarter. With 75 and 80 per cent acetone, the separated spots were in the middle half of the chromatogram; this location of the spots is to be preferred. Table 2 lists the R_F values for 75 per cent acetone. The corresponding R_F values for separations with 80 per cent acetone are about 0.10 higher.

It is of interest to note that rhothane moves faster than methoxychlor in the dimethylformamide–petroleum ether system, whereas the reverse is true with soybean oil–aqueous acetone.

SUMMARY

DDT, rhothane, and methoxychlor are separated by paper chromatography with soybean oil as the immobile solvent and aqueous acetone as the mobile solvent. *o,p'*- and *p,p'*-DDT are separated from each other, and from rhothane and methoxychlor, with dimethylformamide (immobile) and petroleum ether (mobile).

REFERENCES

(1) MITCHELL, L. C., *This Journal*, **36**, 1187 (1953).
(2) *Ibid.*, **35**, 920 (1952).
(3) *Ibid.*, **36**, 943 (1953).
(4) *Ibid.*, **35**, 928 (1952).
(5) MITCHELL, L. C., and PATTERSON, W. I., *ibid.*, **36**, 553 (1953).
(6) MCFARREN, E. F., *Anal. Chem.*, **23**, 168 (1951).
(7) MEIGH, D. F., *Nature*, **169**, 706 (1952).

[6] Refined soybean oil was used in these experiments; other refined oils (other than mineral oil) are probably equally satisfactory.

ANALYSIS OF COMMERCIAL SODIUM LAUROYL SARCOSINATE

By HELENE IWASENKO (Research Laboratories of Bristol-Myers Products Division, Hillside, N. J.)

Commercial sodium lauroyl sarcosinate usually contains sodium laurate, which imparts a bitter, soapy taste.

The sarcosinate content can be determined by titration with 0.004 *M* cationic detergent using dichlorofluorescein as indicator.[1] Sodium laurate, however, will also react with the cationic detergent and thus cause erroneous results.

Attempts to separate the two by means of organic solvents failed because they, as well as the other salts of lauroyl sarcosine and lauric acid, seem to have practically the same solubility and the same holds for the acids themselves. The latter, however, do possess different solubilities in a solution of diammonium hydrogen phosphate, the lauric acid being insoluble. This permits a separation, and the following procedure is recommended.

METHOD

REAGENTS

(a) *Recrystallized quaternary ammonium chloride, M.W. 400.*—For a 0.004 *M* soln weigh accurately 0.3200 g and transfer to a small beaker. Dissolve completely, transfer to a 200 ml volumetric flask and make to vol.

(b) *Dichlorofluorescein.*—Dissolve ca 0.05 g in 3 ml of 0.1 *N* NaOH. Add 50 ml of distd H_2O. Filter if necessary. Keep in a stoppered bottle.

(c) *Diammonium hydrogen phosphate.*—C.P.

(d) *Chloroform.*—Reagent grade.

PREPARATION OF SAMPLE SOLUTION

Dissolve 1.000–1.1500 g of sample in 100–150 ml of distd H_2O and warm on a steam bath for a few min. If the soln is clear, transfer to a 250 ml volumetric flask and make to vol. (Soln I). Transfer a 50 ml aliquot of Soln I to another 250 ml volumetric flask and make to vol. (Soln II).

If cloudiness appears during the prepn of Soln I, discard the sample, re-weigh a 0.2200–0.2300 g portion, dissolve in ca 100–150 ml of distd H_2O, warm on the steam bath, transfer (cloudiness and all) to a 250 ml volumetric flask, and make to vol. (Soln III).

TITRATION

Pipet 10 ml of Solution II (or III) into a 50 ml graduated, glass-stoppered cylinder. Add 15–20 ml of distd H_2O, followed by 2–3 drops of indicator and 10 ml of $CHCl_3$. Shake well. Add standard cationic detergent from a 10 ml microburet almost to the end-point. Avoid shaking; invert the cylinder gently. (Near the end-point the greenish-yellow color of the water layer changes to pink). Titrate to the first appearance of the pink color in the $CHCl_3$ layer. Observe color

[1] *This Journal*, 36, 1165 (1953).

in transmitted light. Titrate at least 3 aliquots and take the av. This reading represents the sum of sodium lauroyl sarcosinate and sodium laurate (if the latter is present).

SEPARATION

Transfer 50 ml of Solution I to a small beaker. Warm on the steam bath for a few min. and add 3 ml of 3 *N* HNO_3. Do not use a stirring rod. Cool in the ice box for 10–15 min., filter through a fluted No. 40 filter (use a policeman) and discard the filtrate.

If, as mentioned above, cloudiness appears when the 1.0 to 1.15 g sample is dissolved, re-weigh the exact quantity used to prep. Solution III above, dissolve in about 100–150 ml of distd H_2O, warm on steam bath, add 3 ml of 3*N* HNO_3 and proceed as above.

In the original beaker dissolve ca 2 g of diammonium hydrogen phosphate in ca 20 ml of distilled water. Turn the filter paper with pptd acids inside out and immerse it in the diammonium hydrogen phosphate soln. With the aid of the policeman, break the paper and incorporate any particles adhering to the sides of beaker. Filter thru No. 40 filter into 250 ml volumetric flask. Rinse the beaker with small portions of H_2O and transfer to filter. Repeat operation until the vol. in the flask is almost 250 ml. Make to vol., mix, and titrate a 10 ml aliquot as described under "Titration." This reading represents the sodium lauroyl sarcosinate alone. The difference between the first and the second titration is a measure of the sodium laurate present.

1 ml of 0.004 *M* quaternary ammonium chloride = 0.001172 g of sodium sarcosinate
= 0.000888 g of sodium laurate

EXPERIMENTAL

Possible interference by lauric acid in the titration after the separation was checked as follows:

Quantities varying from 0.1 to 0.5 g of lauric acid, C.P., were placed in beakers with 2 g of diammonium hydrogen phosphate, dissolved in 20 ml of water, and allowed to stand for 15 minutes. The solutions were then

TABLE 1.—*Recovery of sodium lauroyl sarcosinate*

SAMPLE NO.	Na-LAUROYL SARCOSINATE		RECOVERY
	CALCULATED	FOUND	
	g	*g*	*per cent*
1	0.2107	0.2133	101.2
2	0.2270	0.2227	98.1
3	0.2137	0.2133	99.8
4	0.2097	0.2093	99.8
	Average recovery:		99.7
	Max. variation:		1.6

filtered and titrated with 0.004 *M* cationic detergent. In all cases the first drop produced a distinct pink coloration in the chloroform layer.

In order to check the recovery of sodium lauroyl sarcosinate after the separation, a series of tests were made on samples which other analytical methods had indicated to be from 95.3 per cent to 99.0 per cent pure. The results are shown in Table 1.

Next, known amounts of sodium laurate were added to a sample of sodium lauroyl sarcosinate. Table 2 shows the recoveries by the method described.

TABLE 2.—*Recoveries of laurate and sarcosinate*

SAMPLE NO.	SARCOSINATE			LAURATE				
	CALCULATED	FOUND	RECOVERY	ADDED		FOUND		RECOVERY
	g	g	*per cent*	g	*per cent*	g	*per cent*	*per cent*
1	0.1124	0.1149	102.2	0.0503	30.90	0.0488	30.00	97.1
2	0.2613	0.2672	102.3	0.1009	27.80	0.0959	26.47	95.0
3	0.2314	0.2353	101.7	0.0472	16.94	0.0444	15.93	94.1
4	0.2112	0.2138	101.2	0.0237	10.08	0.0231	9.83	97.5
5	0.2139	0.2133	99.7	0.0069	3.12	0.0073	3.30	105.7
6	0.2139	0.2133	99.7	0.0069	3.12	0.0073	3.30	105.7
7	0.2139	0.2128	99.5	0.0046	2.10	0.0041	1.87	89.0
8	0.2139	0.2133	99.7	0.0046	2.10	0.0037	1.69	80.0
9	0.2046	0.2077	101.0	0.0023	1.11	0.0016	0.77	69.3
10	0.2139	0.2204	103.0	0.0023	1.06	trace	—	—

Next, the possible correlation between bad taste (soapy, bitter) of the sodium lauroyl sarcosinate and the amount of sodium laurate present was investigated. Samples of commercial sodium lauroyl sarcosinate, judged organoleptically as "bad" and "good" were analyzed for sodium laurate content. The results are given in Table 3.

