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ANNOUNCEMENT

SIXTY-THIRD ANNUAL MEETING, 1949

The 63d Annual Meeting of the Association of Official Agricultural Chemists will be held October 10 to 12, 1949, inclusive, at the Shoreham Hotel, 2500 Calvert Street, N.W., Washington 8, D. C.

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REPORT ON RIBOFLAVIN*

(FLUOROMETRIC AND MICROBIOLOGICAL METHODS)

By HENRY W. LOY, Jr. (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the meeting of the Association last year, it was recommended that the fluorometric method for the assay for riboflavin, as described at that time,[1] be studied collaboratively during the coming year. In this method, which combines features of those described by Najjar,[2] Arnold,[3] and Rubin, *et al*,[4] the riboflavin is first extracted from the sample with dilute hydrochloric acid and a clear filtrate prepared in a manner similar to that used in the tentative microbiological method.[5] The final sample solution is acidified with acetic acid and treated with potassium permanganate and hydrogen peroxide. The riboflavin content is then calculated from the degree of fluorescence of the sample solution, of the sample solution plus an added amount of riboflavin, and of these solutions after reduction of the riboflavin with sodium hydrosulfite.

The collaborators in the fluorometric study were asked to assay two samples, No. 1, enriched flour, and No. 2, dried brewers yeast, by the specified fluorometric method, other fluorometric methods in which they might be interested, and the tentative microbiological method.

Fifteen collaborators, including the Associate Referee, submitted results obtained by the collaborative fluorometric method. Seven of these laboratories submitted results by other fluorometric methods and nine by the tentative microbiological method.

Comparisons between the averages of the results submitted for the different methods are shown in Table 1. Approximately 60 per cent of the values reported for each method were within a range of ± 10 per cent of the average value obtained by the respective method. However, there was a wide divergence of some of the results reported for each method. Although many laboratories obtained acceptable results with any one method, there appeared to be no obvious explanation, except in two cases, for this wide divergence of some of the results. Therefore, the study did not lead to the recommendation of a fluorometric method for adoption this year, but it is expected that further studies will prove profitable.

Although good results may be obtained with the present tentative microbiological method,[5] it was believed by some collaborators that

* This report was presented on Tuesday, October 21, 1947, at the last annual meeting and should have been printed with other reports on Vitamins in the August (1948) Journal.

[1] *This Journal*, **30**, 392 (1947).
[2] *J. Biol. Chem.*, **141**, 355 (1941).
[3] *Cereal Chemistry*, **22**, 455 (1945).
[4] *Ind. Eng. Chem., Anal. Ed.*, **17**, 136 (1945).
[5] *This Journal*, **30**, 79 (1947).

further consideration should be given to improvements that may be made in this method. Accordingly, those collaborators who were able to undertake additional work were asked to study a method that is similar to the present tentative microbiological method except for modifications in the preparation of the sample solution and of the photolyzed peptone and yeast supplement solutions used in the basal medium. Also, modifications

TABLE 1.—*Average potency in riboflavin*

(Comparison of results obtained with the different methods used by the 15 reporting laboratories)

NO. OF COLLABORATORS REPORTING RESULTS	SAMPLE NO. 1—ENRICHED FLOUR (*Mg. of riboflavin per lb. of sample*)				
	FLUOROMETRIC METHODS		MICROBIOLOGICAL METHODS		
	COLLABORATIVE	OTHER	TENTATIVE	COLLABORATIVE	OTHER
15	1.51				
7	1.53	1.37			
9	1.54		1.46		
4	1.41		1.42	1.34	
5	1.47		1.45		1.48

NO. OF COLLABORATORS REPORTING RESULTS	SAMPLE NO. 2—DRIED BREWERS YEAST (*Mg. of riboflavin per g. of sample*)				
	FLUOROMETRIC METHODS		MICROBIOLOGICAL METHODS		
	COLLABORATIVE	OTHER	TENTATIVE	COLLABORATIVE	OTHER
15	0.043				
7	0.047	0.045			
9	0.043		0.042		
4	0.041		0.042	0.037	
5	0.038		0.040		0.037

were suggested for the handling of the culture and for the size of test tubes used in the assay.

The collaborators in the microbiological study were asked to assay the same two samples that were used in the fluorometric study. Four of the laboratories submitted results obtained by the tentative and collaborative microbiological methods, and five by the tentative and other microbiological methods.

Comparisons between the averages of the results submitted for the different methods used in the microbiological study also are shown in Table 1. Closer agreement in the results between the laboratories was obtained with the collaborative microbiological method than with the tentative microbiological method, but, since only four laboratories submitted comparative results, the data were considered to be insufficient to lead to the recommendation of any changes to be made in the tentative

method. Except for the collaborative microbiological method, the range of values reported for each method was about the same as the range of values reported in the fluorometric study. Again, there appeared to be no obvious explanation for the wide divergence of some of the results. Although the microbiological study did not lead to the recommendation of any changes in the present tentative method, it is believed that further studies may do so.

COLLABORATORS

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H. C. Schaefer, Ralston Purina Co., St. Louis, Mo.

The Associate Referee wishes to express appreciation to the collaborators and to their organizations for their cooperation in this study.

RECOMMENDATIONS*

It is recommended—

(1) That further work be conducted on the fluorometric method that was the subject of this year's study.

(2) That further consideration be given to improvements that may be made in the present microbiological method.

* For report of Subcommittee A and action of the Association see *This Journal*, **30**, 44 (1948).

CONTRIBUTED PAPERS

COMPARISON OF METHODS FOR THE DETERMINATION OF CAROTENE

By JAMES V. DERBY, JR., and JAMES B. DEWITT, Chemists
(Fish and Wildlife Service, U. S. Department of the Interior, Patuxent Research Refuge, Laurel, Maryland)

In the course of determining the carotene contents of various plant tissues commonly utilized as browse by forest animals, it was observed that widely divergent results were obtained when the analytical procedures were varied according to some of the commonly accepted methods. In order to establish the most suitable procedure, a series of carefully controlled experiments, involving the analysis of replicate samples by different methods, was conducted.

At least three fundamentally distinct procedures have been advanced for the extraction of carotenoid pigments from plant materials. One of these, as exemplified by the methods of Wall and Kelley (1) and Moore and Ely (2) utilizes a Waring Blendor or similar device to allow extraction by cold solvents. A second procedure, such as that prescribed in *Changes in Methods of Analysis* (3), depends upon a hot extraction with low-boiling hydrocarbons, or a mixture of hydrocarbons and a polar solvent. The third method involves a preliminary digestion of the plant material with hot alcoholic potash, followed by extraction with petroleum ether or other solvents. These three procedures were followed in the present series of experiments.

PROCEDURE

A. Preparation of Sample: A quantity of dehydrated alfalfa leaf meal, obtained from commercial sources, was ground to pass a 40-mesh screen, placed in a closed container, and stored in the refrigerator. Portions for analysis were removed and weighed immediately prior to assay. At least five analytical samples were examined by each procedure in the study.

B. Extraction of Pigments: The following procedures were used for the extraction of carotenoid pigments:

1. Method of Wall and Kelley (1): A 2–5 gram sample of the alfalfa leaf meal was extracted with 200 ml. of a 5:3 mixture of 95% ethyl alcohol and petroleum ether (B.P.—35–65°C.) in a Waring Blendor for 10 minutes. The extract and finely divided plant material were transferred to a fritted glass filter connected to a suction flask, and washed with alternate portions of alcohol and petroleum ether until a colorless filtrate was obtained. The alcohol was removed from the extracts by washing with water in a separatory funnel, and the petroleum ether extracts were concentrated on the steam bath, dried by filtration through anhydrous sodium

sulfate into a 100 ml. volumetric flask, and adjusted to volume with petroleum ether.

2. Modified A.O.A.C. Procedure (3): A 2–4 gram sample was extracted in a Bailey-Walker apparatus for one hour with 30 ml. of a 30% solution of acetone in petroleum ether. The extracts were transferred to a 100 ml. volumetric flask, and made to volume with petroleum ether.

3. Alcohol Potash Digestion: A 2–5 gram portion of the alfalfa leaf meal was placed in an Erlenmeyer flask with 100 ml. of 12% alcoholic potash, and digested on the steam bath for 30 minutes. The volume was held constant throughout the digestion by the addition of 95% alcohol. The digestion mixture was cooled to room temperature, transferred to a 500 ml. separator funnel, and the flask rinsed with 100 ml. of distilled water. The rinsings were added to the digestion mixture in the funnel, and the resulting solution extracted with 50 ml. portions of petroleum ether until a colorless extract was obtained (5 or 6 extractions). The extracts were combined in another separator, and 200 ml. distilled water added, care being taken not to shake or swirl the funnel during the process. After two minutes the aqueous layer was discarded, a second portion of 100 ml. water added, and the funnel swirled gently. After separation of the layers (2 minutes), the aqueous layer was removed, 25 ml. of water added, and the funnel shaken vigorously for 30 seconds. Additional extractions with 25 ml. portions of water were continued until the aqueous extracts were neutral to phenophthalein. After the final extraction, the funnel was allowed to stand for five minutes, any residual water removed, and the petroleum ether extracts concentrated to approximately 25 ml. under vacuum on the steam bath. The extracts were then filtered through anhydrous sodium sulfate into a 100 ml. volumetric flask, and made to volume with petroleum ether.

4. Saponification of Extracts: In order to determine the effect of saponification, an aliquot of the solution from (2) was taken, the solvent removed under nitrogen on the steam bath, and the residue treated as in (3).

C. Chromatography: All extracts were chromatographed on a column composed of a 1:3 mixture of magnesium oxide (Westvaco #2641) and Hyflo-Super Cel (Fisher). The column was prepared under suction in an absorption tube measuring 22×100 mm. fitted with a fritted disc of medium porosity. The adsorbent was packed to a depth of approximately 75 mm., and a 1 cm. layer of anhydrous sodium sulfate added. Following the addition of the extract solution, the chromatogram was developed, and carotene eluted, with a 5% solution of acetone in petroleum ether. The eluates were adjusted to desired volumes with petroleum ether.

D. Optical Measurements: The optical densities of the purified pig-

ments were determined at 5 mμ intervals between 400 and 500 mμ, using a Beckman spectrophotometer.

DISCUSSION

When the extinction coefficients ($E^{1\%}_{1cm}$) of the carotene solutions were plotted against wave length (Fig. 1), it was apparent that the results obtained by the four procedures differed both qualitatively and quantitatively. All of the curves show maxima in the neighborhood of 450 and 475

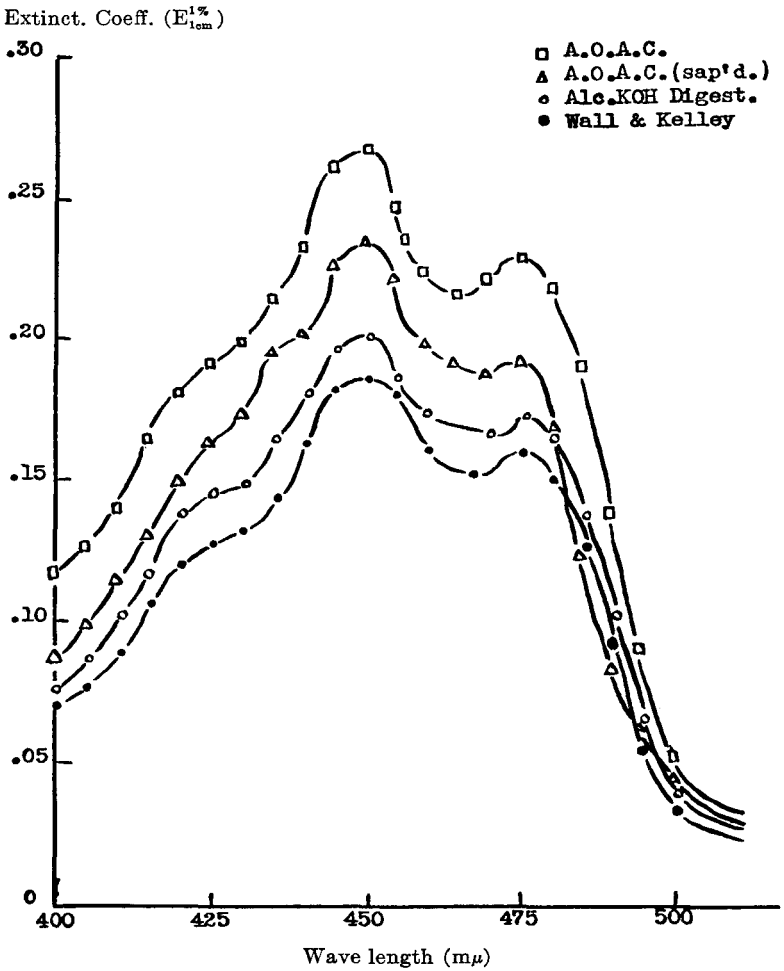

FIG. 1.—Extinction Coefficients of Carotene Solutions Obtained by Different Procedures (Averages for 5 assays by each method)

millimicrons, and in this respect, are typical for solutions of beta-carotene. However, marked differences in the shapes of the curves may be noted at points of secondary inflection, such as 420, 435, and 465 mμ, indicating that the extracts differed in the amounts of other carotenoid pigments. If the ratios E_{430}/E_{450}, E_{466}/E_{450}, and E_{478}/E_{450}, are calculated (Table 1), it will be seen that these curves correspond to that given by Harper and

TABLE 1.—*Ratios between extinction coefficients at different wave lengths*[1]

PROCEDURE	$\frac{E_{430}}{E_{450}}$	$\frac{E_{466}}{E_{450}}$	$\frac{E_{478}}{E_{450}}$
Mod. A.O.A.C.	0.755	0.81	0.83
Mod. A.O.A.C. (sap'd)	0.725	0.76	0.725
Alcoholic KOH Digestion	0.740	0.845	0.845
Wall & Kelley	0.705	0.81	0.81
(Theoretical)[2]	0.71	0.80	0.875

[1] Based upon averages of the results of five determinations by each method.
[2] *Cf.* Harper and Zscheile (4).

TABLE 2.—*Carotene determined in dehydrated alfalfa leaf meal by different methods*[1]

PROCEDURE	CAROTENE (MICROGRAMS/GRAMS)	
	450 mμ[2]	436 mμ[3]
Mod. A.O.A.C.	109.25	108.16
Mod. A.O.A.C. (sap'd)	95.47	98.98
Alcoholic KOH Digestion	80.29	82.91
Wall and Kelley	75.07	72.86

[1] Averages of the results of five determinations by each method.
[2] Calculated by the procedure in *Methods of Analysis* (5).
[3] Calculated by the procedure given in (3).

Zscheile (4) for pure beta-carotene. On the basis of these ratios, the solution obtained by the Wall and Kelley procedure would appear to contain the highest percentage of pure beta-carotene, although its total content of carotenoids is lower than that of any of the other methods. However, the degree of significance which may be attached to these variations in the values of these ratios was not determined, and it might be assumed that the pigment determined by each procedure was essentially beta-carotene.

As shown in Figure 1, the extinction coefficient at 450 mμ for the solution from the modified A.O.A.C. procedure is 43 per cent higher than that for the Wall and Kelley method; 34 per cent higher than that for the alcoholic potash method; and 13 per cent higher than that for the saponified extracts. (This apparent superiority of the modified A.O.A.C. is shown in Table 2, where the carotene contents of the solution were calculated by two different procedures.) It will be noted that the amount of

carotene found (based on the calculation at 450 mμ), by the modified A.O.A.C. procedure is 45 per cent greater than that found by the Wall and Kelley method. Since, in the Wall and Kelley method and the saponification methods, the procedure for the chromatographic separation of carotenes from other pigments, and for the subsequent spectrophotometric estimation, was the same, it appears that the observed differences in the results of assays on replicate samples were due to varying degrees of efficacy of the extraction technics.

SUMMARY

Replicate samples of dehydrated alfalfa leaf meal were assayed for carotene content by four different analytical procedures. The results obtained by the modified A.O.A.C. method were significantly higher than those obtained by the other procedures.

REFERENCES

(1) WALL, M. E., and KELLEY, E. G., *Ind. Eng. Chem., Anal. Ed.*, **15**, 18 (1943).
(2) MOORE, L. A., and ELY, R., *Ibid.*, **13**, 600 (1941).
(3) "Changes in Methods of Analysis," *This Journal*, **31**, 111 (1948).
(4) HARPER, R. H., and ZSCHEILE, F. P., *Food Res.*, **10**, 84 (1945).
(5) *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945) **36.11**, p. 604.

A STUDY OF METHODS FOR THE DETERMINATION OF SUGAR IN CROP PLANTS*

By DOROTHY R. WALDRON, C. D. BALL, E. J. MILLER, and ERWIN J. BENNE (Michigan State College of Agriculture and Applied Science, East Lansing, Michigan)

For a number of years the authors have been concerned with the determination of sugars in a diversified variety of crop plants, including carrot and parsnip roots, onion bulbs, potato tubers, apple, cherry, pear, blueberry and tomato fruits, fruit tree leaves, and various kinds of grain and forage crops. Because of the many problems encountered in sampling, reducing to small pieces and mixing such materials, in order to obtain representative portions for analysis, and in extracting, isolating, and evaluating the sugar contained in them, an investigation was undertaken to test the efficiency and reliability of certain practices when applied to the determination of sugars in different types of plant tissues. Carrot roots, onion bulbs, spinach leaves, tomato fruits, and young brome grass were selected as representative crop types, and comparative values for total (reducing plus hydrolyzable) sugar in samples of each were obtained by

* A joint contribution from the Department of Agricultural Chemistry and the Kedzie Chemical Laboratory. Published with permission of the Director of the Agricultural Experiment Station as journal article no. 946 (n.s.).

use of the combinations described below of (a) two methods for extracting the sugars from the tissue, (b) two different extracting solvents, (c) two methods of hydrolysis, and (d) four methods for evaluating the amounts of sugar. An account of the investigation and the results obtained are presented in this paper.

EXPERIMENTAL

The determination of sugar in a given crop involved six general steps. A brief description of the performance of each step follows:

1. Selecting the sample from the field.—The first step in the analysis of each crop was to select from the field a sample of sufficient size to insure that it represented the average composition of the entire crop. Amounts

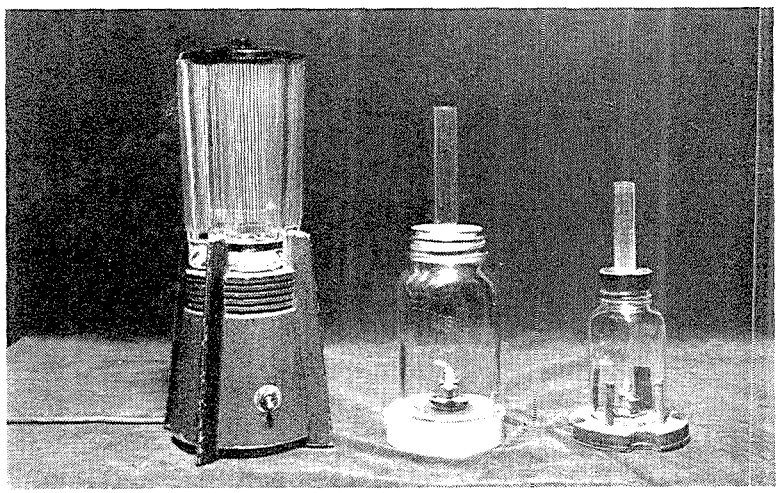

FIG. 1.—Special vessels devised for use with a Waring Blendor.

selected for this purpose were necessarily governed by the extent, uniformity, and nature of the crop concerned.

2. Reducing to small pieces and mixing the field sample.—

(a) Carrot roots and onion bulbs—Ground with food chopper and mixed by hand.

(b) Spinach leaves and young brome grass—Cut as finely as possible with hand shears and mixed by hand.

(c) Tomato fruits—All fruits in the entire field sample were cut in halves and thin transverse sections were cut from one half of each fruit for each analytical portion. Obviously no mixing was necessary with this practice.

3. Weighing the portions for analysis.—The balance used was sensitive to 0.05 gm. The weights of portions used for analysis varied with the

sugar content of the tissue as indicated by preliminary experiments. In general they were comparable to those given below:

(a) Carrot roots	15 gms.
(b) Onion bulbs and tomato fruit	20 gms.
(c) Spinach leaves and young brome grass	15–25 gms.

4. Extracting the sugar from the tissue.—

(a) A Waring Blendor equipped with special vessels (Fig. 1), which were prepared in the authors' laboratory (1, 2), was used for comminuting

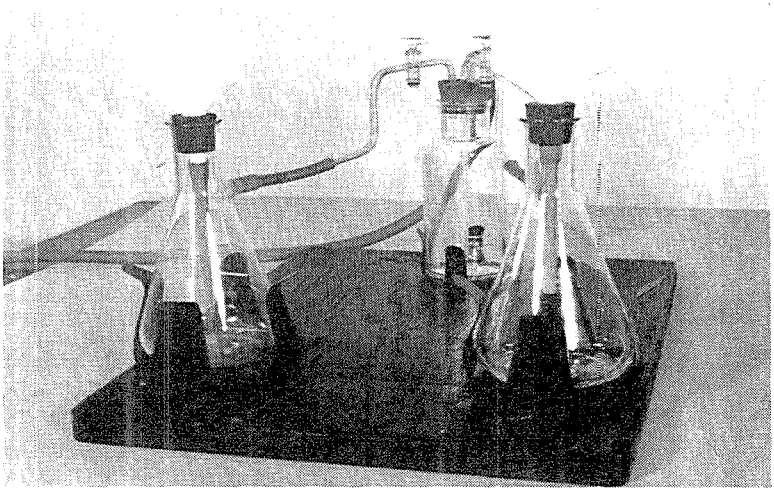

FIG. 2.—A convenient filtering arrangement.

the weighed portions of each tissue in the presence of the extracting solvent. The smaller vessel pictured was used with carrot roots, onion bulbs and tomato fruits; the quart fruit jar with the more bulky samples of spinach leaves and brome grass. Small amounts of calcium carbonate were added to the samples before they were blended, to neutralize the plant acids. The blending time was 4–9 minutes depending upon the nature of the tissue. Distilled water was used as the extracting solvent in one series of determinations and approximately 80% ethanol in another. In the latter series the amount of water present in the fresh tissue was taken into consideration in diluting 95% ethanol to the desired concentration.

(b) Other portions of each tissue were extracted for 10 hours in Soxhlet extractors with such volumes of 95% ethanol that the water present in the samples diluted the concentration of ethanol in the final solutions to about 80% by volume.

5. Filtering the extracts.—All filtrations were made with Büchner funnels using suction. The filtering arrangement shown in Figure 2, also devised in the authors' laboratory (3), was used throughout the work. With the aqueous extracts transfers and washings were made with distilled water, and with the alcoholic extracts 80% ethanol was used for these purposes. With some tissues much difficulty was encountered in filtration especially with the aqueous extracts. Therefore, "filter-aid" was used[1] to facilitate this operation. Although the use of this material aided filtration to some extent, in many cases it was necessary to use disks cut from filter cloth instead of filter paper to make filtration possible. The alcoholic extracts from the Soxhlet extraction required no filtration.

6. Evaluating the amounts of sugar in the extracts.—The alcohol was removed from the alcoholic extracts by evaporation on the steam bath. The protein was precipitated from all extracts with lead acetate, and excess lead was removed with potassium oxalate except with aliquots to be titrated with ceric sulphate, in which cases disodium orthophosphate was used for this purpose. Separate aliquots were hydrolyzed with hydrochloric acid and invertase, respectively. The invertase solution used was obtained from Difco Laboratories, Inc., Detroit, Mich. The amounts of total sugar present in aliquots of the extracts from each sample and each treatment were evaluated by four different procedures: *viz.*, (a) The gravimetric method and (b) the volumetric thiosulfate method, respectively, of the Association of Official Agricultural Chemists (4); (c) the cuprous titrimetric method of Shaffer and Hartmann (5); and (d) the ceric sulphate titrimetric method of Hassid (6).

DISCUSSION

Certain manipulative operations in the analytical procedures used warrant a brief discussion. Use of the Waring Blendor for extracting sugars from plant tissue as described possessed advantages in economy of time and labor compared to the use of Soxhlet extractors or hand mortars, respectively, for this purpose. Trouble encountered in filtering the extracts of certain plant tissues, however, appeared at first to be a serious disadvantage of the practice, but the use of 80% ethanol instead of water as the extracting solvent for some tissues, the substitution of filter cloth for filter paper, and the addition of filter-aid helped to mitigate this difficulty.

It was found to be most advantageous to mix the filter-aid with the extract immediately before filtration was begun. The amount required varied from 1 to 5 grams, depending upon the kind of tissue being analyzed. Experiments showed that the filter-aid used by the authors did not adsorb perceptible amounts of sugar.

[1] Johns Manville Hyflo Super-Cel.

In the gravimetric procedure Selas filtering crucibles of No. 10 porosity were used in most cases for removing the cuprous oxide from the final solution. Such crucibles were convenient and ordinarily very satisfactory for this purpose. The rate of filtration was usually rapid enough to permit the solutions to be filtered within the time schedule required by this procedure; however, with extracts of onion bulbs in which invertase was used the final solutions were extremely difficult to filter. In these cases it was necessary to replace the Selas crucibles with Gooch crucibles containing very thin pads of asbestos.

In using the ceric sulphate titration method for evaluating sugars as published by Hassid (6) the tissue extracts were clarified with finely divided carbon before they were titrated. Hassid recommended that the clarification process be carried out before hydrolysis; however, the authors noted in preliminary work that if the tissue extracts were clarified prior to hydrolysis, values for total sugar were markedly lower than those obtained if the extracts were clarified after the hydrolytic treatment. Hence, the latter practice was followed in obtaining all data reported in this paper.

The results in Table 1 show that the values for sugars obtained by the four evaluation procedures are generally in good agreement when aliquots of a given extract were subjected to the same hydrolytic treatment. Moreover, it will be noted with the first three crops that for a given method of extraction, and a given method of hydrolysis, duplication of values for sugars in different weighed portions of tissue is excellent for each evaluation procedure. Also, judging from the results with the first three crops, it appears that the solvents and methods of extraction used were equally effective in removing sugars from the tissues and that hydrolysis with hydrochloric acid was as efficient as with the invertase preparation used.

Young brome grass, however, is an exception to these general tendencies, since the percentages of total sugar apparently present in this tissue appears to have been greatly influenced by the solvent and hydrolytic agent used. It will be noted than when water was used as the solvent and hydrochloric acid as the hydrolytic agent the values for sugar are consistently high. The next highest values were obtained with aqueous extracts and invertase, and the lowest values resulted when invertase was used with extracts from the Soxhlet extractors. The same tendencies are evident in the results from spinach leaves, but to a less striking extent.

Apparently these variations in results are due to the fact that brome grass and spinach leaves contained substances which were more soluble in water than in 80% ethanol and yielded reducing sugars more readily when hydrolyzed with acid than with invertase. According to Archbold (7) and Hassid (8) certain water-soluble polysaccharides behave in this manner.

