FIRST DAY

MONDAY—MORNING SESSION

REPORT ON PROCESSED VEGETABLE PRODUCTS

By L. M. BEACHAM (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

The collaborative work reported by the Associate Referee on the acetaldehyde content of frozen peas and asparagus as an index of off-flavor was not fully completed at the time he made his report. Further, he reported difficulty in obtaining samples of homogeneous character for collaborative study. I recommend that further study and work be focused on this problem during the ensuing year.

The Associate Referee's report on catalase indicates that the modified rapid method for measuring catalase activity shows considerable promise. If it is successful it will afford a rapid, simple, and accurate means of measuring such enzyme activity. It is recommended* that further study be given it with the view of developing it into an official method.

REPORT ON QUALITY FACTORS IN PROCESSED VEGETABLE PRODUCTS

ACETALDEHYDE CONTENT AS A MEASURE OF FLAVOR IN FROZEN PEAS AND FROZEN ASPARAGUS

By RODNEY D. LOVEJOY (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

Last year, a brief preliminary report was made indicating that the acetaldehyde content of these frozen vegetables showed promise of being a measure of the "off" flavor associated with under-blanching.

Further work has been done in adapting the procedure for acetaldehyde in the *Methods of Analysis* (Chapter 9, Beverages; Distilled Liquors), to vegetable products. Securing truly replicate samples has been one of the greatest problems in studying the method. It has been claimed that in the same package one pea is likely to differ from another in enzyme activity and consequently in the acetaldehyde content that eventually develops; and that individual spears of asparagus are even more likely to differ. Marked variations in acetaldehyde content and in flavor in different packages from the same lot of both commercial packs and experimental packs have been found. These variables made it evident that something more than merely blanching different batches of peas or asparagus for

* For report of Committee C and action of the Association, see *This Journal*, 35, 49 (1952).

different periods of time would have to be done in order to prepare samples for collaborative study.

On the first attempt at preparing collaborative samples, freshly vined and washed peas were secured from a local plant. These peas were thoroughly blanched in the laboratory and frozen. Several days later some of these peas were partially thawed, ground in a Buffalo mill and split into 2500 g subdivisions. To each sub a known amount of acetaldehyde was added plus water to make the total weight 3750 g. Each sub was mixed in a Hobart mixer, packed into one-half pint cartons, and refrozen.

Later analyses showed that while check results were obtained on individual packages, different packages within the same group varied widely. This indicated that the samples were not thoroughly mixed; and therefore they were discarded.

A later attempt was made to prepare replicate samples by using bulk commercial samples of frozen peas. About 6 lbs. of these peas, enough for one series, were ground in a Buffalo mill. Individual tared cartons were filled about one-half full of the ground material, a measured amount of acetaldehyde was added to each carton, and the carton brought to a predetermined weight with more ground peas. Admittedly, the samples are not homogeneous; but it is hoped this fault may be overcome in the analysis by using the entire sample and thoroughly mixing in a Waring Blendor.

These samples have been sent to several collaborators for analyses using the following method:

DETERMINATION OF ACETALDEHYDE IN FROZEN VEGETABLES

REAGENTS

(a) *Starch indicator.*—1%.—7th Ed., **2.61(d)**.

(b) *Sodium thiosulfate soln.*—0.05 *N*.—7th Ed., **9.15(a)**.

(c) *Iodine soln.*—ca 0.05 *N*.

(d) *Sodium bisulfite soln.—ca 0.05 N in ca 10% ethanol.*—Check this soln against **(c)** so that it is slightly weaker than **(c)**.

(e) *Alcohol (aldehyde free).*—95%.

Check the alcohol for freedom from aldehydes in the following manner: To a 500 ml Erlenmeyer flask add 335 ml of distilled water. To another 500 ml Erlenmeyer flask add 300 ml of distilled water and 35 ml of the alcohol to be tested. To both flasks add 25 ml of the bisulfite soln and allow each to stand for 30 min., shaking occasionally. Then add 25 ml of the iodine soln and quickly titrate with the $Na_2S_2O_3$ soln using 5 ml of the starch soln as an indicator.