TABLE 3.—*Correlation between taste and amount of laurate*

SAMPLE NO.	JUDGED	LAURATE FOUND (PER CENT)
1	bad	6.7
1	bad	7.0
1	bad	6.7
2	bad	16.2
3	good	1.7
3	good	0.9
4	good	trace
4	good	0.42
5	good	trace
6	good	none
7	bad	none

The above indicates a correlation between a bad taste in the sodium lauroyl sarcosinate and a high content of sodium laurate. Apparently, however, a bad taste may result from other impurities as well.

SUMMARY

A method for the estimation of sodium laurate in the presence of sodium lauroyl sarcosinate has been presented.

The method is based on the fact that lauric acid is not soluble in a solution of diammonium hydrogen phosphate, whereas lauroyl sarcosine is. The method utilizes a titration with 0.004 *M* cationic detergent with dichlorofluorescein as the indicator.

THE DETERMINATION AND AVAILABILITY OF LESS SOLUBLE BORATES IN FERTILIZERS*

By J. A. NAFTEL and E. W. FAJANS (Pacific Coast Borax Co., Division of Borax Consolidated, Ltd., Auburn, Ala. and Los Angeles, Calif.)

Within the last two to three decades, trace elements such as boron, copper, iron, manganese, molybdenum, and zinc have been established as essential plant food elements. Many plant disorders have become recognized as trace element deficiencies, and in some states, authorities recommend the use of minimum supplements of trace elements for all chemical fertilizers sold (1). The purpose of this paper is to point out the interest in less soluble trace elements and the need for the development of suitable chemical control methods for such products.

Agricultural scientists have recognized the deficiencies of certain trace elements and have in a general way mapped the soil and crops involved. Recommendations for application of the specific trace element or elements found to be deficient have usually followed. Remedial action involves the use of some source of the deficient trace elements, such as ores or salts, which contain them in an available form. Recently (2–5) interest has developed in the use of compounds containing the trace elements in a less soluble and more stable form, such as slags or frits. There are three main reasons for this new approach. First, although the trace elements are essential and necessary, plants are sensitive to excess concentrations and care must be observed to avoid toxicity. Secondly, many of the soils most deficient in trace elements are the light-textured type, readily susceptible to rapid leaching; hence, less soluble trace elements will persist in such

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soils throughout the growth period of the crop. Thirdly, less soluble trace elements are more likely to supply constant low concentrations of the needed nutrient and thus will be more efficient in terms of optimum crop requirements and desired response.

DEGREES OF SOLUBILITY

The sources of boron may be classified as readily soluble, slightly soluble, and difficultly soluble in water. Examples of the above classes, respectively, are: borax and fertilizer borate, both now widely used; colemanite, a calcium borate ore in which interest is increasing; and slags or borosilicate frits. An analogous series of chemical compounds might exist or be prepared for the other commonly recognized trace elements.

Recently, a number of soil scientists (2–4) have pointed out the advantages of using less soluble forms of boron for fertilizer. As a result of extensive investigations at the South Carolina Agricultural Experiment Station, Page (4) recently reported to the Association of Southern Control Officials that colemanite was "superior to the more soluble borates as a source of boron" on certain sandy soils. The superior quality of colemanite was attributed to slower rate of solution, more constant rate of supply of boron, and lesser effects from moisture conditions. In this paper, attention is also called to the need for, and probable utility of, borosilicates of much lower solubility which have shown promise in limited tests.

The less soluble forms of trace elements will need further extensive evaluation in laboratory, greenhouse, and field tests to determine the rates of application, availability to plants, and residual effects in the soil. These less soluble trace elements will require the revision of current chemical methods of control. The development of less soluble sources of trace elements will be facilitated by the early development of suitable and accurate chemical methods of analysis. This problem has received the attention of chemists in some laboratories (6–7), and methods are already in use which appear promising for determining an index of availability for the use of the agronomist.

TOTAL B_2O_3 CONTENT

Water soluble borates.—Methods of determination of B_2O_3 described in *Official Methods of Analysis* of the A.O.A.C., 7th ed. (1950), were designed mainly for mixed fertilizers which contain water-soluble borates such as borax and fertilizer borates. The "Identical *p*H Method" for the determination of borax in mixed fertilizers, given as *Method I* on page 19, is considered by some as the most suitable; it is understood that it has been used in many laboratories with satisfactory results. *Method II*, given

on page 20, is not in favor because it entails a distillation procedure, and it has been demonstrated by Taylor (8) and also by others (9) that low results are generally obtained if the boron is separated from other impurities by distillation as methyl borate. For the analysis of borax or fertilizer borates which contain high amounts of borax and are free from phosphates and ammonium salts, the method given by Scott (10) is recommended.

Less soluble borates (*e. g., colemanite*).—Recently, considerable interest has been shown in colemanite as a source of boron. For the analysis of pure colemanite, the method given by Scott (10) is again recommended. The "Identical *p*H Method" is entirely satisfactory for the analysis of mixed fertilizers containing colemanite. In this method hydrochloric acid is used for bringing the fertilizer materials into solution; this will decompose the colemanite with the formation of calcium chloride and boric acid. The amount of acid recommended in A.O.A.C. *Method I* is sufficient to decompose all the colemanite present, since it will readily dissolve as long as the solution remains acidic.

It should be mentioned that a procedure using hot water as extractant cannot be used for B_2O_3 determinations in fertilizers which contain colemanite or other sparingly soluble borates. If no acid is used for dissolving the sample, a substantial percentage of colemanite remains undissolved. In the "Modified Virginia Method" (11), a sample of 2.5 g is taken. If it is assumed that the fertilizer contains 4 per cent of a borate, this would be equivalent to 0.1 g. On suspending a 0.1 g sample of colemanite (ground to minus 65 mesh) in 125 ml of water and boiling for 10 minutes as recommended in this procedure, it was found that only 44 per cent of the colemanite was dissolved. Using 0.5 g of colemanite (minus 20 plus 40 mesh) only 7.6 per cent of the B_2O_3 was dissolved. For this reason this method cannot be considered for the analysis of fertilizers containing colemanite, and it is recommended that the A.O.A.C. *Method I* ("Identical *p*H Method") be used.

Water insoluble borates.—Several aluminum borosilicate products which contain boron and other trace elements such as iron, copper, manganese, zinc, and molybdenum in a very sparingly soluble form are already in production. Such products may be produced as frits, which means that the essential trace elements are fused together with silicates to form a glass. So far, only a limited amount of experimental work has been done on methods of analysis of such agricultural frits, but there is considerable information available on the determination of B_2O_3 in glass and enamel frits. Since the chemical composition of these products is very similar, the same procedures can be used for analysis. In all procedures for the determination of B_2O_3 in silicates, it is essential to decompose them first by fusion with 5 to 6 times their weight of soda ash. Scott (12) gives a method for the determination of boric oxide in silicates, enamel, etc., in which the

boric oxide is separated from the other constituents (after soda ash fusion and solution in HCl) by distilling it as methyl borate. As mentioned earlier in this paper, our experience is that the distillation method generally gives low results. Scott (13) gives another method for the determination of B_2O_3 in glass. In this procedure, the finely ground glass is fused with an excess of soda ash. The melt is then taken up with dilute HCl, heated to boiling, and dry precipitated calcium carbonate is added in moderate excess to precipitate aluminum and other metals which may be present. After filtration and removal of CO_2, the B_2O_3 is finally titrated by the standard mannite indicator method. There is, however, a certain danger that in the analyses of frits containing very high percentages of silica and low percentages of boron, a certain amount of the silica will remain in solution even after the calcium carbonate treatment and may be titrated together with the B_2O_3; in consequence, the result for boron may be too high.

In view of this, and also because the procedure as such would not be satisfactory for the analysis of fertilizers which contain phosphates, we favor a method which is essentially the same as the "Identical *p*H Method" (A.O.A.C. *Method I*), except that initially the sample is fused with soda ash. Hollander and Rieman (14) have published such a method in which the sample is first fused with soda ash and dissolved in hydrochloric acid; the *p*H is then adjusted with sodium hydroxide to 5.0–5.5 to precipitate aluminum, iron, and similar elements along with most of the silica. After filtration, the B_2O_3 is finally titrated by the "Identical *p*H Method." These workers recommend the removal of phosphate by the addition of a slight excess of silver nitrate at *p*H 5.5.