The variable results obtained with young brome grass and spinach

TABLE 1.—*Results*

Per cent of total sugar in crop plants as determined by different methods of extraction and evaluation[1]

KIND OF CROP	METHOD OF EXTRACTION[2]	METHODS USED TO EVALUATE SUGAR							
		A.O.A.C.				SHAFFER-HARTMANN		CERIC SULPHATE TITRATION	
		GRAVIMETRIC		VOLUMETRIC					
		A[3]	In[4]	A	In	A	In	A	In
Carrot roots	I	7.70	7.71	7.68	7.67	7.29	7.52	7.95	7.79
	I	7.63	7.56	7.63	7.50	7.33	7.44	7.75	7.78
	II	7.39	7.39	7.34	7.27	7.08	7.18	7.46	6.90
	II	7.39	7.43	7.32	7.35	7.04	7.20	7.12	6.96
	III	7.29	7.42	7.19	7.02	7.26	7.20	7.90	7.64
	III	7.39	—	7.37	7.34	7.33	7.33	8.13	7.69
Onion bulbs	I	4.80	4.93	4.51	4.53	4.88	4.89	4.61	4.45
	I	4.87	5.06	4.50	4.55	4.92	4.94	4.71	4.45
	II	4.75	4.87	4.52	4.38	4.83	4.86	4.64	4.34
	II	4.61	4.74	4.36	4.49	4.83	4.80	4.87	4.28
	III	4.57	4.35	4.45	4.31	4.60	4.42	4.31	4.40
	III	4.74	4.50	4.57	4.42	4.78	4.48	4.43	4.50
Tomato fruits	I	2.36	2.26	2.39	2.34	2.37	2.34	2.41	2.20
	I	2.36	2.47	2.33	2.36	2.39	2.34	2.35	2.17
	II	2.44	2.44	2.34	2.41	2.46	2.44	2.29	2.28
	II	2.30	2.32	2.40	2.39	2.44	2.45	2.35	2.28
	III	2.38	2.31	2.34	2.34	2.31	2.26	2.23	2.26
	III	2.35	2.47	2.31	2.24	2.20	2.14	2.25	2.23
Spinach leaves	I	.58	.64	.51	.48	.50	.49	.45	.44
	II	.51	.48	.48	.45	.49	.48	.44	.45
	III	.45	.47	.44	.44	.47	.46	.47	.44
Young brome grass	I	2.21	1.66	2.17	1.82	2.20	1.77	2.20	1.70
	II	1.72	1.36	1.67	1.47	1.68	1.48	1.53	1.35
	III	1.39	1.22	1.34	1.13	1.47	1.29	1.42	1.24

[1] The values in each horizontal line are from separate aliquots of the same extract of one weighed portion of sample. In the vertical columns each value represents a different weighed portion of each tissue. Values given for spinach and young brome grass are averages of results from duplicate aliquots of the same extract.

[2] I = Waring Blendor, distilled H_2O as solvent; II = Waring Blendor, 80% ethanol as solvent; and III = Soxhlet extractor, ethanol as solvent.

[3] Hydrolyzed with HCl. Five ml conc. acid to 100 ml aliquot, heated in boiling water bath exactly 10 min. (Results same as with 15–20 hours at room temp.)

[4] Hydrolyzed with invertase.

leaves typify the complexities likely to be encountered in the determination of sugars in crop plants and emphasize the need for sufficient preliminary work with each different crop to insure that the values obtained with a given analytical procedure actually represent the sugar content of the tissue.

Only values for total sugar are given in this paper, but it is obvious that reducing sugars present in the tissue extracts could have been evaluated in aliquots not subjected to hydrolysis.

CONCLUSIONS

(1) A Waring Blendor equipped with improvised blending vessels was convenient and effective for extracting sugars from the crop plants analyzed.

(2) Water and 80% ethanol could be used interchangeably for extracting sugars from some crop plants, but for others extraction with water gave higher results.

(3) Under the same conditions of extraction and hydrolysis, the four methods used for evaluating reducing sugars gave approximately the same results for total sugars in each crop plant analyzed.

(4) The use of carbon for clarification of extracts prior to hydrolysis was found to give lower results for total sugars than when the carbon was used subsequent to such hydrolysis.

(5) The complexities encountered in the determination of sugars in crop plants necessitate sufficient preliminary work with each different crop to insure that the value obtained with a given analytical procedure represents the true sugar content of the tissue.

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THE DETERMINATION OF THE SULFIDE-SULFUR CONTENT OF CALCIUM SILICATE SLAGS IN RELATION TO THEIR NEUTRALIZATION VALUE*

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A knowledge of the sulfur content of agricultural slags is of importance both because of its value as a plant nutrient and its effect upon the chemical evaluation of the neutralization capacity of the slags. Where the neutralization value of a slag is computed from the analyses of Ca and Mg, such value is subject to a minus correction for the *total sulfur* content of that slag. If, however, the neutralization capacity of the slag is obtained through *titrative procedures*, this value is subject to a minus correction for the *sulfide sulfur* content only.

The sulfide correction problem was dealt with briefly in our early contribution on titrative procedures for calcium silicate slags (6, p. 332) and subsequently in a report on liming materials (7, pp. 296 and 306). Schollenberger (3) had suggested that "automatic" correction for sulfide sulfur be provided through oxidation by means of hydrogen peroxide in the dissolvent acid. This proposed modification was considered of doubtful value towards improving the accuracy and as an undesirable complication for a routine procedure. The titration to *p*H 5.2 using Bromcresol green, therefore, was recommended for adoption for the determination of the neutralization value of blast furnace slags, with the annotation: "Without correction for sulfide content" (2, p. 306). It was suggested, also, that a deduction of 3.5 per cent calcium carbonate-equivalence from the titration value might be admissible as a reasonable mean value for sulfide content. In some instances, however, it may be desirable to obtain a more exact correction which would be based on the sulfide content of the slag.

The objectives of the present investigation were to acquire information relative to the sulfide and sulfate content of various representative agricultural slags and to evolve a simple and accurate procedure for the determination of their sulfide content.

EXPERIMENTS ON THE JOINT DETERMINATION OF SULFIDE CONTENT AND NEUTRALIZATION VALUE

The most convenient procedure for the determination of the sulfide content would seem to be the collection of hydrogen sulfide that is evolved in the process of dissolution of the slag in the determination of its neutralization value. The apparatus required to carry out this process was:

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11–13, 1948.

a 60-ml separatory funnel fitted into a 250 Erlenmeyer flask; a 25×150 mm test tube provided with inlet and outlet tubing connected to flask by means of 6-inch length of 3/16-inch rubber tubing. In the preliminary dissolution of the slag at room temperature, the reaction flask was shaken either intermittently by hand or continuously by mechanical device and the absorbent tube was attached to the flask by means of a rubber band, which was removed when the flask was placed over a Bunsen burner, so that the absorbent tube could be extended for immersion in cold water in a 600-ml beaker.

Dissolution in Presence of Air

Charges of one-half gram of slag were placed in 250-ml Erlenmeyer flasks; the sides of flasks were washed down with about 5 ml of water, the stopper that carried the separatory funnel and the outlet tube was fitted tightly into the reaction flask. A charge of 25 ml of a 2 per cent cadmium sulfate solution was placed in the absorbent tube, which was connected to flask as described. Standard acid, 35 ml, was introduced into the flask through the separatory funnel while the suspension was being swirled. Further agitation was by hand at one minute intervals, or continuously on a shaking machine for 10 to 15 minutes. The funnel was rinsed with 10 ml of water, which was drawn into flask. The contents then were boiled 10 minutes by means of a small Bunsen burner. The rubber connections then were snapped off from the cadmium sulfate tube quickly to prevent the backing-up of the cadmium sulfide suspension.

Dissolution of Charge in Atmosphere of Carbon Dioxide

Since sulfide oxidation by air in the dissolution train was deemed a possible source of error, a parallel series of slag dissolutions were made in air, and in an atmosphere of carbon dioxide, in the same manner and with the same apparatus as described above. The carbon dioxide was introduced after the slag charge had been wetted, and was led through the train for 5 minutes at the rate of 500 ml per minute prior to dissolution of the slag by the acid.

Sulfide Sulfur Evolved from Slags through Dissolution in 0.5 N Hydrochloric Acid

The separatory funnel was rinsed, the neutralization value of the slag was determined by the titration procedure of the flask to *p*H 5.2 using Bromcresol green (7), and the evolved sulfide value was measured by the acidity engendered in cadmium sulfate absorbent tubes.

The eleven samples of representative blast furnace slags analyzed for sulfide content were furnished by the Standard Slag Company of Youngstown, Ohio. They had been used in the testing of the simplified titration procedure to *p*H 5.2 against Bromcresol green, as reported in 1946 (7),

"without correction for sulfide content." The results obtained by dissolution in 0.5 *N* acid are given in Table 1. The calcium carbonate-equivalence of the sulfide content ranges from 1.7 to 4.2 per cent and gives a mean value of 2.50 per cent. The sulfide values are reproducible within 0.2 ml titration, or 0.2 per cent calcium carbonate-equivalence, which is satisfactory in the evaluation of the neutralization capacity of slags. The few

TABLE 1.—*Sulfide sulfur evolved from blast furnace slags through dissolution in 0.5 N HCl, and determined through titration of the $CdSO_4$ absorbent*

SLAG*	TITRATIONS, ML OF 0.1 *N* NaOH PER 0.5-G CHARGE									
	BY DISSOLUTION IN ATMOSPHERE OF CO_2					BY DISSOLUTION IN AIR				
	1	2	3	AV.	S	1	2	3	AV.	S
					per cent					*per cent*
A	3.80	3.80	3.80	3.80	1.22	3.80			3.80	1.22
B	4.25	4.30		4.28	1.37	4.15			4.15	1.33
C	2.50	2.50	2.50	2.50	0.80	2.50			2.50	0.80
D	3.15	3.10		3.13	1.00	3.05			3.05	0.98
E	1.90	1.90		1.90	0.61	1.80			1.80	0.58
F	2.10	2.05		2.08	0.67	2.20	2.00	2.00	2.07	0.36
G	2.40	2.35	2.40	2.39	0.76	2.30	2.25	2.30	2.27	0.73
H	2.15	2.10		2.13	0.68	2.15	2.05	2.05	2.07	0.66
I	2.00	2.00		2.00	0.64	2.05	2.00	2.00	2.02	0.65
J	1.80	1.70	1.80	1.77	0.57	1.70	1.65	1.65	1.67	0.53
K	2.40	2.30	2.30	2.33	0.75	2.35	1.95	2.00	2.10	0.67
Average				2.57	0.82				2.50	0.80

* Supplied by Standard Slag Co., 1945 shipment.

slight increases shown as a result of dissolution being effected in an atmosphere of carbon dioxide were not sufficient to warrant the extra effort.

Completeness of Sulfide Evolutions and Accuracy of the Sulfide Determination

The accuracy of the sulfide procedure was tested by the use of a prepared cadmium sulfide, and by checking the sum of the sulfide value indicated by the evolutions and residual sulfur against the directly determined total sulfur content of eleven slags. The prepared sulfide was obtained by passage of hydrogen sulfide through a water scrubber and into a 2 per cent solution of cadium sulfate. The precipitate was filtered and washed with water until the washings were neutral to methyl orange.

Experiments with Cadmium Sulfide

To effect complete dissolution of the prepared cadmium sulfide for determination of its sulfide value, it was necessary that the hydrochloric

acid be more concentrated than 0.5 *N*. A (1+4) concentration of the dissolvent hydrochloric acid effected dissolution of the prepared sulfide upon heating. The volatility of hydrochloric acid at this concentration necessitated the use of a scrubber between the evolution flask and the cadmium sulfate. A 25×150 mm test tube, half-filled with water and maintained at near boiling, was utilized for that purpose. With these two variations, the apparatus and procedure used for the adsorption of hydrogen sulfide in cadmium sulfide were those described. Usually the dissolution was effected in the presence of the contained air, but in some experiments a hydrogen atmosphere was produced by the inclusion of 1 gram of zinc dust with the cadmium sulfide charge. The cadmium sulfide was analyzed for total sulfide and residual sulfur after sulfide evolution.

TABLE 2.—*Analysis of the CdS prepared and used as absorbent for H_2S*

CdS CHARGE	SULFIDE SULFUR			SULFATE SULFUR		SUM OF $S^{=}+SO_4^{=}$ SULFUR	TOTAL S DETERMINED
	0.1 *N* TITRATION	S-EQUIVALENCE					
		PER DETERMINATION	IN SAMPLE	PER DETERMINATION	IN SAMPLE		
g	*ml*	*g*	*per cent*	*g*	*per cent*	*per cent*	*per cent*
.100	12.40	.01984	19.84				
.100	12.30	.01968	19.68				
.100	12.30	.01968	19.68				
Average			19.73				
.050	6.15	.00984	19.68	.00045[b]	0.90	20.58	
.050	6.20	.00992	19.84	.00045	0.90	20.74	
.050	6.25	.01000	20.00	.00044	0.88	20.88	
Average			19.84		0.89	20.73	
.025	3.10	.00496	19.84				
.025	3.10	.00496	19.84				
.025	3.10	.00496	19.84				
Average			19.84				
.100[a]	12.50	.02000	20.00	.00085[c]	0.85	20.85	20.94[d]
.100	12.60	.02016	20.16	.00085	0.85	21.01	20.82
.100	12.55	.02008	20.08	.00087	0.87	20.95	21.00
Average			20.08		0.86	20.94	20.92

[a] Sulfide evolution of this group was in an atmosphere of hydrogen, all other evaluations were in the contained air.

The corresponding $BaSO_4$ weights were for—

[b] .0033, .0033, and .0032 g
[c] .0062, .0062, and .0063 g } all corrected for a .0008-g reagent blank.
[d] .1532, .1523, and .1536 g

Results from the Use of Prepared Cadmium Sulfide

The triplicate titration values given in Table 2 show good reproducibility for specific cadmium sulfide charges, and precision for charges in the range between .025 and .100 g and those charges embraced the sulfide contents of 1-g charges of blast furnace slag. Slightly higher results were obtained from the .100-g charges of cadmium sulfide, plus zinc dust. This may mean that some oxidation of the sulfide to sulfur did occur in the limited air volume. The highest sulfide value obtained on the prepared product was, however, only 20.08 per cent, which is 2.12 per cent less than

TABLE 3.—*Effect of nascent hydrogen upon the recovery of sulfide sulfur from blast furnace slabs by the evolution method*

SLAGS*	A—BY 0.5 *N*/HCl IN AIR			B—BY HCl, 1+4, IN HYDROGEN†			
	0.1*N* TITRATION	$CaCO_3$ EQ OF S	SULFIDE S	0.1 *N* TITRATION	$CaCO_3$ EQ OF S	SULFIDE S	$CaCO_3$ EQ B—A
	ml	*per cent*	*per cent*	*ml*	*per cent*	*per cent*	*per cent*
A	3.80	3.80	1.22	7.90	3.95	1.26	.15
B	4.15	4.15	1.33	8.80	4.40	1.41	.25
C	2.50	2.50	0.80	5.40	2.70	0.86	.20
D	3.05	3.05	0.98	6.40	3.20	1.02	.15
E	1.80	1.80	0.58	4.85	2.43	0.78	.63
F	2.07	2.07	0.66	6.10	3.05	0.98	.98
G	2.27	2.27	0.73	5.70	2.85	0.91	.58
H	2.07	2.07	0.66	4.80	2.40	0.77	.33
I	2.02	2.02	0.65	5.35	2.68	0.86	.66
J	1.67	1.67	0.53	4.85	2.43	0.78	.76
K	2.10	2.10	0.67	7.30	3.65	1.17	1.55
Average		2.50	0.80		3.07	0.98	0.57

* Supplied by Standard Slag Company, Youngstown, Ohio, 1945 shipment.
† By reaction with zinc dust.

the theoretical value. The sulfate residue from the charge of cadmium sulfide accounted for only 0.9 per cent of the sulfur content of the cadmium sulfide. The sum of the sulfide and sulfate forms of sulfur was 20.94 per cent and is in accord with the 20.92 per cent total content found by the direct determination. The findings reported in Table 2 show the accuracy and precision of the titration procedure for sulfide sulfur, especially when the hydrogen sulfide evolution was accompanied by the evolution of hydrogen.

Although the prepared cadmium sulfide was precipitated under analytical conditions, the precipitate was found to contain cadmium sulfate and approximately 10 per cent of sorbed non-sulfide matter. This does not support the suggestion (5, p. 912) that the gravimetric determination of

the filtered precipitate can be used as a check upon the titration results of the sulfide determination.

Accuracy of the Procedure for the Evolution of Blast Furnace Slags

Because of the apparent incompleteness in the evolution of sulfide from cadmium sulfide in ordinary atmosphere, zinc dust was included in sulfide determinations on the eleven slags, which circumstance precluded simultaneous determinations of the neutralization value and sulfide content on the same charge. Charges of 1 gram per 50 ml of hydrochloric acid (1+4) were used when zinc was included. Apparatus and procedure were as described for the cadmium sulfide, except that preliminary contact in the cold with frequent agitation was adhered to. The sulfide values given in Table 3 were obtained through dissolution with (1+4) hydrochloric acid and nascent hydrogen, in comparisons with dissolution in 0.5 *N* hydrochloric acid under the conditions prescribed for the determination of neutralization value.

Effect of Nascent Hydrogen upon Sulfide Recovery

The results given in Table 3 show that the use of nascent hydrogen gave an average increase in sulfide values of .57 per cent calcium carbonate-equivalence, although some of the increases were as much as 1.55 per cent. Since the use of zinc also introduced a greater acid concentration, the effect of the nascent hydrogen should be differentiated from the effect of acid concentration. Triplicate sulfide determinations on slag K were made by dissolution in hydrochloric acid (1+4) without addition of zinc. The obtained values of 2.2 per cent calcium carbonate-equivalence are in close agreement with those effected by dissolution of the slag in 0.5 *N* hydrochloric acid. Hence, the increase of from 2.10 to 3.65 per cent calcium carbonate-equivalence in slag K is ascribed as an effect of the nascent hydrogen rather than an effect of acid concentration. Apparently, however, the lesser values are not due to oxidation by the contained air, since carbon dioxide displacement failed to effect any material increase in the sulfide values. The chemical composition and structure of the slag are probably the factors that govern sulfide recovery. It is noteworthy that the four charges that showed the least effect from analytical addition of zinc were the water-quenched or air-granulated slags, A, B, C, and D, of "glassy" structure and ready dissolvability by hydrochloric acid in either of the concentrations used. In contrast, the air-cooled slags underwent dissolution slowly and invariably yielded more hydrogen sulfide when the charges were fortified by additions of zinc. Apparently the zinc served to effect reduction of the higher oxides of manganese, and possibly those of iron, which might induce oxidation of the generated hydrogen sulfide and thereby preclude atmospheric oxidation in the apparatus.

Hence, to assure the complete evolution of the sulfide sulfur from an hydrochloric acid-dissolved charge of blast furnace slag, it is deemed imperative to fortify the charge with zinc, or with a metal that will assure dissolution with an adequate supply of nascent hydrogen.

PROCEDURE FOR THE DETERMINATION OF THE SULFIDE SULFUR CONTENT OF CALCIUM SILICATE SLAGS

REAGENTS

(a) *HCl* (1+4).

(b) *Zinc dust.*—C. P. grade, of low lead content.

(c) *Absorbent.*—Dissolve 20 g of $CdSO_4 \cdot 2\frac{2}{3}\ H_2O$ in H_2O and make to 1 liter. Adjust reaction to *p*H 5.6 by means of either potentiometric titration or colorimetric comparisons on a separate 50-ml aliquot and matching a buffer of same *p*H.

(d) *Standard alkali.*—Prepare 0.1 *N* NaOH from CO_2-free NaOH solution and use of CO_2-free H_2O.

(e) *Standard acid.*—0.1 *N* HCl.

APPARATUS

A 250-ml Erlenmeyer evolution flask to fit No. 5.5 stopper, a 60-ml separatory funnel fitted into the stopper and the lower third of its stem drawn to a 2 mm opening and bent upwards at its extremity. The funnel in the stopper is adjusted so that its stem is $\frac{1}{4}$ inch from the bottom of the evolution flask. Two 25×150 mm test tubes are fitted with No. 4 two-hole stoppers and inlet and outlet of 6 mm tubing, which serve to contain jointly the 50 ml of absorbent. The tubes are in series and should be placed in a 600-ml beaker filled with cold water, and the beaker placed upon a tripod on level with the evolution flask. Another test tube of similar size is half-filled with H_2O and clamped in position between evolution flask and absorbent solution to provide trap for HCl vapor.

DETERMINATION

Weigh 1 g of minus 30-mesh slag into the evolution flask; add 1 g of Zn dust and wash down sides with 5–10 ml H_2O; mix contents by means of flattened end of stirring rod and connect the flask. Introduce 50 ml of HCl (1+4) into separatory funnel; open stopcock to allow acid to flow into reaction flask, swirling the contents meanwhile. If necessary, apply pressure to effect complete transfer of acid from funnel. Close stopcock and continue the swirling 5 min. at brief intervals. Apply heat; continue the swirling until boiling starts and then regulate flame to maintain active boiling, although not too vigorous, throughout 10 min. Keep the contents of the HCl vapor-trap near the boiling point throughout dissolution of the slag, then snap off the rubber tubing at the $CdSO_4$ intake quickly, taking care not to allow a backing-up of the contents. Cut off heat, discard contents of the evolution flask and of the scrubber tube, and set up apparatus for the next determination.

Filter the CdS suspension on a 9 cm gravity filter leading into a 250 ml Erlenmeyer flask and wash with H_2O to an overall volume of 100 ml. Add 4 drops of a 0.2 per cent soln of methyl red and agitate vigorously while titrating slowly to the exact orange-yellow tint of the reference soln. That soln comprises 50 ml of the CdS soln diluted to 100 ml, with identical concentration of indicator and contained in a 250-ml Erlenmeyer flask. Should the end point be passed with resultant precipitation of $Cd(OH)_2$, introduce 1–2 ml of 0.1 *N* HCl, and allow to stand until the precipitate disappears and then complete the titration dropwise under vigorous agitation. Divide by 2 the ml of net 0.1 *N* NaOH used to obtain the per cent $CaCO_3$-equivalence of the sulfide sulfur in the sample. The ml of 0.1*N* NaOH×.0016 = g sulfide sulfur per determination; and the latter, times 100 = percentage of sulfide S.

TABLE 1.—*Summations of separately determined occurrences of sulfide sulfur and sulfate sulfur, in comparison with total sulfur determinations on blast furnace slags*

SLAG[a]	TOTAL SULFUR				FRACTIONAL DETERMINATIONS OF SULFUR				SULFUR DISPARITY[d]	$CaCO_3$ EQUIV. OF NON-SULFIDE S CONTENT
	AS $BaSO_4$ FROM 1 GM.				SULFATE S					
	1	2	AV.	S	SULFIDE[b] S	AS $BaSO_4$ FROM 1 GM.	S[c]	SUM		
	g	*g*	*g*	*per cent*	*per cent*	*g*	*per cent*	*per cent*	*per cent*	*per cent*
A	.1010	.1029	.1020	1.40	1.26	.0031	.03	1.29	.11	.44
B	.1115	.1132	.1124	1.54	1.41	.0043	.04	1.45	.09	.40
C	.0680	.0690	.0685	0.94	0.86	.0038	.04	0.90	.04	.25
D	.0815	.0815	.0815	1.12	1.02	.0027	.02	1.04	.08	.31
E	.0790	.0800	.0795	1.09	0.78	.0197	.26	1.04	.05	.97
F	.1241	.1268	.1255	1.72	0.98	.0497	.67	1.65	.07	2.31
G	.0871	.0887	.0880	1.21	0.91	.0162	.21	1.12	.09	.94
H	.0744	.0753	.0749	1.03	0.77	.0156	.20	0.97	.06	.81
I	.0824	.0826	.0825	1.13	0.86	.0136	.17	1.03	.10	.84
J	.1270	.1291	.1281	1.76	0.78	.0696	.94	1.72	.04	3.06
K	.1273	.1311	.1292	1.77	1.17	.0389	.52	1.69	.08	1.88
Average				1.34	0.98		.28	1.26	.07	
Extremes:										
Low				.94	.77		.02			
High				1.77	1.41		.94			

[a] Furnished by Standard Slag Company, 1945 shipment.
[b] Data taken from Table 3, Section B.
[c] S was computed from $BaSO_4$ weights after correction for reagent blank of .0011 g $BaSO_4$.
[d] Sulfur unaccounted for when "summations" were subtracted from determinations of totals.

NON-SULFIDE SULFUR IN SLAGS

Distinction has been drawn between total sulfur content and that in the form of sulfide, in relation to the two methods of analysis that are used for determination of the neutralization value of slags. The slags used in the sulfide methods were used in an effort to establish the actual quantitative differences between the two kinds of sulfur combinations that occur in various slags. Their total sulfur content was determined, and the fractions that remained in solution or in suspension after evolution of sulfide sulfur were determined also, so that the aggregates of the separate determinations would provide overall checks against totals. The total sulfur results given in Table 4 were obtained through aqua regia digestions of 1-g charges, dehydration and removal of silicon dioxide, reduction of ferric iron with alunimum powder, and precipitation as barium sulfate. Residual sulfur was determined through dehydration of residues from the experiments of Table 3, Section B, redissolving and brominating, and then proceeding as for total sulphur.

The sulfide sulfur values for the water-quenched slags are given in Table 4 and are virtually identical with those found as totals. This is in contrast to the disparity between the total and sulfide sulfur values found for the air-cooled slags. The differences range from 1 to 3 per cent of calcium carbonate-equivalence, and are accounted for almost entirely by the sulfates found in the residues. In general, difference between the values for total sulfur and those obtained as summations of the sulfide and residual forms was $0.07 \pm .03$ per cent sulfur. This small difference may be due to incomplete oxidation by the bromination imposed during the evaporation of the sulfide residuals.

ADDITIONAL SLAG ANALYSIS FOR SULFIDE AND SULFATE SULFUR

Samples of blast furnace slags used in previous studies were available for additional sulfide analyses and these were supplemented by 6 electric-furnace phosphate reduction slags. The results assembled in Table 5 substantiate the earlier finding that the sulfur in the granulated blast furnace slags is nearly all in the sulfide form, whereas sulfates account for substantial fractions of the sulfur contained by the air-cooled slags. The sulfur content of the electric furnace phosphate-reduction slags is only about one-tenth that of the sulfur content of the blast furnace slags. The mean sulfide sulfur content of 26 blast furnace slags was found to be 3.35 per cent, in terms of calcium carbonate-equivalence.

EFFECT OF SULFUR CORRECTION UPON THE CHEMICAL EVALUATION OF THE NEUTRALIZATION POWER OF SLAGS

As noted, there are two types of procedure for the chemical evaluation of the liming effectiveness of slags. In the direct analysis, the calcium and

magnesium content is reckoned as of basic character and is balanced against the sum of the equivalents of sulfur, fluorine, and phosphorus, to establish the potential neutralization capacity of the material. This type of analysis may be taken as standard. The other procedure is an adaptation of the simple alkalimetric method with a prescribed titration end-

TABLE 5.—*Sulfur as sulfide and sulfate and their $CaCO_3$-equivalences in blast furnace slags and in slags from electric rock phosphate reduction furnaces*

SLAG			SULFUR AS SULFIDE		SULFUR AS SULFIDE		$CaCO_3$= OF SUMMATIONS
SOURCE	NO.	PROCESSING	S	$CaCO_3$=	S	$CaCO_3$=	
			per cent	*per cent*	*per cent*	*per cent*	*per cent*
Blast furnace[a]	1	Granulated	1.68	3.25	.05	0.15	5.40
	2	"	1.79	5.60	.06	0.18	5.78
	3	"	1.19	3.70	.05	0.15	3.85
	4	Air Cooled	1.15	3.60	.44	1.38	4.98
	5	"	0.98	3.05	.25	0.78	3.83
	6	"	1.09	3.40	.45	1.41	4.81
	7	"	1.18	3.70	.25	0.78	4.48
	8	"	0.66	2.05	.25	0.78	2.85
	9	"	1.26	3.95	.37	1.16	5.11
	10	"	0.93	2.90	.09	0.28	3.18
	A	"	0.82	2.55	N.D.	—	—
	B	"	0.98	3.05	"	—	—
	C	Granulated	0.94	2.95	"	—	—
	D	"	1.19	3.73	"	—	—
Reserve Sample		"	0.88	2.75	"	—	—
Electric	1053	Quenched	0.15	0.48	"	—	—
furnace[b]	1117	"		0.30	.02	.06	.36
	1224	"	0.13	0.40	N.D.	—	—
	1259	"	0.11	0.35	"	—	—
	1286	"	0.19	0.60	"	—	—
	1291	"	0.18	0.55	"	—	—
Average for blast furnace slags				3.48			
Average for 26 blast furnace slags, including those in Table 3, B				3.35			

[a] Supplied by Standard Slag Company, 1942 samples from 1 to 10; 1945 from A to D.
[b] From Tennessee Valley Authority operations at Wilson Dam, Albama.

point to minimize the sorbtion of bases by the precipitates of gelatinous silica and hydrated alumina. In a contribution on slag titrations (6, p. 326) it was pointed out that because of the variant silicon dioxide/aluminum oxide ratio in slags, *such titrations do not attain the accuracy of the standard method of analysis closer than within 1 to 2 per cent.*

Since the official method (1) prescribed for limestone was found inap-

plicable to slags, the simplified procedure of titration to *p*H 5.2 using Bromcresol green was proposed as tentative method for the evaluation of blast furnace slags (7, p. 297). Schollenberger (4) has proposed that the alkalimetric procedure be modified to include special provisions for the oxidation of the sulfur present as sulfides in slag. His procedure requires a more complicated apparatus and a more involved manipulative technique. However, it lays claims to desired improvements, the principal ones being the elimination of interference effects from the silica-alumina precipitate at the titration point of *p*H 8.0, and the automatic correction for sulfide by oxidation by means of hydrogen peroxide prior to the titration.