If the alcohol contains more than 2 p.p.m. of acetaldehyde, proceed as in **19.33(a)**.

PREPARATION OF SAMPLE

1. Determine the gross weight of the package.
2. Allow contents of package to partially thaw.
3. Transfer the entire contents of the package to a Waring Blendor.
4. Determine tare weight of the package.
5. Calculate net weight.
6. Add weight of water equal to net contents to blendor cup.

7. Comminute for 2 min. or more until a homogeneous mixture results.
8. Weigh 100 g of the mixture into a 500 ml standard taper Kjeldahl flask.
9. Add 1–2 drops "DC Antifoam A" or any other inert antifoaming agent.

DETERMINATION OF ACETALDEHYDE

Steam distill 200 ml of distillate into a 500 ml Erlenmeyer flask containing 100 ml of distilled water which covers the end of the delivery tube. The flask is immersed in an ice-water bath. The distillation should be completed in 12–15 min.

Upon completion of the distillation remove the flask from the ice bath and add 35 ml of the alcohol. Pipet 25 ml of the bisulfite soln into the flask and allow to stand for 30 min., shaking occasionally. Add 25 ml of the iodine soln and titrate with the $Na_2S_2O_3$ soln using 5 ml of the starch indicator. In exactly the same manner and at the same time, run a blank determination on 300 ml of distilled H_2O instead of the vegetable distillate. Difference between titrations, in ml of thiosulfate soln $\times 1.1 =$ mg of acetaldehyde in sample. Multiply by 20 to calculate parts per million.

Since all the reports are not in, all the possibilities of this method have not been explored. In several hundred samples of frozen peas and frozen asparagus which have been tasted by from two to six members of the Division of Food, and on which the acetaldehyde has been determined, the agreement betwen the tasters' detection of "off" flavor and the presence of increasingly large amounts of acetaldehyde has been surprisingly good. It is believed that any chemical or other objective methods which will measure poor or "off" flavors would be the most valuable aids in work on quality of foods.

REPORT ON CATALASE IN FROZEN VEGETABLES

PROGRESS REPORT ON THE DEVELOPMENT OF A RAPID METHOD

By BENJAMIN M. GUTTERMAN (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Associate Referee*

For many years now it has been recognized that the ability of frozen vegetables to maintain a normal flavor during proper low temperature storage is related to the level of catalase activity in them. Commercial practice has been to inactivate this enzyme by heating either with steam or hot water. A minute or two at the temperature of boiling water is usually sufficient, but if this blanching, as it is called, proves insufficient to inactivate the catalase, off-flavor will eventually develop. It has never been clearly shown that catalase is the factor which causes the off-flavors discussed by R. D. Lovejoy[1] in his paper, but rather that catalase is merely an index which predicts the production of the off-flavor. It is not attempted, in this study, to correlate the level of catalase activity with either a predictable off-flavor or an already existent off-flavor, but rather to evaluate a rapid method developed in the Division of Food laboratories.

[1] *This Journal*, p. 179.

The new method is considerably faster than the present A.O.A.C. catalase method described in the chapter on Enzymes of the 7th Edition, paragraphs **17.1** through **17.4.**

Obviously a series of samples of a frozen vegetable prepared for collaborative study can rarely approach the homogeneity of true solutions