Ball and Fajans (9) have published a modification of this method which is particularly suitable for analyses of glasses containing small concentrations (0.1–5 per cent) of B_2O_3. They eliminate phosphates with lead nitrate as in A.O.A.C. *Method I*. Both publications show that this electrometric method gives accurate results in the analyses of the B_2O_3 content of glasses.

As most of the agricultural frits are closely related chemically to glasses, and as the "Identical *p*H Method" as developed by Taylor (8) for the analysis of fertilizers has been found very satisfactory, we would recommend the following method for a collaborative study:

PROPOSED METHOD FOR DETERMINATION OF BORON IN FERTILIZERS CONTAINING INSOLUBLE BORATES

Weigh the finely ground sample within 1 mg (1.0 g for up to 0.45% B; smaller samples above that content) and mix thoroly with 5 g powdered Na_2CO_3. Fuse in a Pt crucible for 10 min. Dissolve in 20 ml 6 *N* HCl, and wash the crucible with 30 ml H_2O. Proceed as in the A.O.A.C. *Method I*, Section **2.47**, beginning with the third sentence, except that in the standardization of the NaOH soln described in Section **2.46**, add 8.0 g NaCl, rather than 3.0 g.

Several determinations were carried out on a commercially available trace element frit which contained boron, iron, copper, manganese, zinc, and molybdenum. The final solution was titrated by the "Identical *p*H Method" with and without the addition of known amounts of B_2O_3 to check the accuracy of the method. Results are given in Table 1.

TABLE 1.—*Boron analysis of a trace element frit*

SAMPLE	MG B_2O_3	
	CALCULATED	FOUND
5 ml of frit solution	0.203	0.21
2 ml of frit solution	0.08	0.081
2 ml of frit solution plus 0.056 mg B_2O_3	0.136	0.135
1 ml of frit solution plus 0.028 mg B_2O_3	0.068	0.064

It is understood that this method has been found quite satisfactory by another laboratory which makes a considerable number of analyses of boron frits.

"AVAILABLE" B_2O_3 CONTENT

The methods discussed above will obviously determine only the total B_2O_3 content of a fertilizer. In many cases it may be of interest to know whether the fertilizer contains comparatively readily soluble sodium borates or less soluble boron compounds.

The assumption is made that the rate at which the B_2O_3 in borates becomes "available" to the plant would be in proportion to the rate of solution, and by the same reasoning, the lower this rate of solution the less the danger of toxicity. Considerable work has been conducted on the rate of solution of all types of borates, including some boron frits. Naturally, any measurement of rate of solution will not give an absolute value, as the result will depend entirely on experimental conditions such as fineness of particles, ratio of solids to water, rate of stirring, etc.; however, such experiments are useful as they give the relative rates of solutions and an "index" of availability.

Relative rates of solution were determined as follows: A 10 g sample of the borate was placed in 400 ml of distilled water in a closed, one pint, Ball Mason jar and mechanically agitated in a shaker with a sloshing, rocking action. Ten ml aliquots of the liquid were removed at regular intervals. After filtration, these samples were titrated for their B_2O_3 content by the "Identical *p*H Method." No phosphates were present in any of these samples; therefore treatment with lead nitrate was unnecessary. Figures 1 and 2 give the results of such determinations. The sodium borates, naturally, have a relatively high rate of solution. It will be seen that

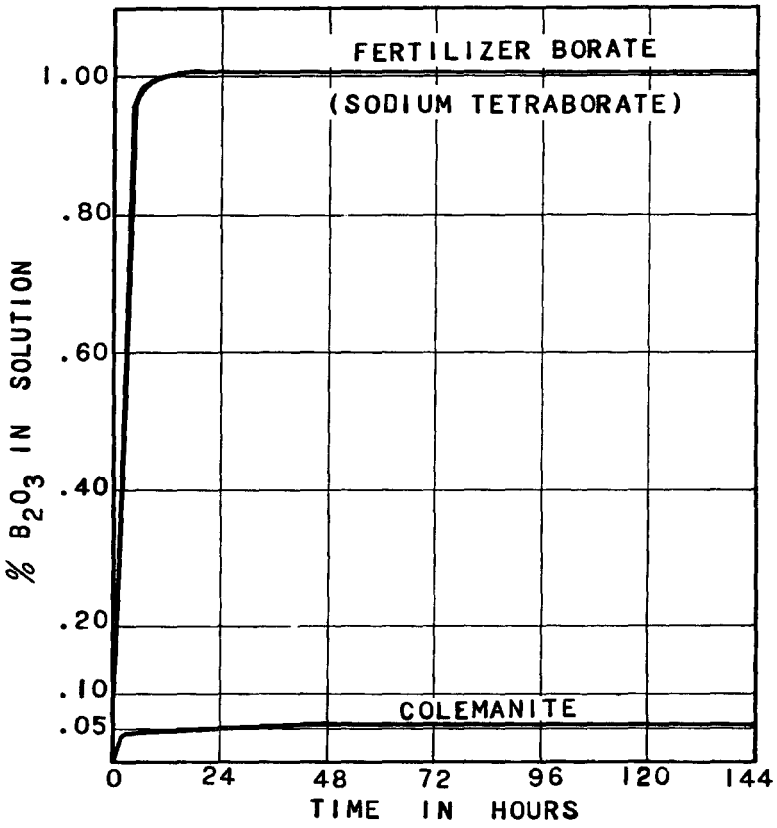

FIG. 1.—Relative rates of solution of fertilizer borate and colemanite.

colemanite, a calcium borate, will dissolve much more slowly, while howlite, a calcium borate containing silica in its molecule, goes into solution even more slowly. The rates of solution of several boron frits are also shown (Fig. 2). It is of interest to point out that plant experiments have shown what seems to be a good correlation between rate of solution and toxicity and/or favorable response by boron-sensitive plants.

In the determinations of the rates of solution of B_2O_3 contained in boron frits, it is essential to use the "Identical *p*H Method," since appreciable amounts of silica are also solubilized. This would be titrated together with the B_2O_3 by the conventional mannite indicator method. It has been found with one product that the indicator method gave a B_2O_3 content about 6 times too high.

Fertilizers containing the less soluble trace elements will no doubt be

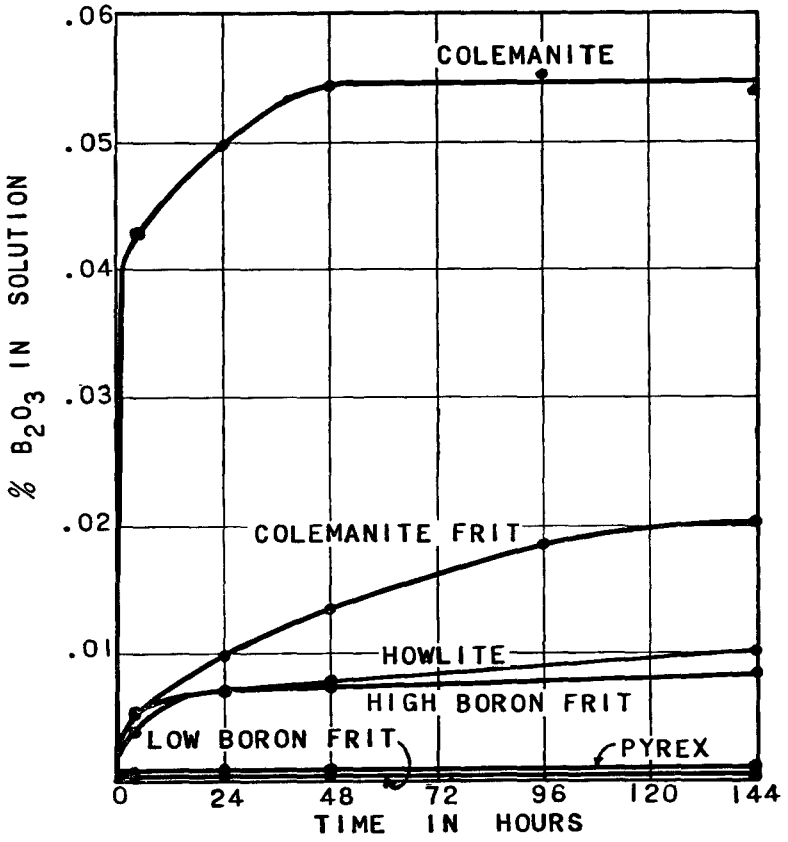

FIG. 2.—Relative rates of solution of boron fertilizers.

offered with guarantees for both the total and the water-soluble contents of contained trace elements. As pointed out above, determinations of total content will offer no particular difficulty, but research and collaborative studies will be required for the development of proper control methods of analysis for the "available" content. It is likely that guarantees or claims that the water-soluble contents of trace elements will be within certain minimum and maximum percentages, when analysed under controlled conditions, will be made.