The analyses presented in Table 6 were for eleven slags by means of six procedures used in previous studies, with corrections for either sulfide or total sulfur content. The criterion of accuracy is the concordance between values given in each column with those reported in Column II. The Bromcresol green ("B.C.G.") results of Column IV show an average deviation of 0.9 per cent, 7 of the values being within 1 per cent and 4 being within 2 per cent. In this laboratory equally good results were obtained through the use of Schollenberger's 2-stage titration method *without sulfide oxidation*. The results in Column VI were obtained by Schollenberger through the use of his own procedure, *with sulfide oxidation*. Those findings register no improvement in accuracy, and show deviations greater than those of any of the other methods under study. Moreover, five of the eleven analyses by Schollenberger show calcium carbonate-equivalence differences of from 2.6 per cent to 4.6 per cent between duplicates, whereas, in the use of the direct titration to *p*H 5.2, such differences were within 0.5 per cent. Hence, the results by the proposed procedure, with inclusion of the step of sulfide oxidation, fall short of the precision obtainable by the simple titrative procedure to *p*H 5.2 by "B.C.G.," where an allowance of a mean value of 3.5 per cent calcium carbonate-equivalence for sulfide sulfur has been made. The titrations to *p*H 5.2 by "B.C.G.," as in Column VII of Table 6, show a mean deviation of only 1.0 per cent. In ten of the eleven findings the deviation is between 1 and 2 per cent, which is within the tolerance inherent to a titrative procedure on a sample from large bulk, as was indicated in a related contribution (6).

PRACTICAL CONSIDERATION AS TO CHOICE OF CHEMICAL PROCEDURE FOR SLAGS

For a product to be classified as a liming material, it is reasonable that a minimal neutralization value be prescribed. However, it is not likely that the salability of a classified liming material would be affected materially by a 2 per cent variation in the determination of neutralization value, so long as the indicated value is well beyond the required minimum.

TABLE 6.—*Neutralization values for blast furnace slags, in terms of $CaCO_3$, as determined by various procedures, with corrections for sulfide sulfur content in the titrative procedures*

SLAG[a]	DETERMINED $CaCO_3$= Ca+Mg MINUS S		TITRIMETRIC DETERMINATIONS									
			III INDIRECT		DIRECT				VI AT *p*H 8.2, WITH "AUTOMATIC" CORRECTION		VII AT *p*H 5.2, AFTER A −3.5%[a] CORRECTION FOR MEAN SULFIDE=	
	I PRODUCER'S	II OURS			IV AT *p*H 5.2		V AT *p*H 8.2					
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A	89.2	+1.1*	88.1	88.3	+0.2*	88.9	+0.8*	89.4	+1.3*	87.5	−0.6*	89.3	+1.2*
B	83.7	+0.3	83.4	83.3	−0.1	83.2	−0.2	82.8	−0.6	83.1	−0.3	84.1	+0.7
C	86.6	+0.3	86.3	86.7	+0.4	87.4	+1.1	87.3	+1.0	87.8	+1.5	86.6	+0.3
D	79.9	+0.2	79.7	79.9	+0.2	80.3	+0.6	80.0	+0.3	80.6	+0.9	80.0	+0.3
E	93.6	−0.7	94.3	95.0	+0.7	94.5	+0.2	94.4	+0.1	94.1	−0.2	93.4	−1.1
F	86.9	+1.1	85.8	86.0	+0.2	87.7	+1.9	87.1	+1.3	83.7	−2.1	87.2	+1.4
G	85.0	+0.9	84.1	84.4	+0.3	86.1	+2.0	85.5	+1.7	84.6	+0.5	85.4	+1.3
H	80.0	−0.6	80.6	80.5	−0.1	80.5	−0.1	80.7	+0.1	81.2	+0.6	79.4	−1.2
I	77.1	(+3.7)	73.4	73.3	−0.1	74.3	+0.9	74.2	+0.8	75.1	+1.7	73.5	+0.1
J	81.5	+0.2	81.3	79.4	−1.9	80.9	−0.4	80.7	−0.6	81.5	+0.2	79.8	−1.5
K	84.1	+0.8	83.3	83.7	+0.4	84.5	+1.2	83.6	+0.3	85.7	+2.4	84.7	+1.4
Average deviation, regardless of sign		0.6			0.4		0.9		0.7		1.0		1.0
Deviation Limits				Number of Deviations									
0 −0.5		4			9		4		4		4		3
0.6–1.0		4			1		3		4		3		1
1.1–2.0		2			1		4		3		2		7
2.1–3.0											2		

[a] Supplied by Standard Slag Company, 1945 samples.

I. Analysis of Ca+Mg by Standard Slag Company laboratories, minus our sulfide determination.

II. Our Ca+Mg analysis after dissolution in 0.5 *N* HCl.

III. Joint titration of Ca+Mg, after elimination of SiO_2 and Al_2O_3 (6, p. 330).

IV. According to procedure, *This Journal*, **30**, p. 297, Method II, with bromcresol green.

V. Our analyses according to Schollenberger's 2-stage titration procedure, but without addition of H_2O_2, *This Journal*, **30**, p. 298, Method III, with phenolphthalein.

VI. Analyses by Schollenberger according to his latest procedure, with his automatic correction obtained through the oxidation of sulfide before the titration, *This Journal*, **30**, 117–124.

* The deviations were from comparisons between the results obtained by each method with those given in Column II.

In case the titration of a liming material should register a value considerably below that minimum, the liming material would be classified as substandard. This would serve to segregate the few slags that might register border-line values within 1 to 2 per cent of the standard requirement, and therefore require a more exact analytical technique to assure correct value and proper classification. Exactness within 2 per cent of the true value of the slag is not attained by the Schollenberger titration procedure or any other titrative method yet tested. That value is obtained only upon careful determination of the contents of calcium, magnesium, and sulfur.

SUMMARY

The evolution and determination of the sulfide sulfur content of slags were investigated to ascertain whether correction should be applied to the titrations used to measure the neutralization value of slags.

Cadmium sulfide was prepared and used to establish precision and accuracy of the titration of the acidity engendered in a cadmium sulfate absorbent.

Upon additions of hydrochloric acid of either 0.5 *N*, or (1+4) concentration, evolution of the sulfide from slags was incomplete in presence of air or in an atmosphere of carbon dioxide. Complete evolution was obtained from the cadmium sulfide and from the slags, however, when the slag plus zinc dust was treated with (1+4) hydrochloric acid. The findings prompted the proposal of a simple method for the evolution and titration of the sulfide sulfur content of calcium silicate slags.

The several proposed procedures for the determination of the neutralization value of blast furnace slags were compared upon basis of precision and accuracy, with special attention given to the merits of the sulfide oxidation as a step in the titrative procedure. The automatic sulfide correction failed to produce the expected improvement in the accuracy possessed by the direct titration procedure to *p*H 5.2, and in the precision attainable by the direct procedure.

It is concluded that the simple titration procedure to *p*H 5.2 by Bromcresol green should be adhered to as an equitable and practical means for the determination of the proximate neutralization capacity of a blast furnace slag.

A correction of 3.5 per cent calcium carbonate-equivalence is suggested as a reasonable deduction for sulfides. A procedure for sulfide determination in slags is presented, however, for occasions where more exact correction for sulfide is required.

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MILK FAT IN MILK CHOCOLATE*

By L. W. FERRIS (Food and Drug Administration, Federal Security Agency, Buffalo, N. Y.)

The problem of the accurate determination of the amount of milk fat in milk chocolate has been before the Association of Official Agricultural Chemists since the early days of enforcement of the Food and Drug Act. At that time two procedures for removing the fat from milk chocolate were under consideration. One was to shake the sample with ether, or petroleum ether, until all fat that could be dissolved was extracted; and the other was to extract the sample for 18 hours in a continuous extracting apparatus with anhydrous ether, interrupting the process at some stage to grind the sample, thus making the fat more accessible to the ether. In commenting on these methods Associate Referee W. L. Dubois (1) in his report to the Association in 1910 noted that lower results were obtained by a straight ether extraction and assumed that merely shaking the sample with ether did not dissolve all the milk fat, since he had found results on a milk powder alone extremely low. He reported analyses of four samples of milk chocolate, the milk fat being estimated from the Reichert Meissl number of the extracted fat. Total milk solids calculated were compared with the amount stated by the manufacturer to be present, but no report was made of the per cent of milk fat actually present.

In 1926, collaborative work (2) was done on one sample of milk chocolate of known composition. The fat for the determination of Reichert Meissl number was obtained by shaking the sample with two portions of petroleum ether. Results of the collaborators show a variation equal to about 36 per cent of the amount of milk fat present.

In 1935, Hillig (3) presented a "unified method" using hydrochloric acid and petroleum ether for the determination of fat in foods. Two determinations were necessary to obtain sufficient fat for one determination of Reichert Meissl number.

In 1945, M. Offutt (4) reported total fat on one sample of milk chocolate by the Hillig method and by a "modified Roese-Gottlieb method," using hydrochloric acid, alcohol, and a mixture of petroleum and ethyl

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 11–13, 1948.

ethers. Offutt recommended that further work be done on these two methods.

W. O. Winkler (5) has suggested two procedures for the complete removal of fat from milk chocolate. One makes use of alcohol and petroleum ether, and the other of ammonia, alcohol, ether, and petroleum ether with massive quantities of solid ammonium chloride and magnesium nitrate to prevent unbreakable emulsions. No analytical results were reported.

It is generally agreed that the Reichert Meissl number, being uniformly high on milk fat and low on other fats, offers the best available criterion for estimating the amount of milk fat in a mixed fat. Efforts in the past have been directed to devising a procedure that would remove all the fat from milk chocolate in as pure a state as possible and, at the same time, yield at least 5 grams of fat: the amount necessary for a determination of the Reichert Meissl number. The procedure of the Association of Official Agricultural Chemists for the separation from milk chocolate of fat to be used for the Reichert Meissl number has always called for a straight ether extraction. The only changes made from the first edition to the present sixth edition have been in the amount of sample to be used, and the substitution of ethyl ether for petroleum ether.

The purpose of this paper is to present evidence that a straight ether extraction on milk chocolate may leave unextracted an appreciable amount of milk fat, and to offer a method for the complete removal of fat from milk chocolate. A single determination of total fat yields a quantity sufficient for duplicate determinations of the Reichert Meissl number.

METHOD

Place 40 g of milk chocolate in a centrifuge bottle, add 100 ml of ether and shake vigorously until the chocolate is well broken up. Centrifuge, and decant or syphon off the ether layer. Make two more extractions with 100 ml of ether each, combine the extracts, and evaporate most of the ether. Add to the residue in the centrifuge bottle 25 ml of 1.4 dioxane and heat for 20 min. in a water bath at approximately 90°C, with frequent stirring. Cool, add 100 ml of ether, stopper, shake well, centrifuge, and decant. Make two more extractions with 50 ml ether, combine the extracts, evaporate the ether, and remove the dioxane completely with a current of air on the steam bath. Dissolve the fat in ether, filter into the flask containing the preliminary ether extract, evaporate ether, dry at 100° and weigh. If any insoluble material appears in the dry fat, filter in a hot oven before weighing out the 5-g sample for the Reichert Meissl number.

RESULTS

In a preliminary experiment, 99 per cent of the total fat present in a sample of milk powder was removed from 6 grams by the dioxane ether treatment. By making a double dioxane and ether treatment, the same completeness of fat extraction was secured on an 18-gram sample, giving sufficient fat for the Reichert Meissl determination. The Reichert Meissl number found was 28.4.

That the fat remaining after a straight ether extraction of chocolate

may be mostly milk fat, is shown by the following results on a commercial sample of "ivory coating," made with whole milk powder, cocoa butter, and flavor:

TABLE 1.—*Results*

METHOD	FAT PERCENTAGE	REICHERT MEISSL NUMBER OF FAT
Straight ether extraction	39.1	5.0
Dioxane-ether on residue	1.0	26.1

The Reichert Meissl number on a sample of chocolate liquor containing no milk fat was 0.1 by the straight ether extraction of fat, and 0.3 on that obtained by the proposed method.

Table 2 gives results on two commercial milk chocolates, five milk chocolate coatings and one "ivory milk coating." The table permits a comparison between the A.O.A.C. method and the proposed method, both as to total fat and milk fat calculated from the Reichert Meissl number.

TABLE 2.—*Comparison of 2 methods (per cent)*

SAMPLE NO.	PRODUCT	MILK FAT*	TOTAL FAT		MILK FAT†		INCREASE BY PROP. METHOD	
			A.O.A.C. METHOD	PROPOSED METHOD	A.O.A.C. METHOD	PROPOSED METHOD	TOTAL FAT	MILK FAT
1	Milk Chocolate	—	32.6	33.9	4.9	6.0	1.3	1.1
2	" "	—	33.4	33.6	5.3	5.4	0.2	0.1
3	Milk Chocolate Coating	—	33.4	33.5	3.3	3.4	.2	.1
4	" " "	—	32.1	32.4	2.8	3.1	.3	.3
5	" " "	—	30.5	30.8	3.2	3.6	.3	.4
6	" " "	3.8	37.2	37.6	3.9	4.1	.4	.2
7	" " "	4.0	32.7	33.2	3.9	4.2	.5	.3
8	"Ivory Milk" Coating	7.2	38.9	39.8	6.5	7.2	.9	.7

* Calculated from manufacturer's formula.
† Calculated from Reichert Meissl number of fat by formula (6).

CONCLUSION

It is shown that a straight ether extraction of milk chocolate as in the A.O.A.C. method leaves unextracted a small amount of fat, which is principally milk fat. A method is presented which is rapid, easy in manipulation, and effects a more complete extraction of the fat. In one instance slightly over one per cent more of milk fat was obtained by the proposed method.

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DETERMINATION OF WATER INSOLUBLE FATTY ACIDS IN DRIED EGGS*

By FRED HILLIG (Food Division,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

The development of acids in eggs has been recognized as accompanying certain types of decomposition. Dried eggs prepared from liquid eggs which had become sour before drying were found to contain volatile and lactic acids.[1] Acidity of the ether extract has also been applied to dried eggs, and directions for its determination have been included in the methods of the A.O.A.C.[2] The acids determined by the latter method include all of the non-volatile fatty acids, as well as the major part of any lactic acid that may be present. However, the method does not measure those acids which are present in eggs as an ether-insoluble compound, such as inorganic salts. A method for the determination of water insoluble fatty acids (hereinafter referred to as WIA) has already been proposed for cream and butter.[3] This method has now been applied to dried eggs. The procedure follows:

DETERMINATION OF WIA IN DRIED EGGS

Weigh 2 g of the prepared sample into a 100 ml beaker and make into a uniform paste with a small quantity of water, using a heavy stirring rod. Transfer the material to a 250 ml centrifuge bottle with water, using a total of 25 ml for the entire operation, and shake vigorously. Rinse the beaker with 20 ml of alcohol, transfer the rinsings to the centrifuge bottle, shake vigorously, and add 50 ml of ether.

Proceed as directed in the method for cream and butter.[3]

RECOVERY EXPERIMENTS

The quantities of oleic and palmitic acids, in alcoholic solutions, shown in Table 1, were transferred to centrifuge bottles containing 2 g of authentic, sound, dried eggs, reconstituted with water, and the WIA were determined by the above method. Table 1 shows the very satisfactory recoveries obtained.

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 11–13, 1948.
† W. B. White, Chief.
[1] Lepper, H. A., Bartram, M. T., and Hillig, F., "Detection of Decomposition in Liquid, Frozen, and Dried Eggs," *This Journal*, **27**, 204 (1944).
[2] *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945). Sec. **23.29–23.32.**
[3] Hillig, F., "Determination of Water-Insoluble Fatty Acids in Cream and Butter," *This Journal*. **30**, 575 (1947).

TABLE 1.—*Recovery of oleic and palmitic acids added to 2 g of dried eggs*

SAMPLE NO.	WIA ADDED			WIA FOUND		RECOVERED
	OLEIC	PALMITIC	TOTAL	TOTAL	NET*	
	mg	*mg*	*mg*	*mg*	*mg*	*per cent*
1	282	256	538	533.8	525.4	97.7
				540.4	532.0	98.9
2	141	256	397	388.0	379.6	95.6
				397.4	389.0	98.0
3	282	128	410	403.2	394.8	96.3
				396.4	388.0	94.6
4	70.5	64.0	134.5	142.1	133.7	99.4
				142.8	134.4	99.9
5	70.5	32.0	102.5	112.2	103.8	101.3
				112.6	104.2	101.7
6	35.3	64.0	99.3	108.0	99.6	100.3
				109.1	100.7	101.4
7	28.2	25.6	53.8	59.9	51.5	95.7
				58.1	50.7	94.2
8	14.1	25.6	39.7	47.9	38.5	96.9
				47.7	39.3	99.0
9	28.2	12.8	41.0	48.2	39.8	97.1
				49.9	41.5	101.2
10	56.4	25.6	82.0	91.5	83.1	101.3
				91.5	83.1	101.3

* The blank on each sample was 8.4 mg (average of 4 analyses).

The method was also applied to a number of the dried egg samples which were the subject of the previous study.[1]

The data given in Table 2 show that dried edible eggs contain small quantities of WIA. No material increase in WIA was observed when edible liquid eggs were held for one day under refrigeration, or for short periods at high temperature before drying; or when they were frozen, and then thawed before drying. Overheating in drying did not appear to increase the WIA. There was also no decided increase when lower grade current receipt eggs were held for 18 hours at 85°F.; but, when addled eggs and mixed rots were added to different batches of the same type of eggs and held for from 18 to 23 hours, there was a decided increase in the WIA, one sample inoculated with mixed rots having an acid content of almost 11 per cent. That limited amounts of certain types of decomposed eggs may

TABLE 2.—*WIA in authentic dried† eggs*

SAMPLE NO.	GROUP 1—EDIBLE EGGS	ODOR LIQUID EGGS	WIA
			mg/100 g
A-1	Better grade current receipts	none	415
A-2	Better grade current receipts	none	420
A-3	Better grade current receipts held 22 hours at 35°F.	none	380
B-1	Lower grade current receipts	none	540
B-2	Lower grade current receipts	none	915
B-3	Lower grade current receipts held 20 hours at 35°F.	none	530
B-4	Lower grade current receipts held 6 hours at 85°F.	"off"	443
RF-1	Current receipts frozen and thawed for 24 hrs. at 70°F.	none	193
RH-2	Current receipts frozen and thawed for 48 hrs. at 70°F.	none	478
RH	Current receipts overheated by drying at 510°F.	none	230
	GROUP 2—EGGS HELD AT 85°F.		
B-5	Lower grade current receipts held 18 hours	sour	728
RO-2	Lower grade current receipts with 2.5% added odorless addled eggs held 22 hours	sour	2,270
RO-3	Lower grade current receipts with 5.0% added odorless addled eggs held 18 hours	sour	4,483
RR-2	Lower grade current receipts with 2.5% mixed rots held 23 hours	sour	5,065
RR-3	Lower grade current receipts inoculated with mixed rots	sour	10,780
	GROUP 3—CONTAINING ADDED INEDIBLE EGGS		
RO-1	Current receipts with 5% odorless addled eggs	none	2,420
RR-1	Current receipts with 5% mixed rots	none	950
RN	Current receipts with 10% moldy	moldy	855
RB	Current receipts with 0.3% black rots	putrid	298
RU	Current receipts with 2% musty	musty	343
BL	Weak yolk and blood ring eggs	none	540

† The description of the preparation of these samples has previously been given.[1]

be present in dried eggs without being detected by the method, is shown by the data obtained on Group 3 eggs.

WIA IN COMMERCIAL DRIED EGGS

Data obtained on eggs dried on a commercial scale and known to be of good quality are given in Table 3.

The WIA content is within the range found for the authentic edible eggs in Table 1.

SUMMARY

An adaptation of the method for the determination of water-insoluble fatty acids in cream and butter is proposed for the determination of these

TABLE 3.—*WIA in commercial dried eggs of edible quality*

SAMPLE NO.	WIA	SAMPLE NO.	WIA
	mg/100 g		*mg/100 g*
1	325	12	205
2	350	13	280
3	550	14	290
4	540	15	120
5	485	16	185
6	225	17	120
7	265	18	155
8	255	19	330
9	230	20	195
10	205	21	55
11	590		

acids in dried eggs. It has been shown that small amounts of WIA are found in authentic dried edible eggs, but that when liquid eggs are allowed to become sour before drying, the WIA in the dried product are increased several fold. Commercial dried eggs of acceptable quality were found to contain WIA in the range found for authentic dried edible eggs.

SUCCINIC ACID IN DECOMPOSED EGG PRODUCTS

By HENRY A. LEPPER and FRED HILLIG (Division of Food,* Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

During the course of the studies by Claborn and Patterson[1] which resulted in the methods for the determination and identification of lactic and succinic acids, it was observed that dried eggs, prepared from raw material known to be decomposed, contained significant amounts of succinic acid. It is known that succinic acid results from certain types of microbial decomposition in foods,[2] coming either from carbohydrate breakdown, or from the decomposition of aspartic and glutamic acids, themselves the products of protein hydrolysis. It thus seemed in order to test this new and very specific method for succinic acid as an index of the various types of decomposition known to occur in eggs, before or after breaking out. In all of the experiments described below, the Claborn and Patterson method for succinic acid was followed.

Shell eggs of edible, marketable quality were purchased on three different occasions. A dozen eggs were broken out, well mixed, and immediately analyzed for succinic acid. In no case was any detected. These samples are

* W. B. White, Chief.
[1] *This Journal*, 31, 134 (1948).
[2] Porter, J. R., *Bact. Chem. & Physiol.*, 1946, pp. 810–813, 815, 862 and 864.

identified as 1, 2, and 3, respectively in Table 1. Sample No. 3 was so chosen that each egg had a substantial amount of dirt from the nest adhering to its shell.

The liquid egg mix from sample No. 3 was permitted to stand at room temperature ((approximately 85°F.). When observed 22 hours later, it

TABLE 1.—*Succinic acid in liquid and dried eggs*

SAMPLE NO.	DESCRIPTION	SUCCINIC ACID
		mg per 100 g[*]
1	Shell eggs, edible marketable quality	none
2	Shell eggs, edible marketable quality	none
3	Shell eggs, edible marketable quality (dirty shells)	none
4	Sample 3 held 22 hours at 85°F.—sour	9.8[1]
5	Sample 3 held 46 hours at 85°F.—sour	19.0[1]
6	Shell eggs—mixed rots	3.3[1]
7	Shell eggs—white rots	13.2[1]
8	Shell eggs—black rots	24.4[1]
9	Shell eggs—addled, no odor	none
A-1	Dried eggs from better grade current receipts	none
A-2	Dried eggs from better grade current receipts	none
B-4	Dried eggs from lower grade current receipts, held 6 hours at 85°F.—not sour	none
B-5	Dried eggs from lower grade current receipts, held 18 hours at 85°F.—sour	56[2]
RO-2	Dried eggs from lower grade current receipts, plus 2.5% addled odorless eggs, held 22 hours—sour	38[2]
RO-3	Dried eggs from lower grade current receipts, plus 5% addled odorless eggs, held 18 hours—sour	44[2]
RR-2	Dried eggs from lower grade current receipts, plus 2.5% mixed rots, held 23 hours—sour	130[2]
RR-3	Dried eggs from lower grade current receipts, inoculated with mixed rots—sour	118[3]

* The analytical results reported in this table were obtained by H. V. Claborn, formerly of the Food and Drug Administration.
[1] The identity of succinic acid was confirmed by the crystalline form of its barium salt.
[2] The isolated succinic acid was identified by conversion to its p-toluidide.
[3] The isolated succinic acid had the same m.p. as authentic acid.

was found to be in a state of active decomposition, being covered with foam and having a pronounced sour odor. The decomposition must have started some time before. Analysis of a sample from the mix showed that substantial amounts of succinic acid had developed at this stage. The remainder of the mix was permitted to stand 24 hours longer at room temperature at which time the foaming and sourness had definitely increased. This advancing decomposition was reflected in an increased succinic acid

TABLE 2.—*Market shell eggs*

SAMPLE NO.	PURCHASED IN	GRADE	NO. BAD	SHELL COLOR*	LOCALITY OF PRODUCTION	REMARKS
75-166-7 H	San Francisco, Calif.	AA	0	W	Petaluma, Calif.	
75-176 H	San Francisco, Calif.	AA	0	W	Petaluma, Calif.	
75-175 H	San Francisco, Calif.	A	16	W	Petaluma, Calif.	7 days old when collected; 14 days in transit.
72-104 H	Los Angeles, Calif.	AA	0	W		Also examd. frozen.
72-180 H	Los Angeles, Calif.	A	9	W		7 days old & 7 days in transit.
78-661 H	Seattle, Wash.	AA	0	B	Woodinville, Wash.	Day-old farm stock.
78-662 H	Seattle, Wash.	AA	0	W	N. W. Wash.	Store stock
86-365 H	Denver, Colo.	AA	0	W	nearby Denver	
86-367 H	Denver, Colo.	A	0	W	15 mi. west Denver	
86-368 H	Denver, Colo.	A	0	W	40 mi. east Denver	
65-297 H	Philadelphia, Pa.	A	0	W	nearby Pa. eggs	
65-298 H	Philadelphia, Pa.	B	3	W & B	Minnesota eggs	10 days old—also examd. frozen.
65-299 H	Philadelphia, Pa.	C	4	W & B	Minnesota eggs	10 days old.
89-664 H	Baltimore, Md.	A	0	W	Harford Co., Md.	Also examd. frozen.
85-374 H	Baltimore, Md.	B	0	W	Harford Co., Md.	
85-375 H	Baltimore, Md.	C	3	W	Harford Co., Md.	Also examd. frozen.
92-866 H	Boston, Mass.	A	0	B	Mass. eggs	Also examd. frozen.
92-802 H	Boston, Mass.	B	0	B	Conn. eggs	Also examd. frozen.
92-803 H	Boston, Mass.	C	0	B	Maine, N. H., & Vt. eggs	
7-815 K	Buffalo, N. Y.	A	0	W	Erie Co., N. Y.	Also examd. frozen—not over 8 days old.
7-816 K	Buffalo, N. Y.	B	0	W	Erie Co., N. Y.	Also examd. frozen.
6-701 K	Buffalo, N. Y.	C	10	W	Erie Co., N. Y.	
9-935 K	New York, N. Y.	A	0	W	South Jersey	
9-936 K	New York, N. Y.	B	1	W	Callicoon Center, N. Y.	
9-938 K	New York, N. Y.	C	1	W	Sullivan Co., N. Y.	
55-498 H	Atlanta, Ga.	A	0	W	Cumming, Ga.	
54-899 H	Atlanta, Ga.	B	1	W	Jackson, Ga.	Also examd. frozen.
54-900 H	Atlanta, Ga.	B	11	W	Ellijay, Ga.	