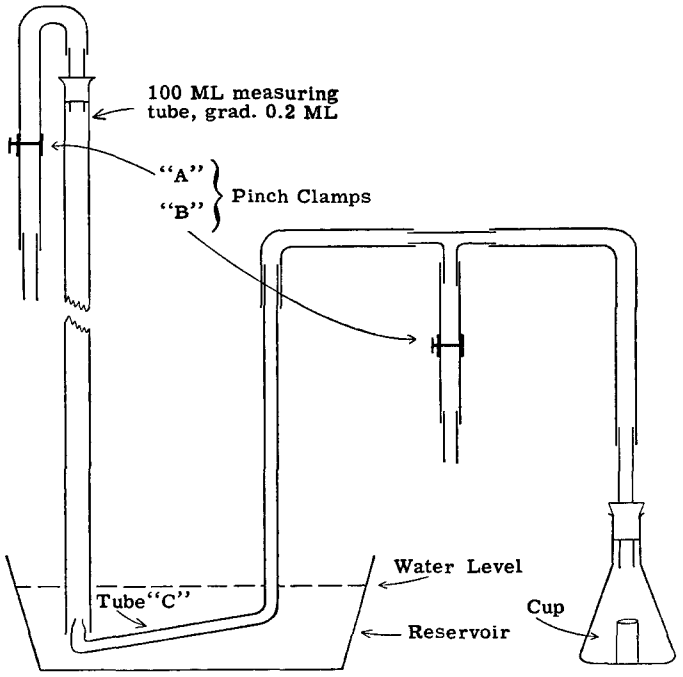

FIG. 1.—Apparatus for determining catalase activity.

or mixtures of small finite particles. Blanching and cooling of vegetables such as peas, which were used for this study, often leaves much to be desired in obtaining the same heat treatment for each and every pea. It has been noted, in the past, that one or two partially blanched peas in an otherwise adequately blanched sample will give elevated results not consistent with other samples from the same mass of the material.

The procedure for preparing samples for collaborative study was to collect some peas at a local canning plant and transport them to the laboratory for processing. The entire lot was divided into five equal batches. One batch was merely frozen and stored, without having been subjected to previous heat treatment. The other extreme was a batch blanched in boiling water for 30 minutes. Each of the remaining batches received different periods of heat treatment within the extremes described. Each

was then immediately cooled and packed 4–5 ounces per package and frozen in a circulating air freezer at minus 10°F. The packages were then distributed among various collaborators.

The method the collaborators were requested to test was one based on principles long in use by the industry. For years industry and control laboratories have determined catalase activity for production control purposes by treating ground samples of the vegetable with 3% hydrogen peroxide in a Smith fermentation tube, or even in an open container. For quality control procedures in the plant this almost always serves the purpose. However, there is an inherent chance of error due to entrapped air, or an error in interpreting what is a maximum allowable quantity of evolved gas. A refinement of this method of evaluating catalase activity by measuring the gas evolution was described by Thompson.[2] His apparatus is constructed on the manometer principle and, within the limits of normally acceptable amounts of gas evolution, serves its purpose. A modification of this apparatus has been used with success in our laboratory, but it was found to be inadequate without corrections, when measuring elevated catalase activity.

The principle of the Smith Fermentation tube method and the Thompson apparatus were therefore combined and the apparatus developed as described in the accompanying diagram. This apparatus is simple, inexpensive, and easily set up. In addition, the method is much simpler and more rapid than the A.O.A.C. catalase method and therefore is applicable to plant control procedures as well as to laboratory studies.

The method is as follows:

RAPID DETERMINATION OF CATALASE IN FROZEN VEGETABLES

REAGENTS

3% hydrogen peroxide.—(May be prepared by diluting 30% hydrogen peroxide.)
Calcium carbonate.

PREPARATION OF SAMPLE

Determine gross weight of package.

Permit contents of package to thaw sufficiently so that they may be removed from the package (the removal may be facilitated by holding the cover firmly on the package and sharply rapping the underside of the package with a hard object).

Transfer contents of package into a large graduated cylinder (500 ml).

Weigh empty package to determine tare weight.

Calculate weight of net contents.

Add sufficient water to the graduated cylinder containing the vegetable so that the total volume in ml of both the vegetable and the water is equal to 4 times the weight of the vegetable in grams.

Transfer contents of cylinder to blendor cup and add 1 g of calcium carbonate.

Comminute for 3 min.