The above method for rate of solution of B_2O_3 is offered for consideration as an index of solubility which may characterize the less soluble borates from the agronomic standpoint. On the other hand, the control chemists may rely upon methods for total and water-soluble B_2O_3 content.

Perhaps the A.O.A.C. will study collaboratively the methods of analysis

outlined above, or other methods which may be proposed. In due course, suitable chemical methods of control for the less soluble trace elements will undoubtedly be developed.

ACKNOWLEDGMENTS

The experiments on methods of analysis and rates of solution were made by Dr. N. P. Nies, Mr. J. D. Stone, and Mrs. M. Stone, in the laboratories of the Pacific Coast Borax Co.; Dr. L. M. Stahler collaborated by directing that phase of the work.

REFERENCES

(1) Reeve, E., Prince, A. L., and Bear, F. E., *N. J. Agric. Expt. Sta. Bull.*, **709** (1944).
(2) Winsor, H. W., *Soil Sci.*, **69**, 321 (1950).
(3) Page, N. R., and Paden, W. R., *Soil Sci. Soc. Amer. Proc.*, **14**, 253 (1949).
(4) Page, N. R., unpublished paper presented at the meeting of the Southern Association of Feed and Fertilizer Control Officials, Clemson College, Clemson, S. C., June, 1953.
(5) Wear, J. I., and Wilson, C. M., in press.
(6) Unpublished data, Chemical Research Lab., Pacific Coast Borax Co., 1953.
(7) Unpublished data, Chemical Research Lab., Ferro Corp., 1953.
(8) Taylor, D. S., *This Journal*, **33**, 132 (1950).
(9) Ball, G., and Fajans, E., *Trans. Soc. Glass Tech.*, **35**, 93 (1951).
(10) Scott, W. W., *Standard Methods of Chemical Analysis*, 5th ed., N. H. Furman, Ed., D. Van Nostrand Co., Inc., New York, 1939, p. 174.
(11) Berry, R. C., *This Journal*, **36**, 623 (1953).
(12) Scott, W. W., *Standard Methods of Chemical Analysis*, 5th ed., N. H. Furman, Ed., D. Van Nostrand Co., Inc., New York, 1939, p. 164.
(13) *Ibid.*, p. 2167.
(14) Hollander, M., and Riemann, W., *Ind. Eng. Chem., Anal. Ed.*, **18**, 788 (1946).

DETERMINATION OF SULFAQUINOXALINE IN MEDICATED FEEDS

By C. R. Szalkowski and W. J. Mader (Analytical Laboratories, Chemical Division, Merck & Co., Inc., Rahway, N. J.)

Sulfaquinoxaline is an effective chemotherapeutic agent for the prevention and control of certain poultry diseases (4, 5, 7, 8). Upon long standing under adverse storage conditions, samples of medicated feeds containing sulfaquinoxaline frequently suffered an apparent loss of sulfaquinoxaline when analyzed by the A.O.A.C. procedure (1, 6, 11). The binding of sulfa drugs by protein has been well established, as has the

specific binding of sulfaquinoxaline (2, 9, 10). Bankowski and Johnson (2) found that the plasma proteins of chicken blood bind the commonly employed sulfonamides in the following increasing order: sulfaguanidine, sulfanilamide, sulfamerazine, sulfamethazine, sulfathiazole and sulfaquinoxaline. On this basis it was felt that possibly a portion of the sulfaquinoxaline in medicated feeds was being bound to the protein of the feed in such a manner that the sulfaquinoxaline was available to the chicken as a coccidiostat but was not extracted quantitatively by the dilute caustic solution (1, 6).

A study was made of this phase of the assay. Table 1 shows the data obtained on unmedicated feed, laboratory prepared medicated feed, and an unmedicated feed to which was added a solution of sulfaquinoxaline. This study shows the following:

1. Unmedicated feed contains a substance which will analyze by the colorimetric procedure of Bratton and Marshall (3) as sulfaquinoxaline. (Unmedicated feeds contained 0.0015 to 0.0047 per cent apparent sulfaquinoxaline.)

2. When a solution of sulfaquinoxaline is added to an unmedicated feed, recoveries average around 97 per cent. (This is consistent with the findings of Dux and Rosenblum (6), who obtained recoveries within 2 per cent of make-up in preparations equivalent to 0.01, 0.10, 1.5, and 15 per cent sulfaquinoxaline.) However, if the color due to the feed blank is deducted a recovery of only about 87 per cent is obtained.

3. On carefully blended laboratory feeds recoveries low by as much as 10 per cent have been obtained and if the color due to the blank is deducted the recoveries are lowered by as much as another 10 per cent.

Attempts were made to improve the recovery by the caustic extraction procedure, but without success. For example, the amount of caustic solution was varied and volumes of 2.5, 5, 10, 25, and 50 ml of 0.5 *N* sodium hydroxide were used for extraction of the sulfaquinoxaline. Increase in

TABLE 1.—*Recovery of sulfaquinoxaline by A.O.A.C. method*

SAMPLE	SQ ADDED	SQ FOUND	RECOVERY	
			UNCORRECTED	CORRECTED
	(*per cent*)		(*per cent*)	
Unmedicated Feed A	0	0.0047	——	——
Unmedicated Feed B	0	0.0020	——	——
Unmedicated Feed C	0	0.0015	——	——
Unmedicated Feed D	0	0.0025	——	——
Feed D+SQ Solution	0.025	0.0243	97.2	87.2
Feed D+SQ (Blended)	0.025	0.0222	88.8	78.8
Feed D+SQ (Blended)	0.0506	0.0459	90.7	85.8
Feed D+SQ (Blended)	0.0400	0.0371	92.7	86.5

sodium hydroxide concentration had a tendency to lower the result for sulfaquinoxaline.

The use of continuous extractors with acetone or ether increased the recoveries somewhat but not enough to warrant their use. Heating alkaline solutions of the feed decreased the recovery of sulfaquinoxaline.

The one approach which did increase the recoveries of sulfaquinoxaline was the simple expedient of digestion of the feed with ficin, a proteolytic enzyme which occurs in the latex of the tropical tree Ficus. (Ficin has been used for some time as a protein digestant in the brewing, meat, cheese, textile, and leather industries.) The ficin digestion releases the "bound" sulfaquinoxaline and enables a more quantitative determination of the drug.

Other proteolytic enzymes such as pepsin, papain, and pancreatin were also tried. Pancreatin showed activity but required close control of conditions.

The procedure using ficin as developed in our laboratory is as follows:

FICIN DIGESTION METHOD FOR SULFAQUINOXALINE IN FEEDS

REAGENTS

(Unless otherwise indicated, all reagents are reagent grade.)

(a) *Ficin soln.*—A freshly prepared 0.1% aqueous soln (Merck).

(b) *Sodium hydroxide soln.*—0.5 *N*.

(c) *Sodium nitrite soln.*—A freshly prepared 0.2% aqueous soln.

(d) *Ammonium sulfamate soln.*—A 1.0% aqueous soln.

(e) *N(1-naphthyl)-ethylenediamine soln.*—A freshly prepared 0.2% aqueous solution of N(1-naphthyl)-ethylenediamine-dihydrochloride (Eastman-Kodak).