TABLE 2.—*Continued*

SAMPLE NO.	PURCHASED IN	GRADE	NO. BAD	SHELL COLOR*	LOCALITY OF PRODUCTION	REMARKS
23-215 K	New Orleans, La.	A	1	B	Kansas	
23-068 K	New Orleans, La.	A	2	W	Kansas	
23-072 K	New Orleans, La.	B	7	W	Kansas	
27-816 K	St. Louis, Mo.	A	0	W	St. Louis Co., Mo.	
27-817 K	St. Louis, Mo.	B	0	W	New Haven, Mo.	
27-818 K	St. Louis, Mo.	B	2	W	St. Marys, Mo.	
69-046 H	Chicago, Ill.	A	0	W	Forrest, Ill.	Also examd. frozen.
69-043 H	Chicago, Ill.	B	0	B	Forrest, Ill.	
69-044 H	Chicago, Ill.	C	0	B	Forrest, Ill.	
24-752 K	Minneapolis, Minn.	A	0	W	Forest Lake, Minn.	
24-920 K	Minneapolis, Minn.	B	8	W	Northfield, Minn.	
24-737 K	Minneapolis, Minn.	B	1	W	Cedar Mills, Minn.	
21-380 K	Kansas City, Mo.	A	0	W	within 150 mi.—K. C.	
21-381 K	Kansas City, Mo.	B	4	W	within 150 mi.—K. C.	
21-382 K	Kansas City, Mo.	B	2	W	within 150 mi.—K. C.	
84-283 H	Cincinnati, Ohio	A	0	W	west central Ohio	
84-284 H	Cincinnati, Ohio	B	1	W	west central Ohio	
84-285 H	Cincinnati, Ohio	C	2	W	west central Ohio	

* W = white; B = brown.

content. The results of the two samples of mix are given as Nos. 4 and 5, respectively, in Table 1.

To study the occurrence of succinic acid in eggs which become decomposed in the shell, a supply of rejects were secured from the candling operations of a dealer in shell eggs. The individual eggs were broken and classified into four groups as to condition. The first three groups cover the types of decomposed eggs usually encountered, namely mixed rots, white rots, and black rots. Each of these types contained succinic acid (samples No. 6, 7, and 8, respectively). The fourth group contained eggs which on breaking showed a ruptured yolk more or less addled into the white. However, they had no odor. Such eggs are usually high in bacteria and will develop an offensive odor of decomposition if allowed to remain in the shell. No succinic acid was detected in this group (sample No. 9).

The application of the method to dried eggs was studied on a series of authentic samples manufactured from liquid eggs of known condition. The results are included in Table 1. Two samples, representing a good commercial grade of shell eggs, promptly dried after breaking out, showed no determinable succinic acid (samples No. A-1 and A-2). Another sample (No. B-4), manufactured from the lowest grade of current receipt eggs, which were broken and held six hours at 85°F. before drying, also showed no succinic acid. There was no decomposition evident in this sample, either in the liquid or in the dried state. However, dried egg sample No. B-5, made from the same liquid magma as B-4 after it was allowed to stand an additional 12 hours at 85°F before drying, until obviously sour and unfit for food, showed large amounts of the acid. Sample Nos. RO-2, RO-3, RR-2, and RR-3 were prepared by inoculating liquid eggs from four separate lots of lower grade current receipts of shell eggs as indicated in the tabulation, and drying the magma after it had been held long enough to become sour.

To demonstrate further the absence of detectable amounts of succinic acid in edible marketable shell eggs, samples were purchased on the retail market in sixteen different cities. Food and Drug Administration inspectors in each city were requested to purchase three samples of 2 dozen eggs each, representing respectively the best, the medium, and the lowest grade of eggs available on the local market. Thirty-seven samples consisted of white shell, and seven of brown shell varieties; two samples were white and brown (one dozen each). The eggs were packed to obviate breakage and sent, properly sealed and identified, together with the records of collection, to Washington for analysis. Upon receipt, the eggs from each sample were broken and the contents individually noted and smelled. Eggs of inedible condition or quality were rejected. The edible eggs in each case were then composited, thoroughly mixed, and analyzed for succinic acid. No succinic acid was found in any of the 46 samples.

Without exception the samples containing a substantial proportion of inedible eggs were either of the lower grades or were known to be a week old, with an additional exposure to summer temperature because of delay in transit. It is a fair assumption that the eggs from such samples that were classified as edible were on the verge of becoming inedible, since they were of comparable age and had been exposed to the same conditions as the inedible eggs in the sample. It is thus significant that even such eggs gave no evidence of succinic acid development.

With a number of samples the egg magma, after withdrawal of the sample for analysis, was immediately frozen and held at 0°F. for from two to three months. Ten such samples of frozen eggs were thawed and analyzed, and in no case was succinic acid detected. The grades indicated on the carton in which the eggs were purchased, or on the inspector's collection report, were found to be as stated. The details of the samples discussed above are summarized in Table 2. The grades assigned are those established by the U. S. Department of Agriculture.[3]

SUMMARY AND CONCLUSIONS

Succinic acid was not found in shell eggs of acceptable, edible quality or in frozen or dried eggs prepared from such shell eggs. It is formed during some processes of decomposition of eggs, either in the shell or after separation from the shell. This evidence of decomposition is demonstrable in dried eggs.

Appreciation is extended to M. T. Bartram of the Food and Drug Administration for assistance in identifying and classifying the eggs according to the type of decomposition, and to W. I. Patterson for helpful suggestions during the course of these studies. The cooperation of the Food and Drug Inspectors in the collection of authentic samples is also appreciated.

WATER INSOLUBLE FATTY ACIDS IN CREAM AND BUTTER*

By FRED HILLIG[1] and S. W. AHLMANN[2] (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

INTRODUCTION

There are three principal sources of supply of cream for butter making: (1) whole milk delivered direct to the creamery by the farmer, where it is separated and churned, (2) cream separated on the farm where, in some

[3] U. S. Standards for Quality of Individual Shell Eggs, Federal Register, 13, 1359 (March 16, 1948).

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[1] Food Division, (W. B. White, Chief), Washington, D. C.
[2] Cincinnati Station (K. L. Milstead, Chief).

TABLE 1.—*Acids in cream and butter*

SAMPLE IDENTIFICATION	CREAM				BUTTER				
	INCUBATION PERIOD	ML 0.1 *N* ACID PER 100 G			ML 0.1 *N* ACID PER 100 G				
		ACETIC	BUTYRIC	LACTIC	TOTAL	ACETIC	BUTYRIC	LACTIC	ACIDITY OTHER THAN LACTIC AND VOLATILE
	Days								
A	0	0.1	None	Trace	1.8	0.6	None	None	1.3
	14	26.9	11	153	35.1	4.8	None	29.0	1.3
B	0	0.5	None	None	3.4	None	None	None	3.4
	7	28.5	50	132	38.0	5.0	5.1	14.7	13.2
Sweet Cream Butter					5.0	0.1	None	None	4.9
Commercial, #1					19.0	2.3	0.3	6.9	9.5
Commercial, #2					52.0	3.2	2.1	17.3	29.4

cases, it may be held for several days, or even weeks, unrefrigerated, before it is delivered to the cream gathering station to be held until it is collected by the centralizer, and (3) farm separated cream which may be held at the farm until a sufficient quantity is accumulated for direct delivery to the creamery or centralizer.

Lactic acid producing bacteria are always present in such cream, and the quantity and rate at which lactic acid will be formed depends upon the sanitary conditions under which the cream is produced and upon the time and the temperature at which it is held. Normal lactic acid souring has long been recognized by some sections of the industry as desirable in the manufacture of butter, in fact souring is often induced under controlled conditions through the addition of "starters" containing lactic acid producing bacteria. However, it frequently happens with improperly controlled spontaneous souring that other changes occur which render the cream unsuitable for use in the preparation of a food product for human consumption. This latter type of cream, and the butter made therefrom, presents a problem to the regulatory official. This paper is a report of progress on an investigation of the chemical changes taking place in cream constituents during initial lactic acid souring, and in cream in which additional deteriorating changes have occurred.

EXPERIMENTAL

Raw sweet cream was incubated at 90°F., with daily stirring. A portion of the cream was churned at the beginning and another at the end of the incubation period. Volatile and lactic acids were determined on both the cream and butter samples by methods previously described (1, 2). The butter was also analyzed for total acids as follows: 50 grams of butter were weighed into each of two 250-ml centrifuge bottles, 10 ml of water were added and the mixture was gently warmed to melt the butter. The contents of the bottles were neutralized with *N* sodium hydroxide, using phenolphthalein as indicator. The fat was extracted with 50 ml each of ether and petroleum ether. The residues in the centrifuge bottles were made acid with sulfuric acid and sodium tungstate was added. They were then transferred separately to a lactic acid extractor (2) and extracted for three hours. The extracts from the two 50-g portions of butter were combined, about 50 ml of water were added and the material was titrated with 0.1 *N* sodium hydroxide. This gave the total acidity on 100 grams of butter.

Data on 2 samples of cream and butter churned therefrom and on an authentic sweet cream butter and two samples of butter obtained on the market, are given in Table 1.

The sum of the titers of the volatile and lactic acids subtracted from the titer of the total acids gives the acidity due to unidentified acids. In the case of butter A, there was no increase in the acidity due to these

TABLE 2—*Authentic butter—1944*

SAMPLE NO.	VAT ACIDITY AS LACTIC	CLASSES OF CREAM IN CHURN				WIA ON FAT BASIS	MOLD
		0	1	2	3		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mg/100 g*	*per cent*
1	0.14	100				94	0
2	0.32	92	8			100	0
3	0.85	79	21			184	47
4	0.45	71	29			85	33
5	0.77	71	29			100	15
6	0.72	57	43			174	8
7	0.76	53	47			184	45
8	0.56	49	51			88	61
9	0.62	48	52			114	28
10	0.63	42	58			86	32
11	0.76	41	59			101	58
12	0.76	38	62			133	10
13	0.85	34	66			84	20
14	0.77	30	70			100	3
15	0.80	28	72			169	22
16	0.70	21	79			99	11
17	0.75	20	80			88	5
18	0.55	74	24	2		185	10
19	0.56	53	45	2		100	2
20	0.90	44	54	2		399	66
21	0.95	35	63	2		599	59
22	0.83	20	78	2		531	22
23	0.75	15	83	2		374	46
24	0.84	32	65	3		189	68
25	1.05	25	72	3		273	42
26	0.74	25	72	3		523	43
27	1.04	14	82	4		443	47
28	0.95	13	83	4		379	50
29	0.40	70	25	5		75	17
30	0.84	12	82	6		293	84
31	0.93	25	68	7		359	58
32	0.97	10	82	8		191	22
33	0.71	19	71	10		353	37
34	0.80	9	80	11		428	77
35	1.27	29	53	18		269	53
36	0.63	9	73	18		350	97
37	0.66		90	10		133	7
38	1.00	47	49	3	1	380	43
39	—	10	74	14	2	259	91
40	0.95		74	24	2	338	86
41	1.00	8	77	12	3	248	63
42	0.87		71	26	3	546	34
43	1.05		59	37	4	300	83

unidentified acids over an incubation period of 14 days, although large quantities of lactic and acetic acids were formed in the cream, indicating extensive souring. However, butter B showed a marked increase of unidentified acids. The cream from which it was churned at the seventh day of incubation showed lactic and butyric acids in relatively large quantities, butyric acid being the principal volatile acid produced.

These results indicate a relationship between the occurrence of these unidentified acids in butter and the condition of the cream from which it was churned. A study of this acidic material, using the method developed for isolating and determining water insoluble acids (3) (hereafter

TABLE 3.—*Authentic butter—1945*

SAMPLE NO.	CLASSES OF CREAM IN CHURN				WIA ON FAT BASIS	MOLD
	0	1	2	3		
	per cent	*per cent*	*per cent*	*per cent*	*mg/100 g*	*per cent*
1	100				50	18
2	82	18			145	74
3	66	34			140	64
4	61	39			154	40
5	34	66			104	44
6	76	23	1		61	20
7		96	4		163	76
8	2	88	10		315	64
9	5	73	22		374	82
10		71	27	2	543	82
11		61	30	9	565	96

referred to as "WIA"), showed it to consist chiefly of these acids. The method was then applied to commercial creams and butters.

Cream is judged as to condition principally by taste and odor both by the industry and by State and Federal regulatory agencies. In this investigation the classification of creams proposed by Vandaveer and Wildman (4) was followed in establishing the condition of the creams churned into the various butters studied. The description of the classifications used is as follows:

(0) Cream which is either sweet or good clean sour; sound cream which a discriminating housewife would use for butter making in her own kitchen.

(1) Cream which has an indefinite or indefinable "off" flavor or odor (*e.g.*, due to feeds).

(2) Cream in which a decomposition characteristic, ("cheesiness," rancidity, putridity, etc.), is strong enough to be definitely recognizable.

(3) Cream in which the flavor characteristics of decomposition are markedly stronger than creams classified as (2).

TABLE 4.—*Authentic butter—1946*

SAMPLE NO.	VAT ACIDITY AS LACTIC	CLASSES OF CREAM IN CHURN				WIA ON FAT BASIS	MOLD
		0	1	2	3		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mg/100 g*	*per cent*
1	—	100				56	7
2	0.12	100				76	0
3	—	100				78	4
4	0.17	100				93	0
5	0.15	100				104	0
6	0.32	100				115	4
7	—	100				119	0
8	—	100				135	2
9	0.38	55	45			159	2
10	0.50		100			100	1
11	0.45		100			193	2
12	0.68		98	2		133	21
13	0.81	11	87	2		209	51
14	0.90	4	94	2		200	37
15	0.82	6	92	2		261	53
16	0.87	4	93	3		150	10
17	0.95	13	84	3		273	86
18	0.85		97	3		209	79
19	0.80	2	94	4		203	68
20	0.57		95	5		128	21
21	1.14		94	6		315	76
22	0.85	10	84	6		331	79
23	0.94		93	7		394	76
24	0.45	2	88	10		201	2
25	0.80		89	11		269	17
26	0.81		84	16		371	5
27	0.92		80	20		231	81
28	0.77		76	24		248	21
29	1.15		90	9	1	411	93
30	1.00		83	16	1	350	24
31	0.58		90	8	2	260	15
32	1.50		66	32	2	381	2
33	0.85		72	26	2	426	55
34	1.22		73	25	2	448	92
35	1.38		65	30	5	410	100
36	1.05		67	19	14	425	95

During the summer of 1944, 43 authentic churns of butter were prepared in the Middle West. In each case each individual can of cream entering into the churn was classified and the percentage of each class

TABLE 5.—*Storage experiment*

SAMPLE NO.	0°F.		40°F.	
	MONTHS STORAGE	WIA	MONTHS STORAGE	WIA
		mg/100 g fat		*mg/100 g fat*
19	0	203	0	203
	4	204	4	207
	5	233	5	276
	22	277	22	462
8	0	135	0	135
	3	157	3	162
	5	273	5	449
	22	323	22	4427
29	0	411	0	411
	1	388	1	398
	5	400	5	1282
22	0	331	0	331
	1	354	1	347
	5	359	5	405
36	0	425	0	425
	2	401	2	421
	5	410	5	523
	22	458	22	7205
11	0	193	0	193
	2	230	2	235
	5	204	5	210
	22	258	22	295
5	0	104	0	104
	1	104	1	123
	5	109	5	78

in the churn was computed. Data are presented in Table 2 on vat acidity, percentage of the various classes of cream in each churn, WIA, and mold count (5). The samples are listed in the order of the increasing quantities of classes 1, 2, and 3 creams present.

The data show that butters in which no class 2 nor class 3 cream was present contained the lower quantities of WIA, and that when such decomposed cream was present the quantity of these acids was materially increased.

Portions of 14 of these authentic butters were stored at 40°F. for 75 days. There was no significant change in the WIA content during this storage period.

During the summer of 1945, 11 authentic churns of butter prepared under the same conditions as in 1944 were made in creameries located in Cincinnati, Ohio. Analytical data on these butters are given in Table 3. Again the higher figures for WIA were found in the butters churned from vat cream containing some cream classified as decomposed.

During the summer of 1946, 36 authentic churns of butter were simi-

TABLE 6.—*Analysis of sweet cream butter*

SAMPLE NO.	VAT ACIDITY	WIA	MOLD
	per cent as lactic	*mg/100 g fat*	*per cent*
1	0.12	95	0
2	0.12	89	0
3	0.07	109	0
4	0.10	100	0
5	0.11	112	0
6	0.11	98	0
7	0.13	80	0
8	0.19	78	0
9	0.15	88	0
10	0.17	110	0
11	0.18	104	0
12	0.15	142	0
13	0.15	76	0
14	0.13	84	0
15	0.18	226	0
16	0.11	114	0
17	0.15	122	0
18	0.13	128	0
19	0.17	121	0
20	0.16	131	0
21	0.13	115	0
22	0.14	90	0
23	0.12	101	0

larly prepared in creameries located in the Middle West. Analytical data on these butters are given in Table 4.

Here again the higher values for WIA were found in the butters churned from vat cream containing some cream classified as decomposed.

Portions of 7 of the 1946 authentic butters were stored at 0° and 40°F. for periods up to 22 months. WIA were determined at different storage periods, as shown in Table 5.

With the exception of sample 8, there was no significant change in the

WIA content of any of these butters after 5 and in some cases 22 months of storage at 0°F. There was some increase in this sample between the third and fifth months of storage and a further small increase after 22 months, at which time visible mold appeared. At 40°F large clumps of mold appeared in samples 8 and 29 after 5 months storage, and the WIA increased approximately 3 fold. There was no significant change in the WIA content of the other 4 butters after 5 months of storage at 40°F., but, there was a decided increase after 22 months storage at 40°F.

During the summer of 1947, 23 churns of butter were made from sweet cream on a commercial scale. Analyses of these butters are presented in Table 6.

TABLE 7.—*Distribution of WIA between butter and buttermilk*

CLASS OF CREAM	PRODUCT	TITRATABLE ACIDITY AS LACTIC	WIA
		per cent	*mg/100 g fat*
0	Cream	0.12	176
	Butter		158
	Buttermilk		20*
1	Cream	0.66	241
	Butter		219
	Buttermilk		24*
2	Cream	1.09	683
	Butter		700
	Buttermilk		38*
3	Cream	1.54	762
	Butter		741
	Buttermilk		74*

* Mg per 100 g buttermilk.

An experiment was conducted to show the distribution of WIA between butter and buttermilk. One hundred gallons of sweet unpasteurized milk from a single producer were separated, and titratable acidity and WIA were determined on the cream. About 2½ gallons were churned, and WIA was determined in the butter. The balance of the cream was allowed to stand at room temperature (87 to 95°F.) with stirring each day. When Class 1 stage was reached it was again sampled for analysis and about 2½ gallons were neutralized to an acidity of approximately 0.2 per cent as lactic, pasteurized, and churned. This procedure was repeated when the cream reached the Class 2 and 3 stages. The analytical data are given in Table 7.

Most of the WIA were retained in the butter, very little being found in the buttermilk. The sum of the WIA in the butter and buttermilk is of

TABLE 8.—*WIA in cream*

SAMPLE NO.	ORGANOLEPTIC CLASSIFICATION	ACIDITY AS LACTIC	WIA
		per cent	*mg/100 g "as is"*
1	0	0.14	25
2	0	0.15	46
3	0	0.13	40
4	0	—	40
5	0	0.78	44
6	0	0.69	68
7	2	0.88	584
8	2	1.73	323
9	2	1.18	53
10	2	1.51	121
11	2	1.14	179
12	2	1.37	503
13	2	0.95	148
14	2	0.97	477
15	2	0.59	176
16	2	1.17	254
17	2	1.17	26
18	2	1.63	122
19	2	1.71	377
20	2	0.82	27
21	2	1.37	113
22	2	1.09	94
23	2	0.86	148
24	2	1.15	141
25	2	1.39	122
26	2	0.68	195
27	2	0.95	222
28	2	1.10	837
29	3	1.55	496
30	3	0.68	142
31	3	1.55	55
32	3	—	190
33	3	1.53	161
34	3	1.13	207
35	3	1.66	396
36	3	0.92	275
37	3	0.92	192

the same magnitude as that in the cream from which the butter was made.

During the investigation in the summer of 1946 a number of individual cans of cream were organoleptically classified and samples were analyzed for titratable acidity and WIA. The data are given in Table 8.

The creams classified as 0 contained small quantities of WIA. Thirty-one cans were classified as either 2 or 3. Twenty-seven of these contained materially larger quantities of WIA than were found in the 6 cans of sound cream. Only 4 of them contained WIA in an amount comparable to that found in the 6 cans of 0 class cream.

SUMMARY

Data are presented on the determination of WIA in 90 authentic churns of butter. When decomposed cream was present in the churn more WIA were found in the butter, in most cases, than in the butter churned from cream containing no decomposed material. It was shown that WIA in cream, for the most part, are retained in the butter on churning. Individual cans of cream classified as being decomposed usually contained much larger quantities of WIA than cans classified as satisfactory for butter making. There was in general little or no increase in WIA in butters stored at 0°F. for 5 months. Except in those cases in which visible mold appeared, there was no significant change in WIA in butters stored for 5 months at 40° F.

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VOLATILE ACIDS IN CREAM AND BUTTER*

PART I. THE DEVELOPMENT OF BUTYRIC ACID DURING THE PROGRESSIVE DECOMPOSITION OF CREAM

By FRED HILLIG (Food Division,† Food and Drug Administration Federal Security Agency, Washington 25, D. C.)

PART II. BUTYRIC ACID IN COMMERCIAL CREAMS AND BUTTERS

By FRED HILLIG and DOROTHY MONTGOMERY (Food Division,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

INTRODUCTION

The development of acidity in raw cream as it ages is universally known. The individual volatile acids formed during the progressive chemical changes in cream, inoculated microbially through normal handling, and held under controlled conditions of time and temperature, were investigated. Preliminary experiments indicated that some acetic acid is usually formed along with the lactic acid when cream sours spontaneously. Further, the retrogressive changes following the initial souring often result in the formation of butyric acid, occasionally as much as 0.5 per cent, accompanied by the development of objectionable organoleptic characteristics. The presence of butyric acid, in milk which has fermented, has been previously observed (1). Therefore the significance of butyric acid as an indication of decomposition in cream in controlled experiments and in commercial authentic creams, and the butter made therefrom, was studied.

PART I. THE DEVELOPMENT OF BUTYRIC ACID DURING THE PROGRESSIVE DECOMPOSITION OF CREAM

Raw, unpasteurized creams, with no micro-organisms present other than those introduced in good sanitary practice, were held 2–3 weeks at three temperatures: (*1*) in a 32°C. incubator, (*2*) at room temperature (25–27°C.), and (*3*) at a constant 20°C. temperature. Samples were withdrawn at frequent intervals for analysis. Lactic acid and lactose were determined by A.O.A.C. methods (2) as directed in sections **22.8** and **22.24**, respectively. At the beginning of these investigations, the method (3) then available for the individual determination of volatile acids when present together was used. This method, and a modification (4) used later, consists essentially of distilling the acids at constant volume at a uniform rate. The earlier computations were made by the procedure of Dyer (5). Later the Hillig and Knudsen (4) procedure was used. The

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† W. B. White, Chief.

published directions for the preparation of the sample (6) were adapted to cream and butter in the following manner:

Preparation of butter sample.—Weigh 50 g into each of two centrifuge bottles, and proceed as directed in the method for water-insoluble acids in cream and butter (6), beginning with the words "Add NaOH" and continuing through the second washing with mixed ethers for removal of fat. Then remove residual ether from bottles by evaporation on steam bath, transfer contents of both to a single 200 ml volumetric flask with H_2O, add 1 ml H_2SO_4 (1+1) and 10 ml of 10% sodium tungstate soln. Make to mark, shake and filter. Transfer 150 ml of filtrate to distillation flask.

Preparation of cream sample.—Weigh 100 g into a 250 ml volumetric flask, add 100 ml of water and 2 ml of sulfuric acid (1+1), and mix, avoiding vigorous shaking. Add 15 ml of 10% sodium tungstate soln, make to mark, shake and filter through a rapid flowing filter paper. Transfer 150 ml of filtrate to distillation flask.

The changes in the constituents of the creams at the three different incubation temperatures are illustrated by Figures 1, 2, and 3, respectively. As would be expected, the lactose decreased throughout the experiments, since micro-organisms in milk tend to attack carbohydrates first. Especially at the higher temperatures, the disappearance was more pronounced near the beginning, at which time lactic acid was formed more rapidly.

With some creams, the rate of formation of lactic acid was later retarded, the amount in creams 1 and 2 (Fig. 1) even decreasing for a few days. This retardation was accompanied in these creams by a sudden, marked increase in volatile acids. These concurrent changes which occurred at an earlier period at the higher temperatures suggest that the volatile acids may have been formed at the expense of the lactic acid.

Butyric acid, as determined from the distillation constant (percentage of total volatile acid in the first 100 ml of distillate), was the principal volatile acid formed during this upsurge. In the earlier stages this acid was not detectable, the chief volatile acid then being acetic.

At the time butyric acid appeared, the creams were in violent fermentation. A disagreeable flavor, such as would lead the housekeeper to regard it as unfit or inedible cream, had developed. In some creams the flavor resembled that of plain sour cream to which butyric acid had been added. In others it resembled that of creams often described by cream graders in the industry as "cheesy." At this stage, the cream had separated into a dry, friable, spongy layer on the surface with a serum layer underneath similar in some respects to a yeasty type of fermentation, except that the latter is usually slimy rather than friable.

Although lactose appeared the logical source of the butyric acid formed as a result of microbial activities in cream, the butterfat is a potential source, since the butyric acid present as a glyceride could be liberated on hydrolysis by lipolytic enzymes. To check this possibility, after neutralization with sodium bicarbonate to about 0.25 per cent acidity as lactic,

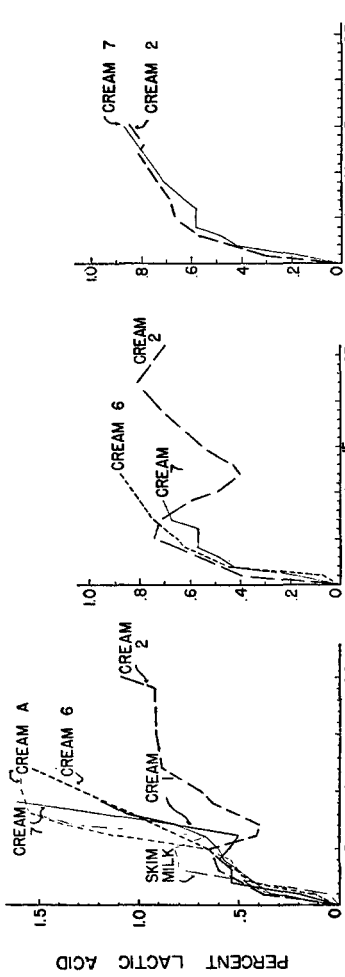

FIG. 1.—Incubated at 32°C.

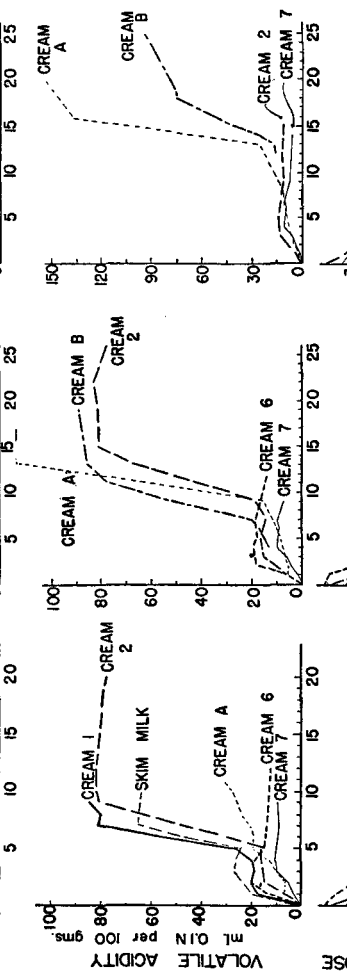

FIG. 2.—Incubated at 25–27°C.

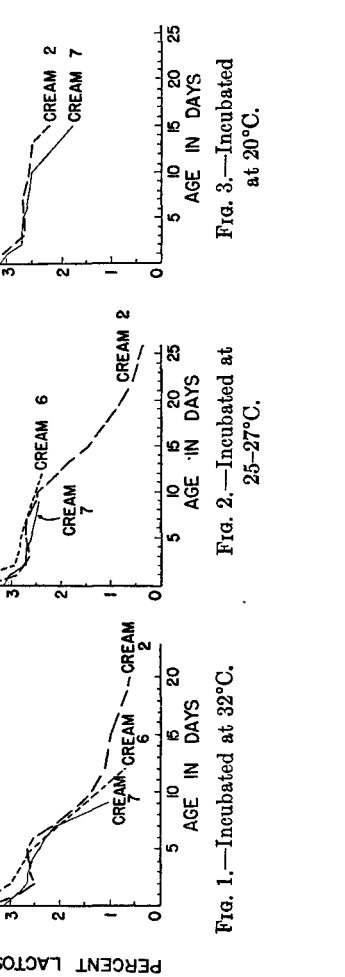

FIG. 3.—Incubated at 20°C.

the creams were churned and the Reichert-Meissl number of the fat from each butter was determined. Further, the distillation constant (percentage of total acids distilled in the first 100 ml of distillate) of the Reichert-Meissl acids was determined by the original method, after transfer of the titrated acids to the distillation flask. Since the total acids in the distilling flask were known, the use of equations was unnecessary.

TABLE 1.—*Reichert-Meisel numbers of butter*

AGE OF CREAM IN DAYS	CREAM 1		CREAM 2		CREAM 5		CREAM 7	
	R–M NO.	DIST. CONST.	R–M NO.	DIST. CONST.	R–M NO.	DIST. CONST.	R–M NO.	DIST. CONST.
Fresh	31.7	82.2	31.2	81.9	30.1	77.8	29.5	81.4
3	31.1	82.5						
5	31.4	81.7	31.1	80.9				
6							30.0	80.5
8	31.6	81.7						
10							29.3	81.0
12					30.0	77.8		
13	31.1	81.5						
14	30.7	80.6						

The results in Table 1 show that both the Reichert-Meissl number and the distillation constant of the Reichert-Meissl acids remained unchanged throughout the progressive decomposition of cream. The butyric acid glycerides in butterfat are significant contributors to the Reichert-Meissl number, and if an appreciable portion of them had been hydrolyzed the number would have been noticeably changed.

In order to test the possibility that the free butyric acid in the cream might have dissolved in the fat in just the right amount to maintain a constant Reichert-Meissl number, some of the butter was neutralized with sodium hydroxide, extracted 3 times with warm water, the residue dried with sodium sulfate, the fat extracted with petroleum ether and dried in a boiling water bath under vacuum. Treated in this manner it was found that there was no change in the Reichert-Meissl values, or in the distillation constant of the Reichert-Meissl acids, of the butter churned from the creams at various stages of incubation.

Further evidence on the source of the butyric acid was obtained on whole and skim milks. Typical results are illustrated by the skim milk curves in Figure 1. The 0.57 per cent of butyric acid found after six days' incubation at 32°C., as determined by the modified distillation method, (4) obviously could not have come from the milk fat. The skim milk contained 0.1 per cent of fat and, as butterfat contains about 3 per cent of combined butyric acid, the maximum theoretical amount from this

source would have been only .003 per cent. Whole milks developed similar concentrations of butyric acid, whereas the total of this acid from the fat of a 4 per cent milk could be only 0.12 per cent.

By postulating that lactose on fermentation will produce 4 molecules of lactic acid, that 1 molecule of butyric acid will be formed from 2 molecules of lactic acid through a series of intermediate reactions, and that 1 molecule of acetic acid will be formed from one molecule of lactic acid, one may test more directly the hypothesis that the volatile acids are formed primarily from lactose. Acetic and butyric acids (calculated from the data used in preparing Fig. 1) have been converted into their assumed equivalent in lactic acid, and these amounts added to the lactic acid remaining as such in the cream. This sum has then been compared with the actual quantity of lactose lost. In Table 2 the results obtained in Cream 2 incubated at 20°.C have been so treated. Similar results were obtained on the same cream incubated at 25–27°C. and at 32°C.

TABLE 2.—*Comparison of loss in lactose and gain in lactic acid*
Cream incubated at 20°C.

AGE OF CREAM DAYS	GAIN IN LACTIC ACID	GAIN IN LACTIC ACID COMPUTED FROM ACETIC ACID	GAIN IN LACTIC ACID COMPUTED FROM BUTYRIC ACID	TOTAL GAIN IN LACTIC ACID	LOSS OF LACTOSE
1	0.308	0.042	—	0.350	0.35
3	0.563	0.118	—	0.681	0.78
5	0.667	0.124	—	0.791	0.77
7	0.685	0.109	—	0.794	0.77
9	0.737	0.102	—	0.839	0.87
12	0.804	0.072	0.074	0.950	0.94
13	0.779	0.051	0.095	0.925	0.93
14	0.817	0.041	0.112	0.970	1.10
15	0.840	0.033	0.131	1.004	1.19

The loss in lactose in all cases closely approximated the "total gain in lactic acid." Thus all the available evidence indicates that the butyric acid found in these creams was produced from lactose through lactic acid as an intermediate step.

To further verify the presence of butyric acid, a relatively large amount of total volatile acids distilled from Cream 2 of Figure 1 were redistilled, 100 ml distillates collected, and each titrated, following the method previously used to determine the highest volatile acid in a mixed distillate (7). From the data, Curve 1 of Figure 4 was drawn. The first 100 ml of titrated distillate obtained in preparing Curve 1 were acidified, transferred to the distilling flask and, various portions of distillate were collected to

prepare Curve 2. The same procedure was followed in preparing Curve 3. Another curve was prepared, but since it coincided with Curve 3, it is not shown. Curve 3 falls below the line given by propionic acid similarly distilled and approaches, but never crosses, the line given by butyric acid.

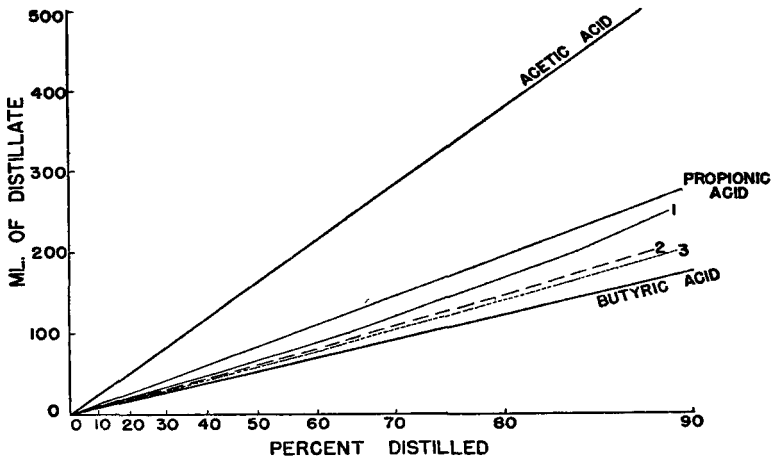

FIG. 4—Fractionation of volatile fatty acids from cream.

It may thus be concluded that butyric acid was the highest detectable volatile acid present in this sample.

PART II. BUTYRIC ACID IN COMMERCIAL CREAMS AND BUTTERS

In the studies described in Part I, it was observed that butyric acid when present in cream, carries over into the hand-churned butter. In order to test this observation on butters more closely approximating the commercial article, a series of small churnings were made, using an electrically driven, roller type churn of 12 gal. capacity. In each case about 4 gallons of cream, all from one producer, were neutralized, pasteurized, and churned, simulating as near as possible the regular commercial process in the details of churning, washing, working, etc. The creams were classified according to the proposed system of Vanderveer and Wildman (8).*

Butyric and propionic acids were determined in these butters by a chromatographic procedure (9). The results are given in Table 3, including grade of cream in the churn and the water-insoluble acids (WIA). A small and variable, but substantial, portion of the butyric acid, when present, usually remained in the butter. Propionic acid, however, was retained in

* See also *This Journal* (p. 743).

only two of the butters made from creams containing it. This acid presumably was lost in the buttermilk and wash water.

No determinable quantity of butyric acid was found in creams suitable for buttermaking. In the creams classed as 2 and 3, both butyric and propionic acids were found—in some cases in rather large quantities.

TABLE 3.—*Distribution of butyric and propionic acids between cream and butter*

SAMPLE NO.	CREAM				BUTTER		
	ORGANOLEPTIC CLASS	ACIDITY AS LACTIC	BUTYRIC ACID	PROPIONIC ACID	WIA	BUTYRIC ACID	PROPIONIC ACID
		per cent	*mg/100 g fat*	*mg/100 g fat*	*mg/100 g fat*	*mg/100 g fat*	*mg/100 g fat*
1	0	0.12	0	0	100	0	0
2	1	0.71	0	0	159	0	0
3	1	0.72	0	0	137	0	0
4	1	1.36	0	0	130	0	0
5	2	1.01	31	5	329	0	0
6	2	1.52	23	3	909	6	0
7	2	0.55	38	4	1362	4	0
8	2	0.88	40	6	1099	4	0
9	2	1.09	171	17	6573	40	0
10	2	1.43	53	6	1517	2	0
11	3	1.44	145	4	694	9	4
12	3	2.12	27	3	1435	0	0
13	3	2.46	176	15	918	4	3

In the companion study on WIA in creams and butters in 1946 (*This Journal*, p. 744), butyric acid was later determined in subdivisions from 37 cans of the cream. The creams were classified and sampled at the creamery, and represented either direct deliveries to the creamery by the producer, or cream picked up by a route driver and delivered to the creamery. The results are given in Table 4, and for convenience, the organoleptic classifications and WIA are repeated. Organoleptically these creams were typical examples of Classes 0, 2, and 3 as encountered in the industry. Class 2 creams had a flavor that is referred to in the industry as "slightly cheesy," or "cheesy." The flavor of the Class 3 creams was either "cheesy" or putrid. Such creams are considered decomposed.

The authentic commercial butters from the WIA studies (*This Journal*, p. 739) of 1945 and 1946, were also analyzed for butyric acid. The results are given in Table 5 with the classification of the creams in each churn. The results for WIA and mold are again included for convenient comparison.

From Table 5 it is apparent that butter churned from vat cream containing some Class 2, or Class 2 and 3 cream, frequently contains butyric acid and usually high WIA or mold count as well.

TABLE 4.—*Analysis of individual cans of cream—Summer, 1946*

SAMPLE NO.	ORGANOLEPTIC CLASSIFICATION	TITRATABLE ACIDITY AS LACTIC	WIA	VOLATILE ACIDS	
				BUTYRIC	PROPIONIC
		per cent	*mg/100 g*	*mg/100 g*	*mg/100 g*
1	0	0.14	25	0.0	0.0
2	0	0.15	46	0.0	0.0
3	0	0.13	40	0.0	0.0
4	0	—	40	0.0	0.0
5	0	0.78	44	0.0	0.0
6	0	0.69	68	0.0	0.0
7	2	0.88	584	9.6	0.0
8	2	1.73	323	27.4	0.0
9	2	1.18	53	3.4	0.0
10	2	1.51	121	26.6	9.3
11	2	1.14	179	50.2	18.3
12	2	1.37	503	16.5	0.0
13	2	0.95	148	63.9	0.0
14	2	0.97	477	10.7	0.0
15	2	0.59	176	16.5	0.0
16	2	1.17	254	0.0	0.0
17	2	1.17	26	0.0	0.0
18	2	1.63	122	48.2	0.0
19	2	1.71	377	56.2	11.3
20	2	0.82	27	0.0	0.0
21	2	1.37	113	12.2	0.0
22	2	1.09	94	6.7	0.0
23	2	0.86	148	6.7	0.0
24	2	1.15	141	53.2	7.0
25	2	1.39	122	36.2	3.3
26	2	0.68	199	49.4	0.0
27	2	0.95	222	8.4	0.0
28	2	1.10	837	28.7	2.1
29	3	1.55	496	160.7	62.0
30	3	0.68	142	94.9	0.0
31	3	1.55	55	3.8	5.2
32	3	—	190	20.7	0.0
33	3	1.53	161	303.6	0.0
34	3	1.13	207	122.8	46.5
35	3	1.66	396	37.1	0.0
36	3	0.92	275	122.4	46.3
37	3	0.92	192	112.5	6.5

TABLE 5.—*Authentic butters*

SAMPLE NO.	VAT ACIDITY AS LACTIC	CLASSES OF CREAM IN CHURN				W.I.A.	MOLD	BUTYRIC ACID
		0	1	2	3			
1945	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mg/100 g fat*	*per cent*	*mg/100g fat**
1		100				50	18	0
2		82	18			145	74	0
3		66	34			140	64	0
4		61	39			154	40	0
5		34	66			104	44	0
6		76	23	1		61	20	0
7			96	4		163	76	0
8		2	88	10		315	64	4
9		5	73	22		374	82	3
10			71	27	2	543	82	6
11			61	30	9	565	96	5
1946								
1	0.12	100				76	0	0
2	0.17	100				93	0	0
3	0.15	100				104	0	0
4	0.38	55	45			159	2	0
5	0.50		100			100	1	0
6	0.68		98	2		133	21	0
7	0.81	11	87	2		209	51	0
8	0.90	4	94	2		200	37	3
9	0.82	6	92	2		261	53	2
10	0.87	4	93	3		150	10	3
11	0.95	13	84	3		273	86	7
12	0.85		97	3		209	79	0
13	0.80	2	94	4		203	68	5
14	0.57		95	5		128	21	0
15	1.14		94	6		315	76	5
16	0.85	10	84	6		331	79	3
17	0.94		93	7		394	76	4
18	0.45	2	88	10		201	2	0
19	0.80		89	11		269	17	0
20	0.81		84	16		371	5	5
21	0.92		80	20		231	81	2
22	0.77		76	24		248	21	0
23	1.15		90	9	1	411	93	3
24	1.00		83	16	1	350	24	2
25	0.58		90	8	2	260	15	3
26	1.50		66	32	2	381	2	0
27	1.22		72	25	2	448	92	7
28	1.05		67	19	14	425	95	2

* The revised distillation method (4) was used on the 1945 samples and the chromatographic method (9) on the 1946 samples.

A test of the application of the butyric acid procedure to commercial butters was made on 23 samples purchased on the open market. The results are given in Table 6, along with results for WIA and mold. Butyric was absent in many of those samples containing the lower amounts of WIA.

TABLE 6.—*Analysis of market butter*

SAMPLE NO.	WIA	BUTYRIC ACID	MOLD
	mg/100 g fat	*mg/100 g fat*	
1	1050	28	64
2	501	23	58
3	733	11	24
4	464	11	22
5	599	13	26
6	969	19	44
7	238	3	88
8	243	3	82
9	264	3	50
10	293	3	50
11	320	3	52
12	251	none	40
13	198	4	87
14	600	16	44
15	964	20	42
16	343	8	18
17	450	9	22
18	99	none	0
19	106	none	0
20	174	none	4
21	181	none	2
22	541	15	22
23	593	15	28

In addition butyric acid was determined on 23 samples of authentic commercial sweet cream butters and no determinable quantities were found in any instance.

SUMMARY

Progressive decomposition experiments on cream show that butyric acid is frequently produced in cream when it reaches that stage of deterioration where it is unfit for human consumption. Results indicate that the butyric acid probably results from the breakdown of the lactose, through lactic acid as an intermediate step, and not from the hydrolysis of the fat and accompanying liberation of the combined butyric acid in

the glycerides. When the acid is present in cream, some is usually carried over into the butter made therefrom.

The proportion so carried over is not important. So long as the amount is within the limit of detection by the method, its mere presence in the finished product is an indication that some decomposed cream was present in the vat mixture from which the butter was churned. No determinable quantity of butyric acid is present in butter churned from sound cream, nor was it found in commercial sweet cream butter.

Grateful appreciation is extended to S. W. Ahlmann, of the Cincinnati Station of the Food and Drug Administration, for his work in classifying all of the commercial creams used in this investigation. Also to those who made the mold counts, especially, W. J. McCarthy of Cincinnati Station, by whom the majority of counts were made. Thanks are also due to W. I. Patterson and H. A. Lepper for their suggestions and assistance during the development of this study.

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DETERMINATION OF 2-AMINOPYRIDINE IN ORANGES

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

The chemical preservative 2-aminopyridine has been proposed as a fungicide for the control of stem-end rot in oranges. The suggested treatment was to immerse the fruit in a solution of the preservative, allow it to remain for a short time, and then rinse it in water.

Because of the toxic nature of 2-aminopyridine, and because it was

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suspected of penetrating into the flesh of the orange (a suspicion that was later confirmed), methods were investigated for its isolation and determination in treated oranges. Ether was selected as the most suitable solvent for isolating this preservative, which is characterized by its extreme solubility in many solvents. A dilute solution of 2-aminopyridine shaken with an equal volume of ether yields only 37 per cent of the chemical to the ether in the first shake-out; but four shake-outs with ether, or a continuous liquid extraction, proved to give satisfactory recoveries of the free base. However, this is true only of the free base; the acid compound is only slightly ether-soluble.

Although most primary aromatic amines can be determined in small

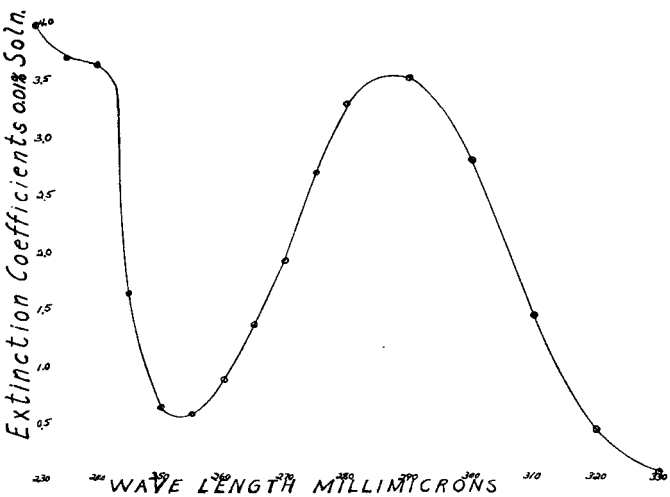

FIG. 1.—Ultra violet absorption spectrum of 2-aminopyridine.

amounts by diazotization followed by a coupling reaction to yield an azo dye adapted to colorimetric measurement, 2-aminopyridine does not respond suitably to this series of reactions; and thus other means were sought for its determination. Methods considered for determining this preservative, after extraction with a selective solvent, were:

(1) Direct titration using a suitable indicator and a standard buffer solution for comparison.

(2) Nitrogen determination of the extracted material. Both of these methods have the obvious disadvantage of non-specificity. However, in studies on orange juice, both appeared to give satisfactory results.

(3) In addition to the above two quantitative methods, the ultra-violet absorption spectrum of 2-aminopyridine, which shows a rather character-

istic maximum and minimum (Fig. 1), furnishes a qualitative confirmatory test capable of detecting many potential interferences.

(1) The reaction between 2-aminopyridine and hydrochloric acid is one of simple addition in equimolecular quantities but, like other reactions of weak bases with strong acids, it requires a rather low *p*H for completion. Since methyl red is usually employed for ammonia determinations, it was the first indicator tested. The results with this indicator were fairly consistent, but were always lower than the theoretical. Recoveries in water solution varied from 96 to 98 per cent of theoretical, and those in orange juice using 4 shake-outs with ether were from 91.5 to 93.4 per cent. Since the color change of methyl orange occurs at a considerably lower *p*H (3–4), titrations were made using this indicator. However, a few determinations showed that the results were too high. Therefore, bromcresol green was selected as an indicator having a *p*H range between that of methyl red and methyl orange.

By use of a series of buffer solutions differing by 0.2 *p*H unit, it was found that the color corresponding to *p*H 4.4 was the optimum end point for satisfactory results, when allowance was made for the water blank. Sample titrations were made to this *p*H by comparison with the standard buffer.

(2) The determination of 2-aminopyridine from its nitrogen content is very satisfactory, and if the result is confirmed by the titration procedure, there is a strong presumption that the isolated material is 2-aminopyridine. Since the ammonia produced gives just double the titration of the original 2-aminopyridine, it is apparent that we are dealing with a nitrogen compound which contains one basic nitrogen atom and one which is not basic. This fact limits considerably the number of possible interfering compounds.

Several semimicro methods of determining the nitrogen were tried to find the most satisfactory one. These were (a) digestion with sulfuric acid, sodium sulfate, and 30 per cent perhydrol (H_2O_2) (1); (b) digestion with sulfuric acid (containing sodium sulfate) and perchloric acid; and (c) a modified Kjeldahl-Gunning-Arnold method using sulfuric acid, sodium sulfate and mercuric oxide catalyst (2). After the first trial or two, the first two procedures were abandoned in favor of the last one (c).

(3) If additional evidence is needed to confirm the identity of the material isolated, the extinction curve in the ultraviolet region of the spectrum obtained on the solution of the compound will supply such evidence. The ultraviolet extinction curve of many chemicals is quite specific and characteristic, and this appears to be the case with 2-aminopyridine (Fig. 1). The position of the maximum and minimum, and the values of the extinction coefficients at these points on the curve, are effective in establishing with finality whether the material analyzed is 2-aminopyridine.

DETERMINATION OF 2-AMINOPYRIDINE IN ORANGES

APPARATUS

Siphon.—Insert two bent glass tubes in a two-hole cork or stopper, one (the mouthpiece) terminating just below the stopper and the other (the siphon tube) long enough to reach the bottom of a centrifuge bottle when the cork with tubes is inserted in the mouth of the bottle. Attach another glass tube to the outside arm of the siphon tube by means of a flexible rubber tube. (The assembly is used to siphon the lower layer from a centrifuge bottle, and the rate of flow is controlled by squeezing the rubber connection.) Prepare a cap for the inner arm of the siphon by boring a hole of the same diameter as the tube part way through a small cork.

PREPARATION OF SAMPLE

Bisect 8–12 oranges and juice with a reamer. Strain the juice to remove pulp and seeds and mix in a "blendor" for about 30 seconds. Transfer 150 ml (measured in graduate) of the mixed juice to a 250 ml centrifuge bottle. Add 50 ml of ether to the bottle, stopper, and shake well for 1–2 min. Place in centrifuge and whirl at about 1800 r.p.m. for 10 minutes. Cap the inner arm of siphon tube; insert it into the bottle, and lower it through the ether layer into the aqueous layer. Then with a glass rod push off the cap and remove the rod. Push the stopper into the mouth of the bottle and lower the end of the siphon to the bottom of the bottle. Start the flow of liquid by blowing, and filter the emerging liquid through a pledget of cotton in a short-stemmed funnel, into a 250 ml centrifuge bottle. Allow as much as possible of the lower layer to siphon over without molesting the ether layer, adjusting the flow, and finally stopping it, by squeezing the rubber connection in the siphon. Discard the ether layer.

To the siphoned liquid, add calcined powdered MgO at the rate of about 1 g per 100 ml. Stir with a glass rod and test at intervals with universal indicator paper.[1] When the liquid reaches a *p*H of about 7.5–8 (indicator paper same color as given by standard buffer soln at *p*H 7.6), add ca a heaping teaspoonful of filtercel, stopper, shake to disperse the filtercel, and immediately centrifuge for 10 minutes. Decant off the supernatant liquid into a 500 ml suction flask, stopper flask, attach to vacuum and evacuate slowly for several minutes to remove most of ether. (Momentarily let in air when the liquid threatens to froth out of the flask.) Proceed with the extraction of the 2-aminopyridine by method A or B.

REAGENTS

(1) *Washed ether.*—Shake 500–600 ml portion of ethyl ether with two 225 ml portions of water in a separatory funnel. Draw off water each time and filter ether through paper.

(2) *Standard 0.02 N hydrochloric acid.*

(3) *Standard 0.02 N sodium hydroxide.*

(4) *Bromcresol green indicator—0.1% soln.*—Rub 0.1 g of the solid with 7 ml 0.02 *N* NaOH in a mortar and make to 100 ml with water.

(5) *Magnesium oxide.*—Fine powder.

(6) *Buffer mixture pH 4.4.*—Prepare M/5 potassium acid phthalate and place 50 ml in 200 ml vol. flask, add 7.5 ml of 0.2 *N* NaOH, and dilute to 200 ml with distilled water.

(7) *2-aminopyridine.*—Recrystallize 2-aminopyridine of Eastman quality from a strong soln in CCl_4, cool, dilute with cold CCl_4, and filter with suction. Wash with cold CCl_4 and allow solvent to evaporate the residue. Dry the residue in vacuum desiccator over H_2SO_4.

[1] Hydrion papers A and B were very satisfactory.

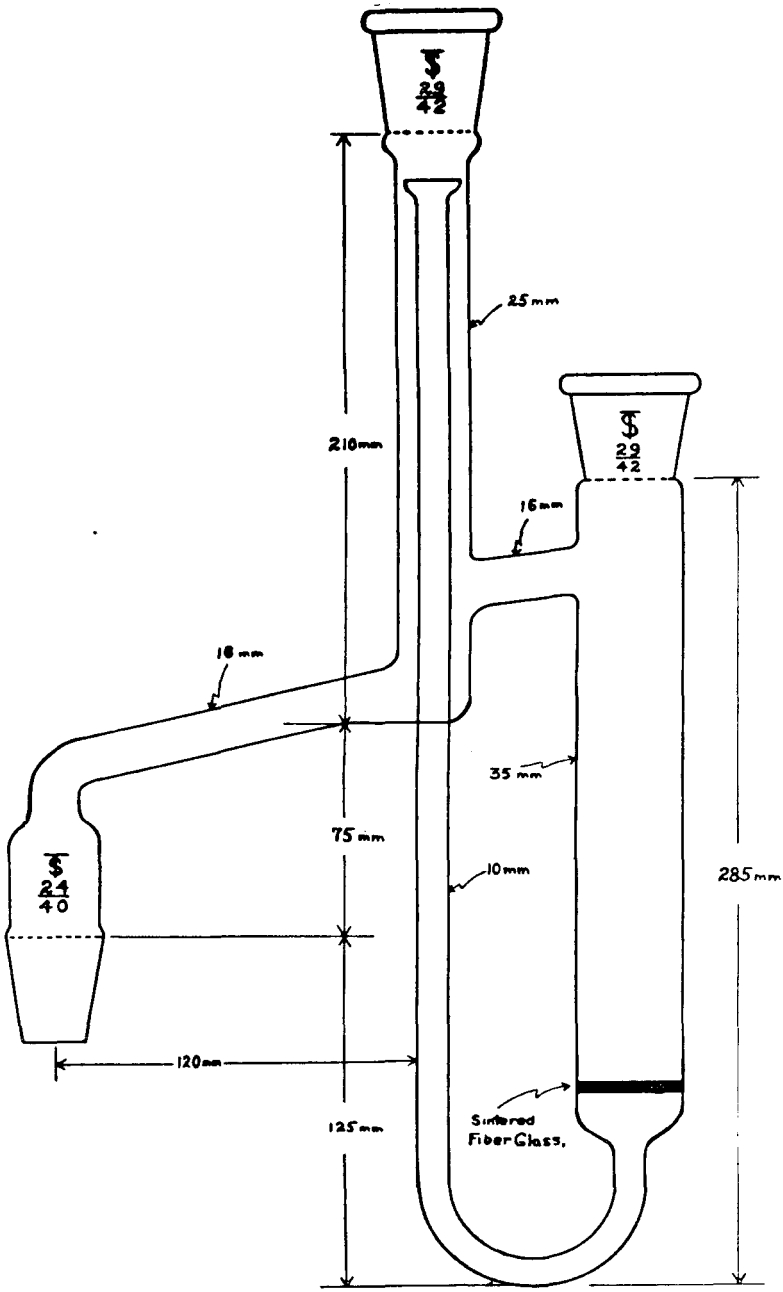

FIG. 2.—Continuous Extractor. Designed by John R. Matchett and Joseph Levine (Bureau of Narcotics, U. S. Treasury Department).