(f) *Sulfaquinoxaline standard soln.*—Weigh 100.0 mg of pure sulfaquinoxaline into a 500 ml volumetric flask, add 5.0 ml of the 0.5 *N* NaOH and make to vol. with distd H_2O. Pipet 25.0 ml of this soln into a 250 ml volumetric flask and dil. to mark with H_2O. Mix thoroly. Each ml of this soln contains 20 mmg of sulfaquinoxaline.

DIGESTION AND EXTRACTION

Weigh an amount of the feed sample equivalent to ca 600 mmg of sulfaquinoxaline into a 250 ml volumetric flask and add 100 ml of the 0.1% ficin soln. Shake for 30 min. Add 5.0 ml of the 0.5 *N* NaOH and shake for an addnl 15 min. Dil. to vol. with H_2O, mix thoroly and let settle

Pipet 50.0 ml of this soln into a 100 ml volumetric flask, add 3.0 ml of concd HCl, shake vigorously and dilute to vol. with H_2O. Filter thru a dry filter, rejecting the first 10–20 ml of the clear filtrate.

COLOR DEVELOPMENT

Pipet 15.0 ml aliquots of the clear filtrate into sep. 50 ml erlenmeyers marked A and B. Pipet 1.0 ml of reagent (c) into each flask, mix well, and allow to stand. At the end of 3 min. pipet 1.0 ml of reagent (d) into each flask, mix well, and let stand for 2 min. Finally, pipet 1.0 ml of reagent (e) into flask A, and 1.0 ml of distd H_2O into flask B. Mix well.

After 10 min. measure the absorbances of the solns in a suitable photoelectric colorimeter or spectrophotometer set at 545 mμ, using a reagent blank as refer-

ence. (The absorbance of A minus that of B gives the absorbance due to sulfaquinoxaline in the sample soln.) Calculate the amount of sulfaquinoxaline present in the feed from the standard curve for sulfaquinoxaline.

If a control feed is available (sample from feed batch before addition of the medication) carry it along simultaneously with the sample. Measure and deduct any color formed from that of the sample.

CALIBRATION CURVE

Pipet 5.0, 10.0, 15.0 and 20.0 ml of the sulfaquinoxaline standard soln (corresponding to 100, 200, 300 and 400 mmg) into sep. 100 ml volumetric flasks. Add 3.0 ml of HCl to each flask and dil. to vol. with distd H_2O.

Pipet 15.0 ml of each of these solns into sep. 50 ml erlenmeyers and develop the color as described above. Plot absorbance readings against mmg of sulfaquinoxaline.

RESULTS

A series of medicated mixtures was blended in our laboratory, using different feeds. These feeds were then analyzed for sulfaquinoxaline by the A.O.A.C. method and by the ficin procedure. Each method was corrected for the color produced by the blank feed used. The results obtained are given in Table 2. Each value represents at least four separate determinations.

Table 3 represents the reproducibility obtained on four different medicated feeds by both the A.O.A.C. method and the ficin procedure. The standard deviation of the ficin procedure is ± 0.8 to ± 3.3 per cent while that of the A.O.A.C. is ± 1.7 to 5.1 per cent.

A comparison of the A.O.A.C. and ficin methods on samples of feed stored at room temperature for 12 months is given in Table 4. The results

TABLE 2.—*Results on laboratory blended feeds*

SAMPLE	SQ ADDED	A.O.A.C. METHOD		FICIN METHOD	
		SQ FOUND[a]	RECOVERY	SQ FOUND[a]	RECOVERY
	(per cent)	*(per cent)*	*(per cent)*	*(per cent)*	*(per cent)*
1	0.0506	0.0459	90.7	0.0498	98.5
2	0.0433	0.0377	87.1	0.0438	101.2
3	0.0150	0.0128	85.3	0.0135	90.0
4	0.040	0.0371	92.8	0.0403	100.8
5	0.0150	0.0122	81.3	0.0143	95.3
6	0.0150	0.0137	91.3	0.0146	97.3
7	0.0100	0.0089	89.0	0.0097	97.0
8	0.0125	0.0086	68.8	0.0112	74.6
9	0.0150	0.0131	87.3	0.0150	100.0
10	0.0163	0.0145	87.8	0.0159	97.5
11	0.0250	0.0227	90.8	0.0236	94.4
12	0.0200	0.0181	90.5	0.0192	96.0

[a] All samples corrected for feed blank. Each result is the average of four determinations.

TABLE 3.—*Reproducibility of methods*

	SQ	AV.	STD. DEVIATION
	(*per cent*)		(*per cent*)
Sample B			
Ficin Method	0.0114, 0.0123, 0.0128, 0.0121	0.0122	±3.3
A.O.A.C. Method	0.0098, 0.0092, 0.0097, 0.0092	0.0095	±2.6
Sample 5B			
Ficin method	0.0184, 0.0184, 0.0188, 0.0186	0.0186	±0.8
A.O.A.C. Method	0.0160, 0.0168, 0.0183, 0.0176	0.0172	±4.6
Sample 9B			
Ficin Method	0.0147, 0.0149, 0.0153, 0.0147,	0.0149	±1.3
A.O.A.C. Method	0.0125, 0.0145, 0.0137, 0.0145	0.0138	±5.1
Sample RP			
Ficin Method	0.0168, 0.0165, 0.0165, 0.0162	0.0165	±0.9
A.O.A.C. Method	0.0154, 0.0147, 0.0150, 0.0147	0.0150	±1.7

on each sample show that the ficin procedure gives a higher sulfaquinoxaline value than the A.O.A.C. method.

Samples of medicated feeds were stored for 10 months in stoppered bottles at room temperature, and at 40°C. Upon analysis by the ficin procedure these samples showed some loss in sulfaquinoxaline. The results are given in Table 5.

ACKNOWLEDGMENT

The authors wish to acknowledge the invaluable assistance of James

TABLE 4.—*Sulfaquinoxaline assays*[a] *of commercial feeds*

FEED	ORIGINAL ASSAY	ASSAYS AFTER 12 MONTHS	
	A.O.A.C. METHOD	A.O.A.C. METHOD	FICIN METHOD
	(*per cent*)	(*per cent*)	(*per cent*)
A	0.0099	0.0057	0.0068
B	0.0093	0.0070	0.0086
C	0.0129	0.0086	0.0124
D	0.0124	0.0070	0.0114
E	0.0121	0.0088	0.0103
F	0.0101	0.0061	0.0104
G	0.0500	0.0225	0.0280
H	0.0131	0.0116	0.0124
I	0.0103	0.0096	0.0096
J	0.0260	0.0201	0.0230

[a] Not corrected for feed blank. Each result is the average of four determinations.

TABLE 5.—*Effect of storage on feeds. Sulfaquinoxaline by ficin procedure*

SAMPLE	ORIGINAL ASSAY	R.T. STORAGE FOR 10 MONTHS	40° C. STORAGE FOR 10 MONTHS
	(*per cent*)	(*per cent*)	(*per cent*)
Unmedicated Feed	0.0048	0.0041	0.0029
Medicated Feed No. 1	0.0151	0.0144	0.0101
Medicated Feed No. 5	0.0181	0.0180	0.0126
Medicated Feed No. 9	0.0154	0.0132	0.0119
Medicated Feed No. 12	0.0236	0.0228	0.0205

Davidson, Jr., formerly of this laboratory, who performed many of the analyses.

REFERENCES

(1) *Official Methods of Analysis, A.O.A.C.*, 7th Ed., 1950, p. 619.
(2) BANKOWSKI, R. A., and JOHNSON, R. E., *Am. J. Vet. Research*, **10**, 282 (1949).
(3) BRATTON, A. C., and MARSHALL, E. K., *J. Biol. Chem.*, **128**, 537 (1939).
(4) DELAPLANE, J. P., *Am. J. Vet. Research*, **6**, 207 (1945).
(5) DELAPLANE, J. P., BATCHELLER, R. M., and HIGGINS, T. C., *North Am. Veterinarian*, **28**, 19 (1947).
(6) DUX, J. P., and ROSENBLUM, C., *Anal. Chem.*, **21**, 1524 (1949).
(7) GRUMBLES, L. C., DELAPLANE, J. P., and HIGGINS, T. C., *Poultry Sci.*, **27**, 169 (1945).
(8) PETERSEN, E. H., *Am. J. Vet. Research*, **9**, 77 (1948).
(9) SCHLENKER, F. S., and SIMMONS, B. K., *ibid.*, **11**, 291 (1950).
(10) SELBER, R. H., and CLARK, I., *Arch. Biochem.*, **10**, 9 (1946).
(11) TYLER, S. W., BROOKE, R. O., and BAKER, W. S., *Anal. Chem.*, **21**, 1091 (1949).