EXTRACTION METHOD A

Pipet 50 ml of the clear liquid into a 250 ml separatory funnel. Extract the liquid 4 times with 100 ml portions of washed ether, alternating between two funnels. Draw off all of the aqueous layer into the alternate funnel (remove liquid in stem by jolting), then pour off the ether extract through the mouth of the funnel, and filter it through a pledget of cotton (moistened with ether) into a 500 ml separatory funnel. After the first and second extractions, rinse the separatory funnel with 5 ml of ether and add the rinsings to the ether extracts.

EXTRACTION METHOD B

Place 100 ml of the clear liquid in a Palkin-type continuous extractor with sintered glass bubble plate,[2] or in the Matchett and Levine type extractor (Fig. 2). The sintered glass plate in either extractor should be of coarse porosity suitable for dispersing gases in liquids (diameter of openings about 150–190 microns). In order to avoid difficulty, the sample and ether should be added in the following manner: If the Palkin-type (sintered glass disc attached to the inner tube) is used, place the sample in the outer tube and fill the tube nearly to the overflow with washed ether. Fill the inner tube with ether by drawing it through the bottom in such a manner that there are no air pockets. Close the top so the tube remains full, and then lower it into the outer tube. (An alternate procedure is to lower it part way and pour ether in the top while it is being lowered to the bottom of the outer tube.)

If the Matchett and Levine type extractor (Fig. 2) is used, first fill the bottom of the U with ether until the level is above the sintered glass plate; then add the sample to the large arm containing the sintered glass plate while simultaneously pouring ether into smaller arm of the U to such a height that the aqueous layer does not pass below the sintered glass plate. After addition of the sample and solvent, close the opening of the large tube.

Extract for 100–120 minutes on an electric hot plate using a 250-ml Erlenmeyer flask containing 125 ml of ether as a receiver. Filter the extract through a pledget of cotton into a 500 ml separatory funnel. Rinse the extraction flask with two 25 ml portions of ether by stoppering and shaking each time, and filter into the ether extract. Finally, pour the contents of the extraction tube into another separatory funnel, draw off the aqueous layer, and filter the ether layer into the ether extract. Proceed with the determination.

DETERMINATION BY TITRATION

To the combined ether extracts in the separatory funnel, add about 40 ml of distilled water and 5–10 ml of 0.02 *N* HCl accurately measured (10 ml of 0.02 *N* HCl is equivalent to 18.82 mg of 2-aminopyridine). Shake well for about 1 minute, allow the layers to separate, swirl the funnel to loosen droplets on the sides, and draw off the lower aqueous layer into a 200 ml Erlenmeyer flask. Give the funnel a whirl, allow to settle, and draw off the aqueous liquid again. Add a second portion of 40–45 ml of distilled water to the funnel and shake well again. Draw off the aqueous layer and combine with the first aqueous extract in the flask. Again give the funnel a whirl, let stand, and draw off any additional aqueous liquid. If the qualitative spectrophotometric test is to be made, make the acid aqueous extracts to a volume of 110 ml and take a 100 ml aliquot for the quantitative determination by titration or nitrogen content. Reserve the 10 ml portion for the spectrophotometric test. Add 3 drops of bromcresol green indicator to the acid extracts, or aliquot, and titrate with standard NaOH (0.02 *N*) until the indicator turns blue. The end point, *p*H 4.4, has now been slightly passed. Bring the soln to *p*H 4.4 with 0.02 *N* HCl by titrating to

[2] The type used was similar to No. 1502 in Scientific Laboratory Glassware Catalog of Eck and Krebs, and that in General Catalog 40, Ace Glass, Inc. No. 6840.

the same color as a similar volume (about 110 ml) of the *p*H 4.4 buffer soln containing 3 drops of the indicator. Titrate 10 ml of the 0.02 *N* HCl (diluted to ca 100 ml volume with water) to *p*H 4.4 with the standard alkali, using the same indicator to obtain the acid equivalent of the alkali at *p*H 4.4. (The titrated soln may be reserved for a nitrogen determination.) Obtain the acid consumed by the sample by subtracting the acid equivalent of the alkali from the total acid used. From the volume of acid consumed, subtract a reagent blank of 0.08 ml for extraction method A, or 0.15 ml for extraction method B, to obtain the acid necessary to react with the 2-aminopyridine present.

Multiply the correct titer (in ml 0.02 acid) by the factor 1.98 to obtain mg of 2-aminopyridine in 50 ml of sample if extraction method A was used.[3] Multiply the corrected titer by the factor 1.882 to obtain mg 2-aminopyridine in 100 ml of sample if extraction method B was used. The values obtained must be multiplied by 1.1 if an aliquot of 100 ml from a total volume of 110 ml was used.

DETERMINATION BY NITROGEN CONTENT

Shake out the combined ether extracts once with about 40 ml of 0.02 *N* HCl, and twice with 40 ml portions of distilled water. (The titrated soln may also be used for the determination.) Add 4 ml conc. H_2SO_4 to the combined acid extracts, evaporate in a beaker (250–400 ml) to about 25–50 ml, and transfer to a 125 ml Erlenmeyer flask. Add to the soln 1.5 g anhydrous Na_2SO_4 and 0.1 g of HgO. Boil down on the hot plate under a hood until the water is removed and the white SO_3 fumes are produced. After the liquid has fumed for several minutes, insert a small short-stemmed funnel in the mouth of the flask. Continue the digestion for 30 min. after the yellow or brownish soln first produced becomes clear and colorless. Cool, and transfer the digest to a 300 ml Erlenmeyer flask with ca 140 ml of water. Add 15 ml of the 50% NaOH soln without mixing, then 5 ml of $Na_2S_2O_3$ soln, connect to the usual trap and condenser and distil.

Receive the distillate in a 200 ml Erlenmeyer flask containing a measured quantity (10–20 ml) of standard 0.02 *N* HCl and 5 drops of methyl red indicator.[4] Distil until about 100 ml of distillate is collected. Titrate the excess acid in the receiver with standard 0.02 *N* NaOH and determine the acid consumed by the NH_3 distilled. Conduct a blank determination on the reagents and subtract the amount of acid consumed in the blank from the titer of the sample. Each ml of 0.02 *N* HCl consumed is equal to 0.2801 mg of nitrogen, or 0.941 mg of 2-aminopyridine. Divide the amount found by 0.95 if extraction method A was used. If a 10 ml portion of the liquid from 110 total was reserved for the spectrophotometric test, multiply the result by the factor 1.1.

CONFIRMATORY SPECTROPHOTOMETRIC TEST

To confirm that 2-aminopyridine is the material being determined, place a portion of the reserved aliquot in a quartz cell and make readings in a spectrophotometer at intervals of 5 or 10 mμ in the ultraviolet region between 230 and 340 mμ. Use .02 *N* HCl, in a matched cell, as blank. From the concentration value of 2-aminopyridine, obtained either by titration or *N* determination, convert extinction readings at each wave length to corresponding coefficients expressed in terms of a .01% concentration and 1 cm cell ($E_{1cm}^{.01\%}$). Compare this extinction coefficient curve with one prepared with a dilute acid soln of pure 2-aminopyridine read in a similar cell. In the preparation of the standard curve it is best to use a standard soln containing 5 mg per 100 ml (.005%) and convert to standard conditions (.01%,

[3] The factor 1.98 is obtained by dividing 1.882 (the mg of 2-aminopyridine neutralized by 1 ml of 0.02 *N* HCl) by the factor 0.95, since only 95% of the chemical is recovered by the 4 extractions provided in the method.

[4] Methyl red indicator 0.1% soln. Dissolve 0.1 g of solid in 60 ml 95% alcohol and make to 100 ml with water.

above) by multiplying extinction values at each wave length by 2. The standard curve is shown in Fig. 1. It shows a minimum at about 253 mμ and a maximum at about 287 mμ.

RESULTS

Some results on titration of water solutions of 2-aminopyridine are given in Table 1.

TABLE 1.—*Titration of 2-aminopyridine with 0.02 N HCl to pH 4.4 using bromcresol green indicator*

PRESENT	FOUND	RECOVERY
mg	*mg*	*per cent*
25	24.9	99.6
25	25.16	100.6
25	24.99	100.0
25	25.03	100.1
25	24.92	99.7
10	9.95	99.5
10	9.95	99.5

It is apparent that this method is quite satisfactory as a titration procedure. The end point is evidently correct for stoichiometrically equivalent amounts of the acid and base.

Table 2 lists results on known amounts of 2-aminopyridine added to orange juice, with closely controlled *p*H of the extraction solution and titration to *p*H 4.4 using bromcresol green indicator.

TABLE 2.—*Titrametric determination of 2-aminopyridine extracted from orange juice by extraction method A*

SAMPLE	2-AMINO-PYRIDINE ADDED	NO. OF ETHER EX-TRACTIONS	HCl CONSUMED	2-AMINO-PYRIDINE FOUND IN EXTRACTS	RECOVERY IN EXTRACTS
	mg		*ml*	*mg*	*per cent*
1	22.73	4	11.52	21.68	95.4
2	47.62	4	24.01	45.18	94.9
3	47.62	4	23.93	45.04	94.6
4	20	4	10.14	19.08	95.4
5	20	4	10.12	19.04	95.2

It is evident that extraction method A gives consistent recoveries of 95 per cent.

Table 3 lists a few similar determinations made on orange juice using the continuous extractor.

The results show that a complete extraction of the total 2-aminopyridine is readily obtained using the continuous extractors with the fritted

TABLE 3.—*Titrametric determination of 2-aminopyridine extracted from orange juice by extraction method B*

ADDED	FOUND	RECOVERY
mg	*mg*	*per cent*
20.0	20.08	100.4
10.0	10.03	100.1
25	24.12*	96.48*
10	9.96	99.6

* Extraction with lactic acid type extractor (3). (No fritted glass bubbler.)

glass bubblers (Fig. 2). For the time specified, extraction is not quite as complete with the lactic acid type of extractor.

The amounts of 2-aminopyridine (in water solutions) calculated from the nitrogen content found by the three digestion procedures mentioned in the introduction, are given in Table 4.

TABLE 4.—*2-aminopyridine calculated from nitrogen content*

SAMPLE	PROCEDURE*	2-AMINO-PYRIDINE ADDED	NITROGEN FOUND	2-AMINO-PYRIDINE CALCULATED	RECOVERY
		mg	*mg*	*mg*	*per cent*
1	(a)	50	12.62	42.37	84.7
2	(b)	50	4.38	14.71	29.42
3	(c)	100	29.79	99.83	99.83
4	(c)	50	14.91	49.85	99.7
5	(c)	50	14.93	49.9	99.8
6	(c)	10	3.054	10.01	100.1

* (a) Digestion with H_2SO_4 and perhydrol.
(b) Digestion with H_2SO_4 and perchloric acid.
(c) Modified Kjeldahl-Gunning-Arnold method.

It is evident that 2-aminopyridine can be accurately determined from the nitrogen content by the modified Kjeldahl-Gunning-Arnold method. Digestion by the other procedures was not satisfactory.

Recoveries of 2-aminopyridine extracted from samples of orange juice by methods A and B are given in Table 5.

The results obtained by the nitrogen method are very satisfactory, and are probably more specific than by the direct titration method. Identical results by the direct method and by the nitrogen determination confirm the presence of an amino compound containing one nitrogen atom which reacts with mineral acid and one which does not so react. The most common food constituent which contains two atoms of nitrogen in the molecule, and which has a somewhat similar structure, is nicotinic acid amide. However, this substance is only sparingly soluble in ether and furthermore, does not titrate with mineral acid under the conditions specified.

TABLE 5.—*Determination of 2-aminopyridine in orange juice from nitrogen content*

SAMPLE	2-AMINO-PYRIDINE ADDED	EXTRACTION PROCEDURE USED	NITROGEN FOUND	2-AMINO-PYRIDINE FOUND IN EXTRACT	RECOVERY
					CORRECTION APPLIED WHEN EXTRACTION A USED
	mg		*mg*	*mg*	*per cent*
1	10	B	2.96	9.95	99.5
2	20	B	5.91	19.84	99.2
3	25	B*	7.22	24.25*	97.0*
4	20	A	5.64	18.94†	99.6

* A lactic acid type extractor (3) was used for this sample.
† Actual figure was divided by 0.95 since extraction method A was used.

SUMMARY

Two methods are presented for the determination of 2-aminopyridine in oranges. Both methods use ethyl ether to extract the chemical from the prepared sample, adjusted to a required *p*H. In the first method, the chemical is determined in the extract by direct titration to *p*H 4.4 using bromcresol green indicator. The nitrogen content of the extracted matter forms the basis for the second method. Data are presented which show both methods to be reliable and accurate.

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DETERMINATION OF REDUCING SUGARS IN FOOD PRODUCTS

COMPARATIVE STUDY OF COLORIMETRIC METHODS

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INTRODUCTION

Colorimetric methods for the determination of dextrose in blood and urine have been widely used. Their application to food products has been rather limited. The Lewis-Benedict and Sumner methods have been used by several investigators in the analysis of dairy products. Jackson (1) has suggested that the Benedict copper method be used for small amounts of reducing sugars but supports his article by no analytical results.

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TABLE 1.—*Error caused when various amounts of dextrose were compared to A standard of 100 mg*

DEXTROSE	THEOR. READING	ACTUAL READING	DEXTROSE FOUND	ERROR
mg per 100 ml			*mg*	*per cent*
		Sumner Method		
300	6.67	6.80	294.1	1.97
200	10.00	9.96	200.8	0.40
150	13.33	13.36	149.7	0.20
100	20.00	19.98	100.1	0.10
75	26.67	26.82	74.6	0.57
50	40.00	40.38	49.5	1.00
		Kingsbury Method		
300	6.67	6.75	296.3	1.23
200	10.00	10.10	198.0	1.00
150	13.33	13.45	148.7	0.81
100	20.00	20.01	99.9	0.10
75	26.67	26.69	74.9	0.13
50	40.00	40.45	49.4	1.20
		Poe-Edson Method		
300	6.67	6.78	295.0	1.67
200	10.00	10.04	199.2	0.40
150	13.33	13.32	150.2	0.13
100	20.00	20.02	99.9	0.10
75	26.67	26.80	74.6	0.53
50	40.00	40.34	49.6	0.80
		Folin Method		
300	6.67	7.82	255.7	14.8
200	10.00	10.42	119.9	4.1
150	13.33	13.62	146.8	2.13
100	20.00	19.93	100.3	0.30
75	26.67	26.94	74.2	1.07
50	40.00	40.98	48.8	2.40
		Folin-Wu Method		
300	6.67	8.02	249.4	16.87
200	10.00	10.34	193.4	3.30
150	13.33	13.67	146.3	2.47
100	20.00	20.05	99.7	0.30
75	26.67	26.83	74.5	0.67
50	40.00	40.87	48.9	2.20
		Benedict Method		
300	6.67	7.53	265.6	11.47
200	10.00	10.21	195.9	2.05
150	13.33	13.54	147.7	1.53
100	20.00	20.04	99.8	0.20
75	26.67	26.84	74.5	0.67
50	40.00	40.73	49.1	1.80
		Lewis-Benedict Method		
300	6.67	7.30	274.0	8.66
200	10.00	9.74	205.3	2.65
150	13.33	13.58	147.3	1.80
100	20.00	19.92	100.4	0.30
75	26.67	26.89	74.4	0.88
50	40.00	40.54	49.3	1.20

The object of this investigation was to compare the various colorimetric methods as to their availability for use in analyzing syrups and fruit products. The authors (2) have reported the use of sodium 2,4-dinitrophenolate as the basis of a reagent for the determination of reducing sugars. For food products this method gave very satisfactory results when compared with the Munson and Walker method. In addition to the above colorimetric method, the following well known methods were studied: Lewis-Benedict (3–4); Folin-Wu (5); Benedict copper method (6); Folin (7); Benedict copper method, new (8); Folin, new (9); Folin-Wu, new (9); Sumner, new (10); and Kingsbury (11).

A number of investigators have found with certain colorimetric methods that the length of the color column of the unknown and standard should be nearly equal. If this condition is not fulfilled, the reading will not be proportional to the percentage of reducing sugar present. According to Rothberg and Evans (12), this condition is not true for the Folin-Wu method. Folin and Dennis (13) and Bierman and Doan (14) state that the same disadvantage is found with the picric acid method of Lewis and Benedict. Sumner (10), however, reports with his methods that readings of 1 mg. of dextrose in the standard would give proportional readings with 0.5 and 2 mg. in the unknown. Benedict (8) claims the same condition to exist with his method. In order to test each of the methods in this respect, various concentrations of dextrose varying from 0.05 to 0.3 per cent were compared with a 1 per cent standard by using each method. With the methods of Folin, Folin-Wu, Kingsbury, and Benedict, the standard and test solutions were diluted to one-fifth because of the intensity of color in the stronger solutions. The results of these comparisons are given in Table 1. Each result is an average of five readings.

In examining the data in the table, it may be noted that the Sumner, Kingsbury, and Poe-Edson methods gave readings which were directly proportional to the amount of dextrose present. The other methods gave results for the higher and lower amounts which were not proportional. For greater accuracy in all methods, it seems better to have the readings of known and unknowns as nearly equal as possible.

A number of syrups and sugars were analyzed by using several of the original colorimetric methods. The results are reported in Table 2. The results for the Munson and Walker (15) method are given for comparison. The data given in Table 2 indicate that all of the methods give fairly satisfactory results. The merits of each method will be discussed later. All data given in Tables 2 to 6, inclusive, are based on duplicate determinations. The percentages of sugar were calculated as dextrose and no method of clarification was used. In Table 6 the sucrose in all samples was inverted with HCl. All other samples were analyzed before inversion.

A number of the older methods were discarded, and a series of samples were run using the latest modification of each of the methods. These

modifications included the new proposed method of Benedict (8), Folin (9), Folin-Wu (9), and Sumner (10).

The comparisons of these various methods together with the methods of

TABLE 2.—*Analysis of syrups for reducing sugars**

FOOD PRODUCT	METHOD					
	MUNSON-WALKER	POE EDSON	SUMNER	FOLIN	LEWIS-BENEDICT	BENEDICT
Pure maple	6.18	6.25	6.25	6.35	6.23	6.13
Pure maple	6.58	6.65	6.60	6.60	6.62	6.65
Pure maple	2.13	2.20	2.16	2.28	2.20	2.28
Pure maple	1.84	1.86	1.83	1.85	1.80	1.80
Pure maple	4.56	4.58	4.55	4.60	4.62	4.56
Pure maple	1.92	2.12	2.12	2.03	2.10	2.03
Pure maple	2.58	2.68	2.50	2.65	2.66	2.72
Maple cake	6.45	6.50	6.51	6.61	6.58	6.49
Maple cake	5.61	5.68	5.68	5.75	5.68	5.71
Maple cake	6.58	6.62	6.63	6.60	6.53	6.60
Maple cake	5.72	5.66	5.70	5.66	5.61	5.76
Maple cake	0.83	0.95	0.90	1.09	0.93	1.02
Maple and cane	2.09	2.22	2.22	2.17	2.20	2.25
Cane and maple	2.56	2.63	2.60	2.63	2.58	2.54
Cane and maple	2.56	2.63	2.67	2.65	2.61	2.65
Cane and maple	6.13	6.26	6.25	6.19	6.32	6.25
Cane and maple	4.53	4.53	4.58	4.50	4.46	4.49
Cane and maple	4.79	4.86	4.82	4.92	4.80	4.82
Cane and maple	1.61	1.66	1.58	1.60	1.53	1.69
Cane syrup	4.65	4.76	4.71	4.76	4.71	4.66

* Results expressed as percentage of dextrose.

TABLE 3.—*Analysis of fruit juices for reducing sugars**

Fruit Juice	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Apple	9.60	9.66	9.64	9.75	9.71	9.71	9.78	9.71
White grape	13.44	13.61	13.64	13.65	13.59	13.59	13.53	13.60
Pineapple	8.96	9.14	9.09	9.14	9.06	9.01	9.11	9.01
Orange	5.12	5.12	5.07	5.05	5.09	5.11	5.05	5.03
Loganberry†	8.88	9.05	9.09	9.17	9.01	9.02	8.97	8.95
Loganberry†	22.36	22.42	22.32	22.44	22.35	22.35	22.48	22.26
Grapefruit†	30.92	31.06	31.00	31.12	31.04	31.12	30.98	31.07

* Results expressed as percentage of dextrose.
† Concentrated, sweetened juices.

Lewis-Benedict, Kingsbury, and Poe-Edson were then made by using other food products such as fruit juices, jellies and flavoring syrups. The results of these tests appear in Tables 3, 4, and 5. The data given in these tables confirm the previous results listed in Table 2. The sucrose in a

TABLE 4.—*Analysis of jellies and jams for reducing sugars**

Food Product	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Apple jelly	16.61	16.55	16.51	16.45	16.51	16.66	16.69	16.53
Grape jelly	23.92	23.90	23.87	23.80	23.91	23.90	23.97	23.84
Raspberry jelly	17.12	17.01	17.10	17.00	17.18	17.01	17.14	17.18
Currant jelly	48.62	48.76	48.84	48.66	48.74	48.76	48.97	48.91
Plum jam	48.90	48.98	48.96	48.73	48.83	49.98	48.73	48.77
Strawberry jam	48.81	48.88	48.87	48.63	48.71	48.76	48.96	48.81
Pineapple-apricot jam	12.05	12.03	11.95	12.00	12.13	12.03	11.83	12.19
Grapefruit marmalade	26.16	26.27	26.22	26.22	26.19	26.27	26.10	26.09
Peach-pineapple jam	19.87	20.08	20.00	20.07	19.98	19.96	20.03	19.78

* Results expressed as percentage of dextrose.

TABLE 5.—*Analysis of flavoring syrups for reducing sugars**

Flavoring Syrup	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Grape	18.16	18.14	18.14	18.11	18.06	18.22	18.12	18.28
Cherry	48.13	48.25	48.09	48.21	48.37	48.29	48.04	48.22
Peach	29.02	29.06	29.01	28.91	28.82	28.93	28.97	29.07
Plum	35.93	36.13	36.09	36.15	36.27	36.19	36.11	36.07
Lemon	4.84	4.87	4.90	4.85	4.80	4.83	4.94	4.91
Orange	15.12	15.29	15.19	15.09	14.98	15.15	14.93	15.16
Grapefruit	18.16	18.29	18.28	18.21	18.26	18.25	18.12	18.26
Raspberry	14.92	14.97	15.13	15.04	14.81	14.99	15.01	15.04

* Results expressed as percentage of dextrose.

TABLE 6.—*Determination of reducing sugars* after inversion of the sucrose*

Food Product	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Syrup	62.65	62.68	62.58	62.58	62.62	62.58	62.82	62.80
Syrup	70.55	70.72	70.42	70.77	70.77	70.42	70.48	70.67
Syrup	66.62	66.76	66.66	66.88	66.80	66.82	66.60	66.71
Syrup	66.82	66.86	66.76	66.72	66.82	66.90	66.92	66.60
Syrup	68.70	68.82	68.68	68.60	68.76	68.70	68.81	68.78

* Results expressed as percentage of dextrose.

number of food products was completely inverted by treating with hydrochloric acid. After neutralization, the reducing sugars were determined as dextrose by each of the newer methods. The results are listed in Table 6.

In food analysis, frequently it is necessary to clarify the sugar solution before the reducing sugar is determined. This process is especially used

with molasses, jellies, and some fruit juices. The first test with clarifying agents was performed on two samples of a 1 per cent dextrose solution. One sample was treated with different clarifying agents and made up to volume. The second sample was untreated but made up to the same volume. The dextrose was determined by the different methods. The standard was set at 20 and the treated samples were read. The results are given in Table 7. As will be noted from these results, all of the clarifying agents

TABLE 7.—*Effect of clarifying agents on colorimeter reading**

Clarifying Agent	Method						
	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Norite	19.94	20.02	20.08	20.06	19.90	19.94	20.04
Alumina cream	20.04	19.96	20.03	20.07	20.23	19.90	20.07
Dry lead subacetate	20.04	20.04	19.94	20.02	20.05	20.07	20.02
Lead sub-acetate solution	20.06	20.07	20.02	20.06	19.96	20.04	20.07
Mercuric nitrate	Ppt.	Ppt.	17.94	17.82	16.17	16.17	Ppt.

* Readings after clarifying. Colorimeter reading of standard dextrose solution set at 20.00.

TABLE 8.—*Determination of reducing sugars* in maple syrup after different clarifying agents were used*

Clarifying Agent	Method							
	Munson-Walker	Poe-Edson	Sumner	Folin	Folin-Wu	Lewis-Benedict	Benedict	Kingsbury
Norite	2.81	2.88	2.85	2.84	2.89	2.82	2.87	2.83
Fullers Earth	2.79	2.86	2.89	2.80	2.83	2.80	2.87	2.80
Lead sub-acetate	2.76	2.85	2.82	2.86	2.81	2.86	2.82	2.85
Alumina cream	2.68	2.77	2.73	2.76	2.78	2.69	2.75	2.74
None	2.79	2.82	2.80	2.78	2.86	2.78	2.84	2.82

* Results calculated as percentage of dextrose.

except mercuric nitrate showed no effect on the accuracy of the seven different colorimetric methods used. These results confirm those reported by Klemme and Poe (16) on the use of clarifying agents in the determination of sugars in bacterial cultures. A sample of maple sugar syrup was used with several of the clarifying agents. The results with such agents are satisfactory, as is shown in Table 8. The values obtained with alumina cream are somewhat lower with several of the methods.

According to Browne and Zerban (17), Bryan, Horne, and other workers report that reducing sugars, more especially levulose, are carried down or occluded in the basic lead acetate precipitate when such salts as tartrates, malates, phosphates, sulfates, etc. are present.

DISCUSSION

The various colorimetric methods which have been proposed for the estimation of reducing sugars in blood and urine have been used in the

present research for the quantitative estimation of reducing sugars in food products. Apparently, it seems that all of these methods could be used for the quantitative estimation of reducing sugars in food products. On closer study, it will be seen that there are a number of disadvantages to be encountered when some of the methods originally proposed for urine and blood analysis are used.

In the first place, with all the methods, with the exception of those of Sumner, Lewis-Benedict, and Poe-Edson, the dilution factor may introduce an error. In all methods, with the exception of the Sumner and the Poe-Edson methods, it is necessary to have two stock solutions from which the working solution is prepared. The reagents for the two above-mentioned methods are each made up as one solution and both have been known to keep for several months. This condition is a distinct advantage in that if only a few determinations are to be made, the reagent can be kept for some time; whereas with some of the other methods the working solution deteriorates in a very short time.

With the Poe-Edson method, the solution after reduction may stand as long as twenty minutes without fading of the color, whereas with a number of the other methods the reading must be taken at once, because otherwise the fading of the color gives inaccurate results.

The use of most clarifying agents did not interfere with the accuracy of the different colorimetric methods.

CONCLUSIONS

(1) The standard colorimetric methods are satisfactory for the determination of reducing sugars in food products.

(2) The common clarifying agents, except mercuric nitrate, do not interfere with the determination of reducing sugars.

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CRYSTALLINE CHARACTERISTICS OF FOUR CAROTENE ISOMERS

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This paper consists of a partial description of the crystallographic and optical properties of the four carotene isomers whose absorption spectra have been reported by E. M. Bickoff, L. M. White, A. Bevenue, and K. T. Williams.[2] This description of these carotinoids is given at this time because it seems unlikely that any more of the unstable carotene isomers will be available in the near future. It is hoped that this partial description will be found useful by others working on these materials.

The isomers were prepared from a commercial carotene product by chromatographic adsorption methods given in detail by the authors cited above.[2] After separation of alpha-carotene, the beta-carotene was isomerized with iodine and then fractionated. The crystals were grown by addition of methanol to a benzene solution of each isomer in a centrifuge tube and allowing the mixture to stand in a refrigerator. After crystals had grown, the tube was centrifuged and the supernatant liquid was decanted. Portions of the solid suspended in a little of the saturated solution were placed on a microscope slide, covered with a cover glass and then examined both before and after the solvent had evaporated. Attempts to determine refractive indices failed, because the crystals were soluble in the index liquids (methylene iodide solutions) except for a potassium mercuric iodide solution whose index was too low in most cases.

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.
[2] Isolation and Spectrophotometric Characterization of Four Carotene Isomers. E. M. Bickoff, L. M. White, A. Bevenue, and K. T. Williams. *This Journal*, **31**, 633 (1948).

The indices are in the neighborhood of 1.7 for sodium light as noted for each isomer below. Pleochroism is strong in most cases although it is not readily observed on thick crystals and varies somewhat with the orientation. Very thin crystals may be pale yellow, thick crystals will be opaque.

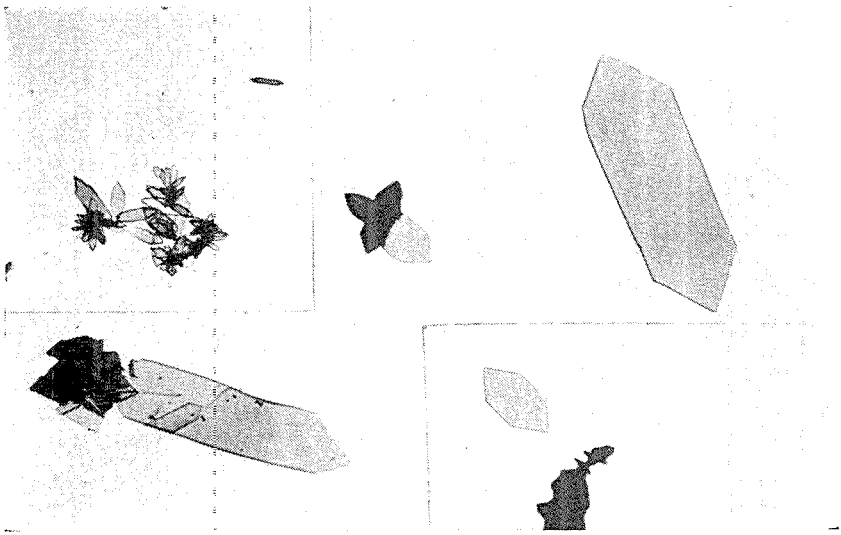

FIG. 1.—All-trans-beta-carotene. 100×.

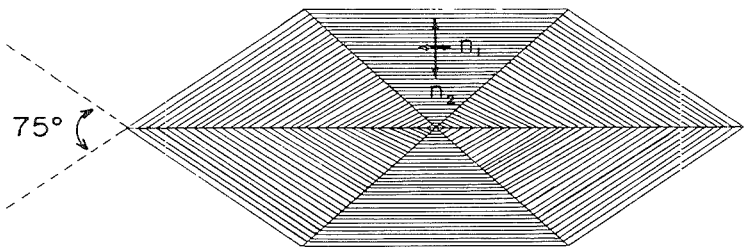

FIG. 2.—All-trans-beta-carotene showing zoning.

Samples of commercial carotene have shown plates of the all-trans-beta carotene type which, if unoxidized, dissolve completely when a drop of benzene is allowed to run under the cover glass during microscopical observation, but if the crystals have suffered partial oxidation an insoluble colorless residue will remain as a "pseudomorph" of the original crystal. The amount of this residue will increase with increase in oxidation. The crystals separating at the edge of the cover glass as the benzene evaporates

are usually platy but may include some patches made up of fine grains in dendritic patterns as the drop dries. Birefringence and pleochroism are strong.

All-trans-beta-carotene.—Photomicrographs of crystals of this isomer are shown in Figure 1. The habit is platy with a tendency to cluster. The crystals are often thin enough to show thin film interference colors. The plates are six sided in outline and from two to four times as long as they are wide. The termination is usually imperfect, often showing a shoulder

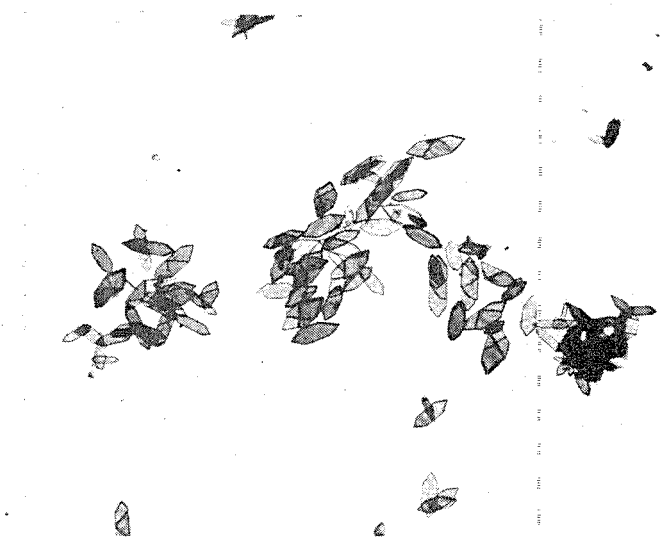

FIG. 3.—All-trans-alpha-carotene. 100×.

at the base of an acute termination, but in some cases the end angle is obtuse. Those crystals which seemed most nearly perfect had a terminal angle of about 75°. Close inspection reveals that these plates are often composed of segments as shown in Figure 2. These segments may be due to twinning. Under polarized light between crossed nicols these segments do not all extinguish together but opposite pairs extinguish together. The pair of segments at the sides of the plate are extinguished when the plane of vibration is parallel to the length of the crystal but a slight rotation is necessary to bring either of the other pairs of segments to extinction. The slow ray (n_1) is lengthwise of the crystal and the pleochroism shows the darkest orange color for lengthwise vibrations, with a lighter yellowish orange for crosswise vibrations (n_2).

The side segments show a higher refractive index than their adjacent segments as judged by the movement of the Becke line at the boundary

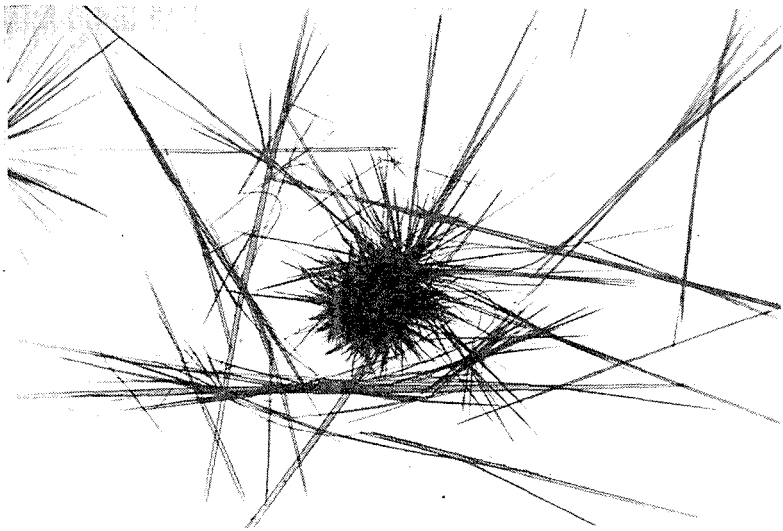

FIG. 4.—Neo-beta-carotene-U. 100×.

FIG. 5.—Neo-beta-carotene-U. 100×. Grown on microscope slide.

within the crystal. The difference is smaller for crosswise than for lengthwise vibrations.

These crystals dissolved in index liquids composed chiefly of methylene iodide. They appeared to be stable in an aqueous solution of potassium

mercuric iodide (Sonstadt's or Thoulet's soln), n = 1.68, but their refractive indices were considerably higher than this value.

All-trans-alpha-carotene.—Crystals of this isomer are shown in Figure 3. These crystals are very similar to those of all-trans-beta-carotene. Thin film interference colors are responsible for the banding seen in many of these crystals. The terminations are more nearly perfect on these crystals as compared to the all-trans-beta, and the zoning, when present appears to be due to the superimposition of several crystals in such a way as to

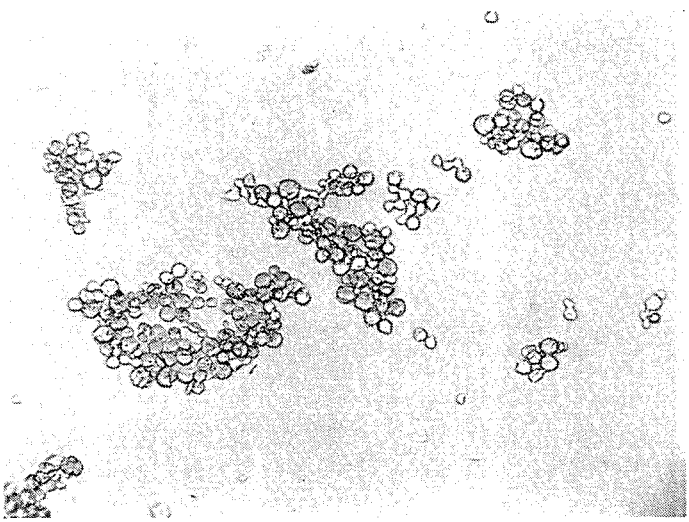

FIG. 6.—Neo-beta-carotene-B. 100×.

give greater thicknesses of some zones than others. The description of unsolvated carotene crystals given by F. G. Kohl[3] appears to be most nearly in agreement with the properties of the alpha and beta isomers reported here.

Neo-beta-carotene-U.—Crystals of this isomer are shown in Figures 4 and 5. These crystals are conspicuously different from the all-trans-alpha or all-trans-beta isomers. They habitually form tapering needles of narrow blades which tend to cluster into loose sheaves and tufts as seen in Figure 5. The crystals in Figure 5 were grown on a slide by addition of methanol to a benzene solution under a cover glass. In polarized light the extinction is parallel. The slow ray is crosswise, corresponding to the strongest absorption which gives a yellowish brown color. For lengthwise vibrations the color is light yellow. Both refractive indices are less than the 1.68

[3] F. G. Kohl, Untersuchungen über das Carotin, Verlag von Gebruder Borntraeger, 1902, Leipzig.

value of the Sonstadt's solution used, contrasting in this property with the all-trans-alpha and all-trans-beta isomers. These characteristics agree with those reported by A. Polgar and L. Zechmeister.[4]

Neo-beta-carotene-B.—Crystals of this isomer are shown in Figure 6. These crystals grow only in spherulitic aggregates whose crystalline nature is revealed under polarized light. The rounded masses are dark red at first but become almost colorless as a result of oxidation after standing in air on the slide for a day or two. Immersion in Sonstadt's solution greatly retards this oxidation. Between crossed nicols the rounded masses show a spherulitic cross in which the slow ray is tangential as determined with a quartz wedge in sodium light. Both refractive indices are greater than 1.68. Many of the rounded masses are partially fused together, sometimes in chains, and some of them are isotropic, indicating that they are still liquid. It is possible that the carotene separates first in oily globules which partially run together before or during crystallization. After oxidation the colorless masses resulting are isotropic.

DETECTION AND DETERMINATION OF COCAINE IN THE PRESENCE OF PROCAINE AND VARIOUS OTHER SUBSTANCES

By JAMES L. YOUNG (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.)

The detection of cocaine, in the presence of procaine, offers one of the most difficult problems confronting the chemist engaged in analysis of samples involved in litigation under the Harrison Narcotic Law.

These two substances are not only soluble in the same solvents, but form crystalline complexes with the "alkaloid reagents," which are so much alike as to defy positive identification.

Consideration of these compounds from the standpoint of decomposition and the subsequent identification of the by-products formed, led to the present approach to the problem.

Cocaine, being the methyl ester of benzoyl ecgonine, yields methanol on hydrolysis with an alkali. Procaine, on the other hand, not being a methyl ester, does not.

The methanol resulting from the hydrolysis of cocaine is determined by Beyer's[1] modification of the Georgia-Morales[2] test, since this method produces a color of such stability that comparison with test standards is practicable.

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[4] A. Polgar and L. Zechmeister, *J. Am. Chem. Soc.*, **64**, 1856 (1942).
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EXPERIMENTAL

Mixtures of cocaine with procaine and varying proportions of other synthetic drugs, as well as the opium alkaloids and such diluents as lactose, starch, and cane sugar, were hydrolyzed and the resulting methanol determined colorimetrically. None of the substances mixed with the cocaine gave positive tests alone, nor did they influence the test in mixtures. Only compounds which yield methanol on mild hydrolysis should present any problem of interference. The sensitivity of the test was ascertained to be approximately 2 mg of cocaine hydrochloride.

METHOD (QUALITATIVE)

REAGENTS

Approximately 2% aqueous solution of NaOH.

Approximately 24% Ethyl Alcohol (by volume).

Potassium permanganate soln:—Dissolve 3 g $KMNO_4$ and 15 ml of sirupy phosphoric acid (85%) in 100 ml of distilled water.

Oxalic-sulphuric acid soln:—Dissolve 5 g of $H_2C_2O_4$ in 100 ml of H_2SO_4(1+1).

Modified Schiff's reagent (Fuchsin-Sulfurous Acid T.S., U.S.P. XIII):—Dissolve 0.2 g of Kahlbaum's rosanaline HCl in ca 120 ml of hot water. Cool, and add 2 g of Na_2SO_3 previously dissolved in 20 ml of water. Add 2 ml of concd. HCl, dilute the soln to 200 ml, and place in the refrigerator for at least 24 hours before using for quantitative determinations. Store in a cool place.

DETERMINATION

Introduce 25–50 mg of the alcohol-free sample into a round bottom long neck distilling flask of ca 25 ml capacity. Add 2.0 ml of NaOH followed by 5 ml of water.

Connect the flask to a small condenser and carefully distill until 2 ml of distillate is collected in a 25 or 50 ml glass stoppered cylinder. (Ground glass joints should be used on all connections.) To the 2 ml of distillate add 0.25 ml of 24% ethyl alcohol and 2.75 ml of water. Add 2 ml of $KMNO_4$ reagent and allow to stand with occasional agitation during 10 minutes. Now add 2 ml of oxalic acid reagent followed by 5 ml of Schiff's reagent. Invert the cylinder three times and tightly stopper at once. Examine the cylinder after one and two hours viewing thru the long axis. A blue or violet color indicates the presence of cocaine in the sample. The intensity of color is a function of the amount of cocaine present.

METHOD (QUANTITATIVE)

Standard cocaine solution:—Accurately weigh 50 mg of pure cocaine hydrochloride and dissolve in 10 ml of distilled water; 0.1 ml of this solution equals 0.5 mg of cocaine hydrochloride.

DETERMINATION

Weigh 25 mg of the sample (freed from alcohol if necessary), introduce into a 25 ml round bottom flask and follow the Qualitative Method with the following modifications. Collect the 2 ml distillate in a 6-inch Nessler tube having a 2 ml marking. Introduce 0.4 ml, 0.6 ml, and 1.0 ml portions, respectively, of the standard cocaine soln into separate distilling flasks and proceed as under Qualitative Method, collecting the 2 ml distillates in 6-inch Nessler tubes. After standing two hours, make trial comparison of the color developed by the sample and by the standards.

(The oxidation of the sample and the standards with the permanganate reagent should be started at the same time.) If the color developed in the sample exceeds that of 1.0 ml standard, a smaller sample must be employed, which can be accomplished by dissolving a suitable weight of the sample in water or 0.02 *N* HCl and using an aliquot for analysis.

For accurate work the final comparison should be made with a series of standards in 0.1 ml increments. If suitable photometric equipment is available, a permanent standard curve may be prepared from extinction readings at 560 millimicrons.

SUMMARY

A method is presented for the detection and determination of cocaine alone, or in mixtures. The alkaloid is hydrolyzed and the methanol resulting from the hydrolysis is determined colorimetrically. The method is accurate, rapid, and requires a minimum of manipulation and apparatus

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MOLD MYCELIA COUNT METHOD FOR CRANBERRY SAUCE

By WILLIAM V. EISENBERG and ALBERT H. TILLSON (U. S. Food and and Drug Administration, Federal Security Agency, Washington, D. C.)

It has been recognized for a number of years that the mold mycelia count method as used for tomato products is also applicable to cranberry products for the purpose of detecting the use of rotten fruit. The method as used by many laboratories has consisted of making a mold count directly on a portion of the gelled sauce.

The writers recently conducted a survey for the purpose of testing this method and establishing the relationship between the mold mycelia count and the condition of the fruit being used. At the outset of this survey it was observed that the direct method of counting was not entirely suitable, since the microscopic field was often too dense to permit clear vision and prompt detection of the mold mycelia in the cellular mass. This condition was observed in making counts at the factory of both the pulp and the finished sauce. The use of a pectin solution for dilution purposes, such as is used in making mold counts of many other fruit products offering the same diffiulty, seemed highly desirable. (See Figs. 1 and 2.) Accordingly, the following method was devised for use in making counts of both the strained and whole berry sauces.

METHOD

(*a*) *Strained Sauce*

Immerse the unopened can of sauce in a boiling water bath for 30–45 minutes, in order to facilitate breaking the gel. Remove can from bath and open carefully to avoid loss of sauce thru sudden release of pressure. Empty contents of can into a suitable sized beaker (1 liter beaker for #2 can). Stir the sauce in order to break the gel. A slow-speed electric mixer (350–450 r.p.m.) may be used for this purpose.

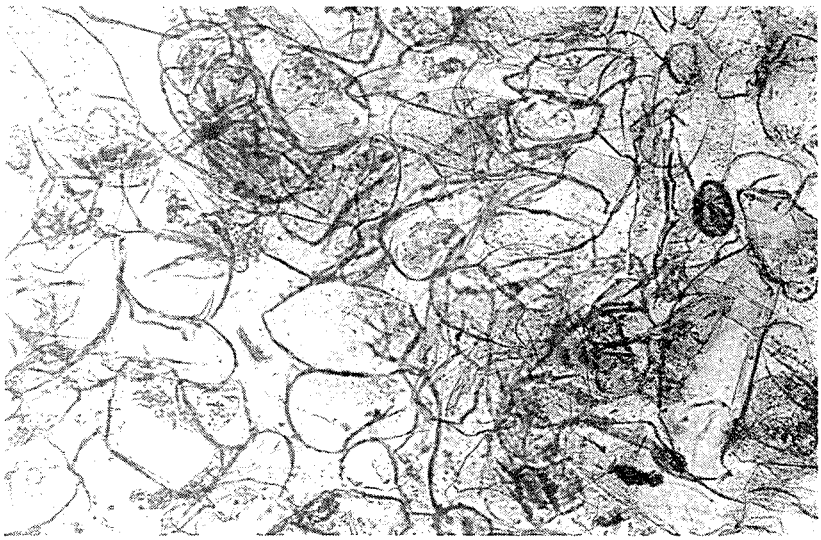

FIG. 1.—Undiluted strained cranberry sauce on mold counting chamber. ×100.

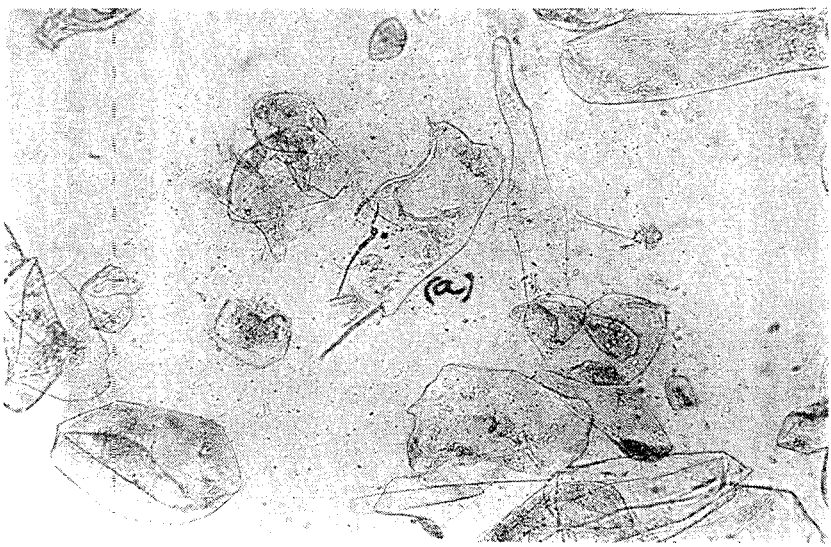

FIG. 2.—Diluted strained cranberry sauce (1+1) on mold counting chamber. ×100. Note mold mycelia along the cell wall and in interior of cell at (a).

Mix thoroly 50 g of the stirred sauce with 50 g of a 3% pectin soln.[1] Make a mold count of this mixture using official mold count method as directed in *Methods of Analysis*, 6th Ed., Sec. **42.57.**

(*b*) *Whole Sauce (Seeds and Skins Included)*

Pulp contents of container (if considerably greater than 1 lb, such as #10 can, remove well-mixed aliquot of 1 lb) thru cyclone with screen openings ca 0.027" in diam. This will remove skins and seeds and prepare a homogeneous pulp for mold counting. Mix 50 g of pulp with 50 g of 3% pectin soln. Make mold count of this mixture as directed in *Methods of Analysis*, 6th Ed., Sec. **42.57.**

Manufacturing plants using this method for control purposes can take samples of the strained pulp or the finished strained sauce from the production line and make their dilutions directly, thus foregoing the need for breaking the gel as directed in the method for strained sauce. The writers have found, however, that control is best exercised on the sorting belt in in order to eliminate rotten fruit.

Collaborative work was undertaken in order to test the methods. Diluted samples were prepared for mold counting and these were rotated among the analysts, each preparing his own slides as directed in the method. These analyses were made of sauces examined during the field survey at various plants and later in the laboratory. The material used consisted of cranberry products sampled in the Cape Cod and the New Jersey areas.

The comparative results are tabulated below:

Comparison of mold count results on same sauces by three different analysts

SAMPLE NO.	MOLD COUNTS			
	WVE	AHT	FRS*	AVERAGE
		Strained Sauce		
1	46	52	—	49.0
2	38	40	—	39.0
3	68	72	—	70.0
4	50	48	—	49.0
5	26	32	—	29.0
6	44	42	36	40.7
7	34	34	40	36.0
8	76	78	76	76.7
9	76	80	74	76.7
		Whole Sauce		
10	10	14	—	12.0
11	4	6	2	4.0
12	14	16	6	12.0

* FRS participated only in the mold counts made in the laboratory.

[1] See *Methods of Analysis*, 6th Ed., Sec. 42.2(b) for preparation of the pectin soln.

These comparative results are influenced by two factors, first, the sampling variation in removing a portion for preparing the counting chamber and the distribution of the mold among the microscopic fields in the counting chamber and second, the personal variation among analysts. The analytical results show good agreement among the analysts. The variation can be accounted for to a large extent by the first factor mentioned above, namely, sampling variation.

The results of this study demonstrate conclusively the validity of this method for the quantitative determination of mold mycelia in cranberry sauce. Concordant results can be obtained by qualified analysts trained in the use of this method.

TESTS ON THE EFFICIENCY OF VARIOUS FILTH RECOVERY PROCEDURES

II. INSECT FRAGMENTS AND RODENT HAIRS FROM FLOUR*

By KENTON L. HARRIS and LILA F. KNUDSEN (United States Food and Drug Administration, Federal Security Agency, Washington 25, D. C)

In August 1940 the Food and Drug Administration issued a mimeographed "Method for the Recovery of Filth from Flour, Revised." This method evolved out of the work of several Administration analysts: W. S. Greene had used saturated salt solution to float rodent hairs and insect fragments from flour. Analysts at the New Orleans Station had used the same principle but had modified the procedure. The Wildman trap flask, employed at that time for tomato products, was used in a saturated-salt-gasoline method described by the Cincinnati Station and the Microanalytical Division. The latter method was rewritten by W. H. King (New Orleans Station), tested and modified by K. L. Harris and W. G. Helsel (Microanalytical Division), and issued in a mimeographed form September 1938. The "Manual of Microanalytical Methods," compiled in 1943 for use by analysts of the Food and Drug Administration, contained this same method, condensed in wording. In addition a second extraction, by the following technique, was called for after the first trapping off in the first trap flask:

"Add 10 to 20 ml . . . gasoline . . . to the trap flask; stir in vigorously; add ca 10 ml saturated NaCl soln; allow to stand 10 to 20 minutes with intermittent stirring of the bottom layer; trap off and filter."

J. F. Nicholson and S. M. Walden (1) (Baltimore Station), in March 1944, reported a pronounced increase in the insect fragment recovery from

* Part I, by Harris and O'Brien, appeared in *This Journal*, 29, 84 (1946).

six samples of contaminated flour when the flour was first digested with an aqueous extract from pancreatin.[1] They concluded that the pancreatin released insect fragments that would otherwise be held down in the flask by the protein; and also because the sticking of insect fragments to protein is unpredictable, that the use of a pancreatic digestion would tend to produce more uniform results. A method based upon their work was added to the U. S. Food and Drug Administration "Manual of Microanalytical Methods."

Working independently, analysts of the Atlanta Station and the Microanalytical Division used saturated salt solution in the trap flask in place of the water recommended in the above method. They felt that the increased specific gravity of the salt solution would aid in the flotation of insect parts.

The following method given in *Methods of Analysis* of the Association of Official Agricultural Chemists, Sec. **42.30** (amended), is based upon the results of all these investigations:

Insects, insect fragments, and rodent hairs. Weigh 50 g of flour into 250 ml beaker. Add ca 60 ml pancreatin soln (prepared as a filtered aqueous extract of 5 g pancreatin per 100 ml H_2O) and stir into smooth paste. Add ca 40 ml of the pancreatin soln (100 ml total) and mix. (Adjust to *p*H 7–8 with ca saturated Na_3PO_4 soln.) Allow to stand ca 15 min, and if necessary readjust to *p*H 7–8. Maintain at 40° for not less than 3 hours. Transfer digested material to liter Wildman trap flask. Add 20 ml of gasoline and mix thoroly. Allow mixture to stand 5 min., fill with saturated NaCl soln, and after 30 min. trap off into 250 ml beaker. Add ca 10 ml gasoline to the material in the trap flask, stir the gasoline into the mixture and after ca 5 min., trap off into the same beaker. Transfer contents of beaker to trap flask and fill with saturated NaCl soln. Stir and after ca 30 min. trap off into beaker[2] and filter thru rapid filter paper, using suction. Examine microscopically.

Aside from the recovery work by Nicholson and Walden the development of the present method for the detection of insect fragments and rodent hairs from flour has been accompanied by no comparative recovery tests. Changes that appeared reasonable, or which increased the ease of handling, or which permitted more rapid microscopic examination by incorporating less flour residue in the floating layer, were simply accepted and used by the analysts doing the great bulk of this type of work. The use of a second trapping has become well established in this laboratory although, aside from the work by Harris and O'Brien (1946) (2), there has been no published data. These analysts concluded: "The second extractions in a Wildman trap flask yield appreciable amounts of filth. In gen-

[1] The first reference to the use of the enzyme pancreatin to release contaminants from foods is in a mimeographed publication "Examination of Bakery Products for Filth," by Harry Shuman, Philadelphia Station, U. S. Food and Drug Administration, February 19, 1941. Although pancreatin has since been widely used in the analysis of food for filth, there has been no previous mention in the published literature of the technique as used by Shuman.

[2] Under "Changes in Methods of Analysis," *This Journal*, **30**, 90 (1947) the following addition has been made to sec. **42.4(a)** as a sixth paragraph: "When the trapped-off material contains an appreciable amount of starch material, add sufficient conc. HCl to make 1–2% HCl, boil, and filter while hot." To preserve the uniformity in the procedures being tested, all trapped-off portions reported in this paper were treated in this manner.

eral, where recoveries are low on the first extraction they are higher on the second. Therefore, the use of two extractions on all samples will give more uniform results than will one extraction." Eisenberg (1947) (3) working with spices found that, when appreciable amounts of filth were found in the first extraction, additional fragments were found in the second, as shown in Table 1.