NOTES

PURIFICATION OF UNSAPONIFIABLE MATERIAL BY CARBON COLUMN CHROMATOGRAPHY

By ARNOLD LADA (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

Without a purification step, the isolation of pure sterol derivatives, such as the acetate, from the crude unsaponifiable fraction obtained from fats and oils is rather difficult. The chromatographic procedure given below has been found useful for such purification.

This method has been formulated for the purification of sterols from 100–200 grams of butter fat. Vegetable oils with high unsaponifiable residue, such as corn oil, require a smaller sample (50 grams) to prevent overloading the column, which results in less pure fractions. Fats and oils with low unsaponifiable residue may require a somewhat different fraction selection. For example, when a 200 gram sample of coconut oil, beef fat, or lard is used, the crystalline material appears in later fractions than with the common seed oils and butter fat. Any oily fraction from the column is rejected.

METHODS

Saponification.—Saponify 50–200 g sample according to Cannon's procedure (1).

Purification of sterols.—To a chromatographic tube (21–22 mm i. d. $\times$24 cm long) add in 4–5 portions a 1+2 mixture of Darco G–60 and Celite 545 to a height of 11–12 cm; after adding each portion, tamp with plunger. Fit a 125 ml separatory funnel as a solvent reservoir to the tube with a rubber stopper. Wet the column by passing 50 ml alcohol through it under 3–5 lbs. pressure. Release the pressure as the last amount of solvent enters the adsorbent. (This procedure applies to all solvents to prevent the column from cracking.)

Dissolve the unsaponifiable residue in a minimum amount of alcohol (10–20 ml) by warming on the steam bath. Add this solution directly to the column and apply pressure. Pass 100 ml of alcohol through the column; discard the eluate. Pass through the column successively the following solutions, collecting the eluate in fractions of 50 ml (fractions 1–6) and 33 ml (fractions 7–9):

Fractions 1 and 2: 100 ml 20% $CHCl_3$ in alcohol (v/v)
Fractions 3 and 4: 100 ml 40% $CHCl_3$ in alcohol (v/v)
Fractions 5 and 6: 100 ml 55% $CHCl_3$ in alcohol (v/v)
Fractions 7, 8 and 9: 100 ml 70% $CHCl_3$ in alcohol (v/v)

Fractions 1 and 2, which contain only negligible amounts of residue, are discarded. Evaporate the other fractions to dryness. Discard the oily fractions (3 and usually 4) and reserve the crystalline portions (fractions 5, 6, and 7 from butter fat and the common seed oils) which contain the largest amount of fairly pure sterol material.

REFERENCES

(1) CANNON, J. H., *This Journal*, **36**, 181 (1953).

VOLATILE ACIDS FROM OXIDATION PRODUCTS OF CHOLESTEROL AND PHYTOSTEROLS

By Arnold Lada (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

Separation of the mixture of volatile acids obtained from chromic acid oxidation of cholesterol and phytosterol in 1+3 sulfuric acid indicated that propionic acid was a degradation product of phytosterols but not of cholesterol. To confirm the observation, several animal sterol fractions isolated from butter fat, beef fat, and lard as well as plant sterol fractions isolated from the common seed oils and commercial sitosterol samples were oxidized. In the oxidation of cholesterol, acetic and butyric acids were always obtained and valeric acid was occasionally found. Phytosterols, however, yielded propionic acid in addition to these acids. To avoid the formation of propionic acid from non-sterol materials in the unsaponifiable fraction of fats, a purification step, such as a carbon column treatment (1) is necessary before oxidation.

Acetic acid was always the predominant acid from both purified plant and animal sterols, and appeared in quantities of 10–20 mg from the column-purified unsaponifiable material from 50 to 200 grams of fat. Propionic acid was present in quantities of 1.0 to 1.5 mg from 100 grams of peanut oil, cottonseed oil, or soybean oil. The amount of propionic acid found was not a linear function of the quantity of vegetable oil in the sample.

In the separation of the acids, threshold volumes were usually: butyric, 8–12 ml (preceded by valeric); propionic, 20–26 ml; and acetic, 35–40 ml.

METHODS

Oxidation.—Dissolve the residues of the crystalline sterol fractions obtained from the carbon column purification (1) in ether, transfer to a 100 ml round bottom flask with a standard taper 19/38 joint, and evaporate to dryness. Add 1.5 g CrO_3 and 30 ml 1+3 H_2SO_4. Connect the flask to a water-cooled condenser (ca 240 mm over-all length; standard taper joints throughout) and heat the flask with an electric heating mantle. Collect 10 ml of distillate in a graduated cylinder immersed in an ice bath, and transfer to a 50 ml beaker; wash the condenser and cylinder twice with 5 ml H_2O and add to the beaker. Make the contents of the beaker alkaline to phenolphthalein with 1 *N* NaOH and evaporate to dryness on a steam bath or a hot plate.

Separation of acids.—Proceed as directed in *Official Methods of Analysis*, 7th Ed., **18.17** (2).

REFERENCES

(1) Lada, A., *This Journal*, **37**, 550 (1954).
(2) *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Washington, D. C., 1950, **18.17**.

THE USE OF POTASSIUM HYDROXIDE AS A FUSION AGENT IN THE DETERMINATION OF THE FLUORINE CONTENT OF VEGETATION*

By L. J. HARDIN, W. H. MACINTIRE, and MARY ELLEN TUBB (The University of Tennessee Agricultural Experiment Station, Knoxville 16, Tennessee)

Several laboratories have advocated alkali fusion of plant ash as a step preliminary to the perchloric acid distillation in the determination of fluorine in vegetation samples (1, 2). In some cases fusion gave higher values, especially on certain types of vegetation, and consequently sodium hydroxide fusion of the plant ash has been adopted generally as a means of effecting decomposition of the silicofluorides and for more accurate recovery of fluorine through distillation.

Results in this laboratory have confirmed the advantage of the NaOH fusion of the ash, especially when the type of the vegetation sample is not known. In fusion trials in which potassium hydroxide was used instead of sodium hydroxide, fluorine recoveries were comparable, and preliminary observations indicated some significant physical advantages in favor of the potassium hydroxide.

To confirm the suitability of potassium hydroxide as a fusion agent, charges of ten samples of seven different vegetations were analyzed in replication. Pellets (5 g) of sodium hydroxide and of potassium hydroxide were used to effect fusion of the ash, and fluorine determination was made by means of the Willard and Winter procedure (3) The results are given in Table 1.

TABLE 1.—*Sodium and potassium hydroxides as fusion agents in the determination of the fluorine content of vegetation ash*

MATERIAL[a]	FLUORINE CONTENT—p.p.m.			
	NaOH FUSION[b]		KOH FUSION[b]	
	INDIVIDUAL RESULTS	AVERAGE	INDIVIDUAL RESULTS	AVERAGE
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Alfalfa—high	66, 64	65	63, 68	66
Alfalfa—low	24, 25	25	24, 23	24
Bermuda—high	45, 47	46	43, 45	44
Bermuda—low	21, 21	21	18, 18	18
Crabgrass—low	22, 18	20	20, 25, 18	21
Fescue—high	66, 60	63	68, 71, 71	70
Fescue—low	27, 28	28	28, 25	27
Small grain—low	18, 18	18	18, 17	18
Orchard grass—high	107, 110	109	120, 98, 98, 114, 112	109
Orchard grass—low	22, 24	23	20, 21, 19	20

[a] "High" and "low" connote relative content of fluorine.
[b] Analytical charges of 5 grams of the dried, ground vegetation were treated with a suspension of CaO, dried, and incinerated. The respective ashed charges then were subjected to perchloric acid distillation after fusion with NaOH pellets, and after fusion with KOH pellets.