TABLE 1.—*Insect fragments and rodent hairs recovered by the first and second extraction of spices**

(Insect parts and rodent hairs from different samples)

INSECT FRAGMENTS		RODENT HAIRS	
1ST EXTRACTION	2D EXTRACTION	1ST EXTRACTION	2D EXTRACTION
17	1	1	0
0	1	1	0
3	0	1	0
3	0	1	0
24	1	0	4
203	33	8	5
19	1	0	2
4	1	1	0
17	3	1	0
5	0	5	1
71	15	1	0
5	1		
23	4		
19	5		
1	0		
38	3		
45	4		
1	0		
3	0		
42	2		

* Eisenberg, 1947.

Helsel and Harris in previously unpublished data (see Table 2) showed significant amounts of filth recovered from corn meal in the second trapping.

These results, plus the common observation in this laboratory of insect fragments floating in the oily layer of the so-called second extraction, leave no doubt but that the second trapping off recovers an appreciable number of insect parts and rodent hairs when they are present in the material being examined.

EXPERIMENTAL

In order to evaluate the other specific details leading up to and tentatively incorporated in method **42.30**, a series of tests were made following

the plan started in Part I, *loc cit.*, p. 84, in which an artifically contaminated lot of flour was used as the basic contributor of the filth to be extracted. A 100-pound batch of patent flour was mixed with a 20-pound lot of flour heavily contaminated with insect fragments, finely ground rodent excreta, and rodent hairs. A preliminary examination of this resulting flour indicated that the insect fragment and rodent hair counts were so high that it would be impractical to count the filth extracted from it.

TABLE 2.—*Rodent hairs and insect fragments recovered by the first and second extractions from corn meal**

RODENT HAIRS		INSECT FRAGMENTS	
1ST EXTRACTION	2D EXTRACTION	1ST EXTRACTION	2D EXTRACTION
38	1	281	30
10	0	502	27
23	5	196	56
5	1	68	26
6	1	55	26
5	0	382	79
5	5	350	111
10	5	274	67
18	6	342	67

* W. G. Helsel and K. L. Harris, unpublished.

For this reason, 7 grams of this dirty flour were mixed with 43 grams of a clean, commercial, general purpose, patent household flour to make the 50 gram portions of flour used in tests of various filth extraction methods.

A. Comparison of various techniques which contributed to the development of the 1945 A.O.A.C. method: section 43.20

Ten 50-gram portions of flour prepared as described above were tested by each of eight variations of the basic method, in such a manner that data was obtained on filth recoveries by the above-mentioned method by 4 variable procedures (1) trap flask size, (2) extraction of medium, (3) pretreatment, with chloroform as given in section **42.29** of *Methods of Analysis*, (4) digestion with pancreatin. The analytical results are given in Table 3 and shown graphically in Figure 1.

1. Trap flask size.—Series 1 and 2, 3 and 4, and 6 and 7 were similar procedures varying within the pairs only by the trap flask size. By means of analysis of variance, a comparison of the recoveries obtained shows that there was no significant difference introduced by flask size. Previous experience has demonstrated that, when handling lower grades of flour, less flour debris will be deposited on the filter paper if a 2-liter trap rather than

TABLE 3.—*Rodent hair and insect fragment recoveries from flour*

SERIES	TYPE FILTH	EXTRACTION PROCEDURE				RESULTS PER 50 GRAM SUBDIVISIONS													
						INDIVIDUAL SUBDIVISION COUNTS										SUMMARY			
		CHLOROFORM	DIGESTION	FLOT. MED.	TRAP SIZE	1	2	3	4	5	6	7	8	9	10	HIGH	AVE.	LOW	S.E. AVE.
		(VAR. 3)	(VAR. 4)	(VAR. 2)	(VAR. 1)														
1	Hairs	No	Pancr.	Water	1	12	20	13	13	19	13	9	21	19	13	21	15	9	1.3
	Insects					194	162	172	201	229	190	163	156	171	154	229	179	154	7.6
2	Hairs	No	Pancr.	Water	2	20	16	20	17	19	10	15	20	25	18	25	18	10	1.2
	Insects					199	169	183	214	174	222	147	230	175	175	230	189	147	8.4
	Hairs	No	Pancr.	Salt	2	9	9	12	13	16	6	10	17	23	11	23	12	6	1.6
	Insects					153	173	171	186	177	143	184	196	185	147	196	172	143	5.7
4*	Hairs	No	Pancr.	Salt	1	11	13	17	9	11	15	16	14	9	16	17	13	9	0.9
	Insects					144	174	173	182	163	173	161	186	149	146	186	165	144	4.7
5	Hairs	Yes	None	Water	2	5	6	7	8	3	0	0	0	0	0	8	3	0	1.0
	Insects					225	224	298	217	241	18	20	0	2	16	298	126	0	39.0
5A	Hairs	Yes	None	Salt	2	0	1	0	0	0	0	0				1	0	0	0.2
	Insects					78	66	35	72	51	97	64				97	66	35	7.4
6	Hairs	No	None	Salt	2	18	8	19	21	15	15	19	15	14	13	21	16	8	1.2
	Insects					159	201	184	219	172	210	181	171	190	160	219	185	159	6.4
7	Hairs	No	None	Salt	1	6	21	11	13	14	13	7	18	14	7	21	12	6	1.5
	Insects					155	188	188	199	162	162	153	184	146	158	199	170	146	5.8
8	Hairs	No	Dil. HCl Paste	Acidified Water	1	13	17	15	16	11	11	15	13	11	13	17	14	11	0.7
	Insects					127	133	121	112	116	112	120	108	183	176	183	130	112	8.5
9	Hairs	No	Dil. HCl-Slurry	Acidified Water	2	15	25	11	9	12	18	13	19	18	19	25	16	9	1.5
	Insects					191	162	187	203	240	240	168	264	242	243	264	214	162	11.4
10	Hairs	No	HCl-Acetone	Acid. Acetone-wat.	Separate Funnel	15	16	17	15	15	4	11	15	11	20	20	14	4	1.4
	Insects					257	278	203	246	286	72	262	249	153	259	286	226	72	21.1
11	Hairs	Directly through #100 Screen				4	8	5	7	11						11	7	4	
	Insects					58	63	50	56	48						63	55	48	

s.e. Ave. = Standard error of the average.
* *Methods of Analysis*, 6th Ed. sec. 42.30.

a 1-liter trap is used. Therefore, since no loss of filth is involved, the use of a 2-liter flask is recommended.

2. Extraction medium.—As tested by an analysis of variance comparing series 1 and 4 and 2 with 3, there is a significantly higher recovery of both insect fragments and rodent hair fragments when water is used in place of the saturated salt solution. This finding is contrary to that expected by analysts who thought that, because of its higher specific gravity, saturated aqueous sodium chloride would float more filthinto the oily layer; but agrees with the reasoning that it is easier to wet (with oil) the filth in liquids of lower surface tension and so enable them to be carried by the oil into the floating layer. Helsel and Harris, in devising a method for the isolation of filth from corn meal, found that a higher insect fragment recovery was obtained by trapping off in 60% ethanol than in 40% ethanol. In spite of the lower specific gravity an unimpaired recovery was obtained in alcohol water solutions as compared with water alone. It therefore seems reasonable to conclude that insect fragments and rodent hairs are more readily wet by oil when in solutions of low surface tension and thus are more completely extracted into the oily floating layer.

3. Pretreatment with chloroform.—Pretreatment according to **42.29** is as follows:

> Weigh 50 g of flour into 250 ml beaker. Add $CHCl_3$ to within ca 1 cm of top of beaker, mix thoroly, and allow to settle at least 30 min. Several times during this period, stir layer that rises to top. Decant soln and floating flour, being careful not to disturb heavy residue in bottom of beaker. Before decanting, take care that floating layer has not become so compact as to render this operation difficult. Wash down sides of beaker with stream of $CHCl_3$ from wash bottle. If so much material remains that it would interfere with subsequent microscopic examination, repeat decanting after adding more $CHCl_3$ until very little flour is left in beaker. Take care not to decant any heavy filth that may be present. Wash heavy residue remaining in beaker with stream of $CHCl_3$ onto ruled filter paper in Hirsch funnel. Transfer filter paper to Petri dish and examine microscopically.

The choice is then optional whether the subsequent analysis under **42.30** for insect fragments and rodent hairs should be carried cut on the same or a new aliquot. Scattered and unreported observations in this laboratory indicated that the flotation in chloroform might make subsequent extractions for light filth erratic. In order to test this, portions of the flour were treated as in **42.29** except that all of the material was decanted into the funnel, dried, and then extracted for insect fragments and rodent hairs.

The results of these tests show that the reliability of the method is markedly diminished when the same portion that is to be extracted for light filth has been previously treated by the chloroform sedimentation procedure. The flours in series 5 (Table 3), which previously had been treated with chloroform were tested in two groups each of five 50-gram portions. Subdivisions 1 to 5 all showed higher insect fragment recover-

ies and lower rodent hair fragment recoveries than other methods. Subdivisions 6 to 10 in series 5 all showed low insect fragment recoveries, and no hairs, whatever were obtained. The same analyst tested both groups, and the same apparatus was used. Moreover, at the same time as each of these groups of five were being tested, another group was being tested by one of the other procedures without resulting in such marked divergence. In addition to these ten portions, seven other tests had been run in conjunction with some preliminary work. The results of these seven analyses are given in Table 3, series 5 A. Although 5 and 5A differ in the flotation medium used, the recoveries are definitely low.

J. F. Nicholson, U. S. Food and Drug Administration, states (personal communication) that in routine testing, he has encountered unexpectedly low results in chloroform-treated samples which he had every reason to believe were contaminated. In instances where he made a repeat examination on separate protions untreated with chloroform, the results were higher.

It seemed possible that the low recoveries in 5A were due to the removal by chloroform of the oleophilic coating naturally present on rodent hairs and insect cuticle. However, insect fragments and rodent hairs which had been extracted for seven hours in running chloroform were subsequently completely recovered from a gasoline-water mixture (no food present). Furthermore, previously unpublished data by Helsel and Harris, as given in Table 4, show higher insect fragment and rodent hair recoveries in corn

TABLE 4.—*Rodent hairs and insect fragments from duplicate 50-gram samples of whole corn meal, with and without previous $CHCl_3$ sedimentation**

No $CHCl_3$	Hairs	19	4	5
	Insect fragments	142	139	100
With $CHCl_3$	Hairs	30	5	8
	Insect fragments	196	234	137

* W. G. Helsel and K. L. Harris, unpublished, 1939.

meal after soaking in chloroform. If the poor recovery in flour is due to the extraction of oils from hairs and insects, then these results on corn meal do not necessarily refute this argument, since there is so much oil in whole corn meal that the relatively crude extraction would be incomplete, although the *improved* recovery still remains unexplained.

4. Pancreatic digestion.—In comparing a flotation preceded by pancreatin digestion with the flotation of undigested flour, the undigested flour was made into a paste in the extraction medium, as would be done with pancreatin, before diluting it and extracting in the trap flask. A comparison of the recoveries obtained in series 3 and 6, and 4 and 7 shows no statistically significant difference in either insect fragment or rodent hair recoveries on the flour used here.

However, Nicholson and Walden (1944) observed (1) that in the handling of routine laboratory samples they had less trouble with the formation of emulsions when pancreatin was used before the trapping operation; and that on flour from which they recovered less filth than might be expected from the previous history of the sample, they were able to approach more closely the expected results by using pancreatin on the repeat analysis. After they started to use a pancreatin-digestion procedure, they noted a decrease in the number of samples which were difficult to handle. Moreover, on the six comparative samples tested by direct flotation and by flotation after pancreatin digestion they found an increased recovery on the pancreatin treated portion.

These tests, reported in Table 5, were made on five samples of soft wheat general purpose flour and one sample of soft wheat second clears (low grade) flour, each from a different mill, and each selected for re-testing with pancreatin because the original results were unexpectedly low. Although these data were obtained by using two different extraction fluids (in addition to the pancreatin variable) the results are significant.

TABLE 5.—*Insect fragments from six different 50-gram samples of flour by two procedures*

Nondigested, sat. salt[1]	116[2]	26	12	94	112	41
Pancr. digested, water	868	127	116	352	134	77

[1] The basic difference was the use or non-use of pancreatin.
[2] Second clears flour; others were general purpose.

In discussing these results and their influence upon recommendations to be made as to the best method to use for flour, it is important to note that certain flours are more difficult to handle than others. A high patent flour, such as was used in the experimental work reported in this paper, contains less bran and protein than the lower patents and low grade flour reported by Nicholson and Walden. It is the author's (H.) experience that the lower grades are the ones which give more flour and bran debris floating into the oily layer and so make filter papers which are more difficult to read. It is his experience also that the use of pancreatin before extracting filth will give filth papers bearing less extraneous cereal debris and so permit more rapid filtration and more rapid microscopic examination, without the need of probing thru cereal tissues that might confuse less experienced analysts.

Thus the use of pancreatin in no way lowers the recovery; it may increase the recovery from some flours; and it permits the more rapid handling of routine samples.

B. Digestion in dilute HCl

The addition of a pancreatic digestion procedure to the method increases the analytical time, and some thought has been given to a procedure that

will rapidly break down some of the proteins and starch. Boiling in dilute hydrochloric acid will accomplish this and has been suggested for several cereal products.[3] Two such procedures are discussed in this section.

1. Digestion in 200 ml 2% HCl:

To 50 g flour in a beaker add sufficient 2% HCl to form a thick paste. Stir until smooth. Add 2% HCl to make total of 200 ml. With constant stirring bring to boil. Cool. Transfer to 1-liter trap flask and extract with gasoline and H_2O in the usual manner.

The results by this method are reported in Table 3, series 8. The hair recovery was good, with the least variable results. The insect fragment count is slightly lower than with the pancreatin procedures.

2. Digestion in 400 ml 2% HCL.

The American Association of Cereal Chemists (Mimeo. 1946) (4) reported a similar procedure but with a larger volume of dilute hydrochloric acid, and with mineral oil added to the boiling mixture. In view of this work, and since the smaller volume of flour had a tendency to adhere to the bottom of the beaker and scorch, the following method was used:

To 50 g flour in a beaker add water and stir into a thin smooth paste. Add H_2O to make 400 ml total H_2O added. Add conc. HCl to make total HCl of water 2%. With intermittent stirring bring to a boil and boil 20 min. Transfer to 2 liter trap flask and trap off with gasoline and water in the usual manner.

This hydrochloric acid treatment with a greater volume of solution' longer boiling time, and larger flask size gave a high recovery of insect fragments (see Table 3, series 9). In fact, a comparison of these results with any of the pancreatic digestion recoveries shows the hydrochloric acid recoveries to be higher. The rodent hair recovery is not significantly different from all of the pancreatin digested series.

C. Extraction in separatory funnel

Walker and Dalby (1947) (5) report on hydrochloric acid-acetone digestion and oil-gasoline extraction of insect fragments and rodent hairs in a separatory funnel. Their method is designed for spices, but some workers reported verbally that it had been used for flour. The method as adapted for flour in this laboratory is as follows.

To 50 g flour in a 1-liter Florence flask add 200 ml acetone in such a manner as to rinse down the neck of the flask. Immediately swirl until flour and solvent are mixed. Add 380 ml H_2O and 20 ml conc. HCl and mix. Add 20 ml light mineral oil. Place under reflux condenser and boil for 20 min. Transfer hot to a 2-liter separatory funnel rinsing flask with ca 200 ml of 1:1 H_2O-acetone. Allow to stand until mixture has stratified. Withdraw floury sediment and clear layer into another separatory funnel leaving the floating oily layer. Add ca 100 ml 1:1 H_2O-acetone to the first funnel and again withdraw floury material to second funnel leaving oily layer. Add 50 ml gasoline to the second funnel, mix vigorously, and allow to stratify. Withdraw and discard sediment from second funnel. Several times add ca 100 ml H_2O-acetone

[3] *Methods of Analysis, A.O.A.C.*, 6th Ed., Sec. 42.28; *This Journal*, 29, 63 (1946).

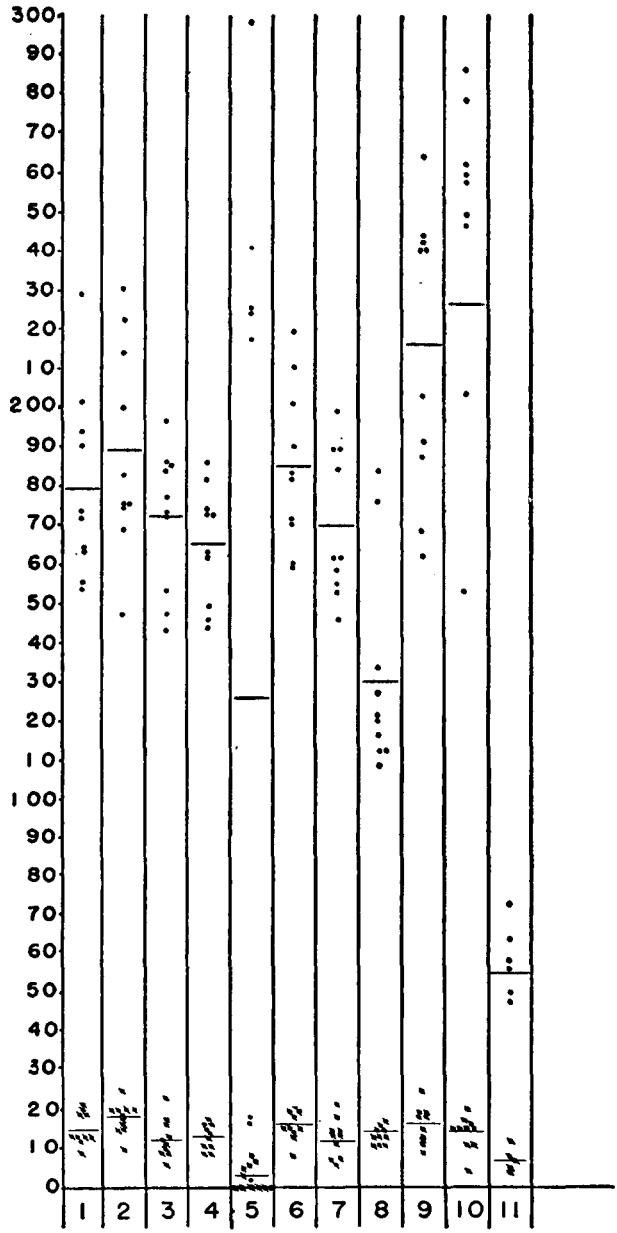

FIG. 1. Distribution of Insect Fragment and Rodent Hair Counts in the Eleven Series of Flours

• = Insect fragment count

\ = Rodent hair count

— Average, respectively, of insect fragment count (upper) and rodent hair count (lower).

and repeat, withdrawing and discarding sediment. Remove oil layer from both funnels to beaker, bring to boil in acid soln and filter.

The results obtained by this method are given as series 10 in Table 3. It is at once apparent that this series 10 procedure gave relatively high recoveries of insect fragments. The disconcerting point is that the method results in such a wide range of counts, subdivision 6 being markedly low, and the standard error of the average for insect fragments being 21.1. However, this procedure shows considerable promise. It specifically reopens the question of comparative recoveries using the Wildman trap flask (Howard, 1935) (6) as compared with a separatory funnel. The Wildman trap flask has been so successfully used to recover filth from such a wide variety of products that its inherent disadvantages often have been overlooked. Further work already initiated in this laboratory, evaluating the present recovery and loss of insect fragments and rodent hairs during the mechanics of trap flask operation, should illuminate this problem. In this regard it should be noted than when flour was digested in dilute hydrochloric acid, as described for series 9, a filth-bearing oily layer would not separate from the mixture in a separatory funnel with the same clear-cut division between oil and flour as was the case in a trap flask.

D. Use of sieves or silk bolting cloths

From time to time various workers in U. S. Food and Drug Administration laboratories have proposed the use of silk bolting cloths or metal sieves to retain insect fragments and rodent hairs and separate them from flour. Since the sieve sizes which they have suggested as being of possible value have approximately the same aperture sizes as the bolting cloths used in flour mills it is self-evident that extraneous material as well as the flour, will pass through.

Tests were made on five 50-gram portions of flour by the following method (Loughrey, 1945): (7)

Add 50 g of flour, with stirring to break up lumps, to soln of 30–35 ml HCl in 800 ml cold water in 1500 ml beaker. Bring to boil, stirring, and boil until thin and translucent. Filter thru #100 screen. Transfer material on the screen to ruled filter paper and examine.

Counts by this method (Series 11 in Table 3) very clearly show that insect fragments and rodent hair fragments in this flour are incompletely retained on a #100 seive, although a relatively higher proportion of the hair fragments are retained. Moreover, the filter papers obtained by this method contained more bran than when gasoline flotation was used, and the papers were more difficult to examine microscopically.

SUMMARY AND CONCLUSIONS

(1) Comparative data are given on the *A.O.A.C. Methods of Analysis*, **42.29** and **42.30** methods. Treatment of flour with chloroform as pre-

scribed by 42.29 markedly lowers the recovery of filth by 42.30. Separate aliquots should be used for each section.

(2) There was no significant difference between the use of a 1-liter and a 2-liter trap flask, nor between pancreatin-digested and non-digested portions. Since the use of a 2-liter flask and the use of pancreatin results in the inclusion of less plant debris in the floating layer and less difficulty with routine samples, they should be used. Their use may be omitted when examining high patent flours.

(3) The use of water in the trapping procedure gave significantly higher recoveries than did the use of salt solution. The method should be so modified.

(4) The second "trapping off" recovers additional filth elements.

(5) The average insect fragment count using large quantities of dilute hydrochloric acid was as high as, or higher than, any of the averages for pancreatin digestion.

(6) Preliminary tests indicate that a new procedure, based upon the work of the American Association of Cereal Chemists, may give markedly improved recoveries. Further investigations are warranted.

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TEST PAPER OF UREASE AND ACID-BASE INDICATOR FOR DETECTION OF UREA

By J. W. COOK (Food and Drug Administration, Federal Security Agency, San Francisco, Calif.)

The Marshall method (1) for urea makes use of urease to produce ammonia and carbon dioxide from urea. The increase in alkalinity of the solution is titrated with standard acid using an indicator which is not appreciably sensitive to carbonic acid. Urease papers, that is, filter paper impregnated with urease solution with subsequent drying, have been used as a convenient and stable laboratory preparation of the enzyme for a number of years (2) (3). Impregnating the urease papers with an appropriate indicator provides a convenient and delicate spot test reagent for

urea in material such as urine-contaminated food products. In the absence of alkaline substances, the test is as specific for urea as is the enzyme employed. The absence of alkaline substances may be established through the use of an auxiliary test paper impregnated with the indicator alone. The test does not require the aid of the microscope as do the common methods for comparable amounts of urea (4). Agar plates with urease and indicator incorporated also give the same delicate test.

METHOD

PREPARATION OF TEST PAPERS

Urease soln.—0.2 g urease Squibb (or other suitable preparation) (2) (3) per 20 ml water. Make the dry powder into a paste by stirring with a drop or two of water and then dilute to 20 ml.

Brom thymol blue soln.—0.15 g brom thymol blue per 100 ml water. Make soln yellow with a few drops of 0.1 *N* HCl.

Preparation of paper.—Immerse pieces of heavy filter paper in the urease soln, remove, and allow the paper to dry partially. Then immerse in the indicator soln, remove, and hang the paper up until thoroly dry. The paper should be yellow. Preserve in a wide-mouth, dark bottle in a cool, dry place. The paper should be stable indefinitely. Prepare blank test papers similarly by impregnating with the indicator alone.

PREPARATION OF AGAR PLATES

Agar soln.—2 per cent. Bring 2 g agar plus 100 ml water to boiling, with stirring, and boil for a few minutes.

Brom thymol blue soln.—Same as for test paper.

Urease soln.—0.2 g per 10 ml. Prepare as for test paper except dilute to 10 ml.

Preparation of plate.—Melt some of the agar soln, filter thru two layers of cheesecloth, and allow to cool somewhat (but not sufficiently to set). Mix 10 ml of agar with 10 ml of urease soln, add 0.5 ml of indicator soln, stir well, pour into a petri dish, and allow to gel. The plates should be yellow. Prepare blank test plate similarly with indicator soln alone.

PROCEDURE

Place minute crystals of urea or urine-stained food material on both the agar-urease and agar-blank, or on moistened test paper and blank paper. (Urea produces a bright blue spot in 10–30 seconds which continues to increase in size for about 20 min. on the urease preparation, but does not produce color in the blank preparations. Alkaline salts produce color in both the urease and blank preparations. When alkaline salts are added to the moistened papers the blue color appears immediately after the alkaline salts are wetted, whereas urea crystals dissolve and seem to disappear; then after a period of 10 to 30 seconds the color appears. The color fades in about one hour.)

DISCUSSION

Agar plates afford a relatively large surface where a large number of determinations can be made at one time. The plate remains moist and is quite convenient for laboratory use. It has the disadvantage of instability of the enzyme, and may not be quite as sensitive as the test papers. The test papers have a great advantage in that the enzyme is in a dry state and therefore stable for long periods, unless exposed to adverse conditions

of moisture, temperature, and light. The test paper can be carried by food inspectors for spot tests of food materials contaminated by rodent urine equally as well as it can be used in the laboratory.

ACKNOWLEDGMENT

The use of the urease-indicator test on paper was suggested by Doris H. Tilden.

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CORRECTIONS FOR VOL. 31, NO. 3

In the report on Separation of Bromides, Chlorides, and Iodides in Drugs, by Newell E. Freeman and Beulah V. McMullen, the equation (4) on page 552, should read as follows:

(4) $X^- + 2O = XO^+$

BOOK REVIEW

The Scientific Principles of Grain Storage. By J. A. Oxley, Northern Publishing Co., Liverpool, England. 96 pages plus references, index; price $1.72.

This book is largely concerned with heat, water, and air transfer in bulk stored grains. It is a phase of the grain storage problem in which Mr. Oxley is an authority and his treatment will be of help to persons engaged in research on this subject, since it points out the various factors concerned in grain storage. The factors themselves are treated with more words than substance.

K. L. HARRIS

NOTE

Note on the Determination of Phosphorus in Organic Compounds

By A. R. WREATH (Victor Chemical Works, Chicago, Ill.)

In the determination of phosphorus in organic compounds the semimicro Parr bomb method yields excellent results on all types of organic compounds, as was reported by Wagner *et al.* (1). This procedure is considerably shorter than that recommended by Hardin and MacIntire (2). We use this combustion method on samples of 50–80 mg and follow the A.O.A.C. volumetric procedure (3) after the excess sodium peroxide has been destroyed and the soln neutralized with HNO_3.

We also use an acid digestion on some samples after first checking the results against those obtained by the Parr bomb combustion. This procedure requires less apparatus and permits the handling of a larger weight of sample. The method is as follows:

Weigh accurately ca 0.5000–1.0000 g sample into a 400 ml beaker. Add 50–60 ml HNO_3 (Sp. gr. 1.42) and boil down to ca 25 ml. Add 15–20 ml $HClO_4$ (60–70%) and evaporate to dense fumes of $HClO_4$ and a colorless soln (Note 1). Cool. Dilute to 250 ml volume, neutralize a suitable sized aliquot, and complete the determination by the A.O.A.C. volumetric procedure (3).

NOTE 1. If the soln turns brown while being evaporated to fumes, remove from hot plate at once and add a few ml of HNO_3. Replace on hot plate and continue. This may have to be repeated more than once. An explosion may result if this is not done.

Both procedures yield excellent results. No trouble has been experienced with the HNO_3-$HClO_4$ digestion. Note 1 was added to the procedure as a precautionary measure. The preliminary digestion with HNO_3 serves to reduce the organic material to a safe level prior to the addition of $HClO_4$.

Some typical results:

	P_2O_5 CONTENT	
	HNO_3-$HClO_4$	PARR BOMB
	per cent	*per cent*
Hexaethyl tetraphosphate[a]	57.6	57.8
Tetraethyl pyrophosphate	49.0	49.0
Tributyl phosphate	33.8	34.0
Tricresyl phosphate[b]	19.0	19.3
Triphenyl phosphate[b]	22.0	22.1

[a] Commercial preparation.
[b] Practical grade.

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