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The comparison shows very close agreement between the values obtained by means of the two alkali fusions. The potassium hydroxide melt was more fluid than the melt of the sodium hydroxide and disintegrated more readily when the cooled mass was taken up with water. Virtually no frothing occurs during the subsequent perchloric acid distillation of the potassium hydroxide fusion, in contrast to the frothing that usually occurs in the analogous distillation of the sodium hydroxide fusion. However, upon addition of the perchloric acid to the potassium hydroxide fusion, abnormal quantities of insoluble salts are formed and persist during the distillation, although this condition exerts no adverse effect upon fluorine recovery, as shown by the comparison in Table 1.

Because of the foregoing considerations and the concordance in the results obtained by means of the two alkalis, it is concluded that potassium hydroxide should be recognized as equal, or preferential, to sodium hydroxide as a fusion agent in the determination of fluorine in plant ash.

REFERENCES

(1) REMMERT, L. F., PARKS, T. D., LAWRENCE, A. M., and MCBURNEY, E. H., *Anal. Chem.*, **25**, 450 (1953).
(2) ROWLEY, R. J., GRIER, J. G., and PARSONS, R. L., *ibid.*, **25**, 1061 (1953).
(3) WILLARD, H. H., and WINTER, O. B., *Ind. Eng. Chem., Anal. Ed.*, **5**, 7 (1953).

PREPARATION OF SILICIC ACID COLUMNS FOR PARTITION CHROMATOGRAPHY

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In preparing a silicic acid column for partition chromatography, the usual directions call for wetting the silicic acid with the immobile solvent (water or aqueous acid solution), using as large a quantity as possible without causing the resultant mix to become sticky when slurried with chloroform or other mobile solvent. This is a time-consuming, hit or miss procedure which can easily result in the use of varying amounts of aqueous medium by different chemists.

During the study of the chromatographic procedure for the separation of malic and citric acids[1] it was observed that threshold volumes for malic acid obtained by the collaborators varied from 70 to 90 ml, while those for citric acid varied from 120 to 180 ml. Since silicic acid readily absorbs water from air, it was thought that the amount of water in the different batches might account for these differences.

Mellor[2] states that silicic acid loses only part of its water when dried at 100°C. and that a temperature of about 500°C. is needed to convert it to anhydrous silica. In the work reported here the moisture content of various batches of silicic acid was determined by heating portions of about one gram in a small crucible over a Fisher burner at low red heat for about 15 minutes. Longer heating gave no further loss in weight.

All lots of silicic acid used were manufactured by Mallinckrodt for chromatographic analysis and were checked for suitability according to the procedure of Ramsey and Patterson.[3]

[1] Ferris, L. W., *This Journal*, **37**, 305 (1954).
[2] Mellor, J. W., *Modern Inorganic Chemistry*, Longmans, p. 949.
[3] Ramsey, L. L., and Patterson, W. I., *This Journal*, **28**, 644 (1945), p. 648; **31**, 139 (1948), p. 143.

To study the effect of the use of different amounts of 0.5 N H_2SO_4 in preparing the column upon the threshold volumes of malic and citric acids, three batches of silicic acid containing different amounts of water were used. A volume in ml equal to half the weight of the silicic acid in grams was used and the threshold volumes of both malic and citric acids were determined. This experiment was repeated using increasing amounts of 0.5 N H_2SO_4 as long as a satisfactory column could be made. Results are given in Table 1.

TABLE 1.—*Effect of increasing amounts of 0.5 N H_2SO_4*

ML 0.5 N H_2SO_4 USED PER 6 G SILICIC ACID	THRESHOLD VOLUME OF MALIC ACID	VOLUME REQUIRED TO ELUTE ALL MALIC ACID	THRESHOLD VOLUME OF CITRIC ACID
Silicic Acid No. 1 (90% SiO_2)			
3.0	60	110	120
3.5	70	130	150
4.0	70	130	160
4.2	90	140	170
4.3	90	150	180
Silicic Acid No. 2 (84% SiO_2)			
3.0	80	130	150
3.4	90	140	170
3.6	100	150	190
Silicic Acid No. 3 (82% SiO_2)			
2.9	70	130	160
3.3	90	150	180

It was observed that considerably more 0.5 N H_2SO_4 could be added to the silicic acid having the lower water content before it became sticky when triturated with chloroform. It was also noted that with increased 0.5 N H_2SO_4, less pressure was required to produce the desired rate of elution, threshold volumes were slightly increased, and a better separation of the two acids was obtained.

It was further observed that when near the maximum amount of 0.5 N H_2SO_4 was used, the sum of the 0.5 N H_2SO_4 and of the water originally present in the silicic acid was equal to 90 per cent of the anhydrous silica in the silicic acid.

This observation is substantiated by results obtained with six different lots of silicic acid and upon three of these after they were exposed to moist air until considerable water was absorbed (1B, 2B, 3B, Table 2). It is therefore possible to calculate the amount of 0.5 N H_2SO_4 required for any lot of silicic acid by the following formula:

$$V = W(1.9A - 1)$$

where

V = ml 0.5 N H_2SO_4 required

W = grams of silicic acid used

and

$$A = \text{ratio } \frac{\text{anhydrous}}{\text{hydrous}} \text{ silicic acid.}$$

This formula was used to calculate the volume of 0.5 N H_2SO_4 to be added to 6 grams of silicic acid (column 3, Table 2).

TABLE 2.—*Application of formula*

SILICIC ACID (LOT NUMBER)	RATIO ANHYDROUS / HYDROUS SILICIC ACID	ML 0.5 N H_2SO_4 ADDED TO 6 G	THRESHOLD VOLUME—MALIC ACID	ML REQUIRED TO ELUTE ALL MALIC ACID	THRESHOLD VOLUME CITRIC ACID
			(*ml*)		(*ml*)
1	.9105	4.4	100	160	190
1B	.8397	3.6	100	150	190
2	.9213	4.5	100	150	190
2B	.8244	3.4	100	150	190
3	.9023	4.3	90	150	180
3B	.8417	3.6	90	150	180
4	.8272	3.4	90	150	180
5	.8444	3.6	100	150	190
6	.8187	3.3	90	150	180

It is noted that the threshold volumes of both malic and citric acids are consistently reproduced with lots of silicic acid that very considerably in water content.

Since the formula indicates approximately the maximum amount of aqueous phase that a batch of silicic acid will hold without becoming sticky when mixed with chloroform, it therefore may also be used to determine the amount of water to add in the preparation of a chromatograph column for separation of fatty acids as in *Methods of Analysis*, A.O.A.C., 7th Ed., par. **18.17.** Even where threshold volumes are not involved and the quantity of aqueous phase is not critical, as in the determination of monofluoroacetic acid, par. **24.79,** the calculation will aid in the preparation of a satisfactory column.

THE ANALYSIS OF MANGANESE ETHYLENEBISDITHIOCARBAMATE COMPOSITIONS*

By M. LEVITSKY and W. K. LOWEN (Grasselli Chemicals Department, Experimental Station, E. I. du Pont de Nemours and Co., Wilmington, Del.)

In a previous publication (3), the junior author discussed the importance of thoroughly dispersing samples of "Manzate"[1] fungicide (based on manganese ethylenebisdithiocarbamate) prior to the addition of hot digestion acid in the conventional carbon disulfide evolution procedure (1, 2) for analyzing dithiocarbamates,

* Presented at the Sixty-seventh Annual Meeting of the Association of Official Agricultural Chemists, Oct. 12, 13, and 14, 1953, at Washington, D. C.

[1] Reg. U. S. Pat. Off. Trade-mark of E. I. du Pont de Nemours & Co., Inc.

and a procedure for accomplishing the desired dispersion was described. Further investigations have revealed an alternative method for obtaining the required dispersion, which is less subject to operator errors, and which reduces the digestion time from ninety to twenty minutes. The modified procedure utilizes essentially the same reagents and apparatus as those described in the original method (3). However, a solution of "Versene" liquid (34 per cent solution of the tetrasodium salt of ethylenediamine tetraacetic acid, obtained from the Bersworth Chemical Co., Framingham, Mass.) is used in place of boiling alcohol to provide the desired dispersion.

METHOD

A sample of Manzate, 0.4–0.6 g, is weighed into the dry reaction flask. Ten ml of Versene liquid is then added and the contents are swirled for 15–20 seconds.[2] At the end of this time, no lumps of Manzate are visible and the sample is usually completely dissolved. The flask is immediately attached to the absorption train, the dropping funnel is inserted and positioned with the delivery tube pointed downward, and the vacuum is adjusted so that a rapid stream of bubbles is drawn through the metering water trap. A 50 ml portion of boiling distilled water is added, followed immediately by 50 ml of hot 9 *N* sulfuric acid. The initial reaction is quite vigorous, and a slow, careful addition of acid is mandatory to avoid "back-up" losses of carbon disulfide. When the entire charge of acid has been added, the mixture is heated to reflux temperature and digested for 20 to 30 minutes; afterwards, the potassium methylxanthate is recovered from the alcoholic potassium hydroxide trap and titrated with standard iodine solution by the conventional procedure.

Application of the Versene dispersion technic provides a significant reduction in the digestion time required to insure complete recovery of carbon disulfide from samples of Manzate fungicide. For one particular formulation, a digestion time of only 10 minutes indicated 75.69 per cent Manzate; this figure increased to 76.01 per cent after a 20 minute digestion, and remained sensibly constant for digestion periods up to one hour. The precision obtained using the modified procedure is equal to that established for the alcohol dispersion method which was described in the previous paper and is illustrated in Tables 1 and 2.

TABLE 1.—*Comparison of results with alcohol and Versene dispersion technics*

SAMPLE	MnEBD	
	VERSENE DISPERSION (20 MINUTE DIGESTION)	ALCOHOL DISPERSION (90 MINUTE DIGESTION)
	per cent	*per cent*
1	86.00, 85.76 85.53, 85.41	85.97, 85.81
2	44.39	44.06

[2] Swirling times up to several minutes may be tolerated without significant decomposition of manganese ethylenebisdithiocarbamate; however, minimum delay between the additions of Versene and hot digestion acid is recommended.

TABLE 2.—*Comparison of routine reproducibility, using Versene and alcohol dispersion*[a]

REPLICATION USING ALCOHOL DISPERSION	REPLICATION USING VERSENE DISPERSION
per cent MnEBD	*per cent MnEBD*
81.37	75.69
81.10	76.01
81.37	75.84
81.77	75.95
82.55	75.97
82.58	75.97
81.91	75.46
81.94	
Mean = 81.81	Mean = 75.84
Std. Deviation = 0.54	Std. Deviation = 0.20
Mean Deviation = 0.41	Mean Deviation = 0.15

[a] Reproducibility comparisons are based on different Manzate compositions.

REFERENCES

(1) CALLAN, T., and STRAFFORD, N., *J. Soc. Chem. Ind.*, **43**, 8T (1924).
(2) CLARK, D. G., BAUM, H., STANLEY, E. L., and HESTER, W. F., *Anal. Chem.*, **23**, 1842 (1951).
(3) LOWEN, W. K., *This Journal*, **36**, 484 (1953).

BOOK REVIEWS

Chemical Methods in Industrial Hygiene. Interscience Manual No. 3. By F. H. GOLDMAN and M. B. JACOBS. Interscience Publishers, Inc., New York, 1953. x+274 pp. Illus., index. Price $3.75.

This volume is one of a series of manuals covering various fields of chemistry and industry. Its purpose, as stated in the preface, is "to describe in detail, wherever possible, many of the analytical methods available to the chemist for the examination of the materials, substances, and samples that are encountered in the field of industrial hygiene. Methods involving the use of field instruments or special laboratory apparatus involving X-ray diffraction, polarography, spectroscopy, and petrology have been omitted."

There are nine chapters dealing with the following subjects: (1) Sampling; (2) reagents and solutions; (3) size distribution of dusts and powders; (4) free silica; (5) inorganic compounds and gases; (6) metals; (7) volatile solvents; (8) organic solvents; and (9) miscellaneous gases, vapors, and mists. Also included are a bibliography and an appendix which lists threshold limit values adopted by the American Conference of Governmental Industrial Hygienists in 1952, a conversion table for gases and vapors, and an outline of sampling and analytical procedures in air analysis.

This volume is not a comprehensive text but is rather a working manual, and should be accepted as such by the industrial hygiene chemist. It records those procedures which, for the most part, have been accepted through long experience, and presents them in simple yet detailed form. A rapid scanning of the bibliography leaves the impression that it may not be as current as desirable; out of 114 references cited, fewer than a dozen are taken from the literature of the past five years. Users of the Threshold Limit Values, also, should remember that these data are revised annually, and although 1952 values are recorded, several important changes have been made in the past year, particularly with respect to some of the commonly used chlorinated hydrocarbons.

In general, the book contains much information that is both useful and practical, and it should be a welcome addition to the library of the industrial hygiene chemist.

W. F. REINDOLLAR

Cocoa. By EILEEN M. CHAT (British Food Manufacturing Industries). Interscience Publishers, Inc., New York, 1953. 302 pp. Illus., index. Price $8.50.

The title of this book may be misleading to those accustomed to present American terminology, as it reflects the older terminology largely used in England and, to some extent, on the Continent. In contrast to our use of the term to mean the manufactured powdered article, in foreign usage the term "cocoa" covers a wide scope, ranging from the plant species and the beans of commerce to the manufactured product.

The book contains some 300 pages and is divided into 12 chapters. It is a rather comprehensive survey of cacao from its culture to final manufacture, and the volume includes discussions of related biological and chemical factors.

The author begins with an abridged history of cacao, including its origin and culture among the Indians before the time of Columbus, and traces its distribution to other parts of the world. There is an ample treatment of the agriculture and the diseases of cacao; a brief discussion of its botannical classification is also presented. From the material given, it is apparent that there is some disagreement among authorities on this question.

Information on insect pests is brief, and of the insects listed as infesting cacao beans, only *Ephestia elutella* appears to be of importance. According to some other authorities, *Araeocerus fasciculatus* and *Corcyra cephalonica* are the more important pests.

A section of the book which follows presents information on the biological and chemical changes which occur in the maturing fruit and in the fermentation and curing processes. Supporting analytical data are included. The processes in the description of the chocolate manufacturing plant which follows are doubtless representative of present British practices which are in general use; however, some of the recent practices in American factories are not covered, particularly in regard to roasting technics and winnowing methods.

A chapter on analysis of cacao products describes briefly or supplies references to methods for the determination of cacao constituents or common adulterants. Methods listed may include some which are not effective, and some good procedures are omitted; but in general, a number are given from which the analyst can select the most reliable. More information can be obtained with the use of the selected references.

W. O. WINKLER

Fruit and Vegetable Juice Production. By DONALD K. TRESSLER and MAYNARD A. JOSLYN. Avi Publishing Co., Inc., New York, 1954. 962 pp. Illus., index. Price $15.00.

A revised and expanded text replaces the now-out-of-date 1939 edition of "Fruit and Vegetable Juices" by Tressler, Joslyn, and Marsh. The text has been completely rewritten with the addition of eleven new chapters as follows: Sterilization, filtration, and chemical preservation; storage changes; jelly manufacture; dehydrated fruit products; volatile flavor recovery; standards and regulations; plant sanitation; quality control; laboratory examination; blended fruit juices; and home preparation and preservation.

The book differs from the older edition in that the authors have the assistance of twenty specialists who collaborated in its preparation. Each collaborator has written a chapter to whose subject he has devoted years of study and investigation. The book is designed for use as a text in food technology courses. It should be equally suitable for reference purposes for all others interested in the chemistry and technology of fruit and vegetable juice production.

The thirty chapters composing the text contain a total of 128 tables and 163 figures. Excellent bibliographies appear at the end of each chapter. Chapter 1 on historical and economic aspects of the juice industry has information in tabular form which shows trends in production and cost of canned, frozen, and fresh juices. Other fundamental chapters of a general nature include: preparation, heat preservation and preservation by freezing, and vacuum concentration. Twelve of the chapters deal with specific fruit and vegetable juices and include all of the currently important commercial products: pineapple, grapefruit, orange, lemon, grape, apple, cherry, tomato, berry, vegetable juices, fruit juice beverages, blended fruit juices and nectars, fruit juice sirups, and fruit juices in jelly making. Other important chapters cover plant design, utilization and disposal of processing wastes, and nutritive value of fruit and vegetable juices.

The authors and collaborators have produced an excellent and authoritative text. The printing and other mechanical features are satisfactory.

R. A. OSBORN