Allow to stand for 2–3 min and filter thru 6″ gauze-backed cotton milk filter.

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[2] R. R. Thompson, *Ind. Eng. Chem., Anal. Ed.*, 14, 585 (1942).

METHOD

Prepare a glass cup of such size and shape that it will hold at least 8 ml, can be placed upright within a 250 ml Erlenmeyer flask (a litmus paper bottle serves admirably), and can be easily upset by shaking the flask. Pipet 8 ml of filtrate into the cup and set the latter, with the aid of a pair of long forceps, inside the Erlenmeyer flask. Pipet 10 ml of H_2O_2 directly into the flask.

Fasten the flask to the rest of the apparatus in accordance with the attached illustration. Open pinch clamp "A" and by the use of suction raise water level to about the zero mark and then tightly close pinch clamp. Open pinch clamp "B" and with the use of gentle air pressure force the water from tube "C" until one or more bubbles rise in the measuring tube.

Quickly close pinch clamp "B" while still exerting positive pressure. Reopen pinch clamp "A" and with suction return water level in measuring tube to the zero mark and hold it there while closing pinch clamp.

Start stopwatch and shake flask for 15 seconds, making sure that the cup has been overturned. Shake again for 5 seconds after 2½ min. have elapsed since starting time. Allow reaction to continue for 3½ min. from the starting time and read change in water level in the measuring tube. Record ml of gas received.

(For accuracy of reading the measuring tube may be made from a 100 ml buret with 0.2 ml graduations.)

Owing to numerous delays it was not possible to get the samples to the collaborators until a short time ago, and as yet insufficient results for evaluating the method have been received from them. Upon the receipt of these results a report will be submitted to the A.O.A.C. The above method holds promise, however, since good reproducibility has been obtained in our own laboratory.

It is recommended* that the rapid method for catalase in frozen vegetables, as described by the Associate Referee, be subjected to further study.

No reports were given on moisture in dried vegetables, or peroxidase in frozen vegetables.

REPORT ON OILS, FATS, AND WAXES

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

There will be no report on spectroscopic methods of oil analysis, although the Associate Referee has made some preliminary studies in this field. The Associate Referee on peanut oil will also be unable to present a report this year.

Collaborative studies on antioxidants were confined to the determination of propyl gallate in oils, and the satisfactory results support the Associate Referee's recommendation that the method should be accepted as first action. The Associate Referee on coal-tar colors in oils has con-

* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 49 (1952).

ducted further collaborative work on the first action method and the results obtained warrant her recommendation that the method be adopted as official.

RECOMMENDATIONS*

It is recommended—

(1) That studies on quantitative methods for peanut oil be continued.

(2) That studies on spectroscopic methods for the analysis of oils be continued.

(3) That further collaborative work be conducted on the method for propyl gallate in oils.

(4) That studies on quantitative methods for the determination of antioxidants other than propyl gallate be continued.

(5) That the first action method for coal-tar colors in oil be adopted as official, and further work on coal-tar colors in oil be discontinued.

REPORT ON COAL-TAR COLORS IN OIL

By Marie L. Offutt (U.S. Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

The modified method for coal-tar colors in oil, which was adopted as first action last year, was submitted for collaboration again this year.

Three samples of oil were sent out. Sample 1 contained Oil Orange SS (FD&C Orange No. 2), and Quinizarine Green SS (D&C Green No. 6). Sample 2 contained Yellow AB(OB) (FD&C Yellow Nos. 3 & 4), and Quinizarine Green SS (D&C Green No. 6). Sample 3 contained Oil Orange SS (FD&C Orange No. 2) with a small amount of added chlorophyll.

The collaborators all reported correctly the coal-tar colors present and seemed to have no difficulty in extracting these colors. Two of the collaborators suggested using more 60% alcohol to separate FD&C Orange No. 2 from D&C Green No. 6 and filtering the alcoholic solution of D&C Green No.6 before acidifying and dyeing.

The Associate Referee wishes to express her grateful appreciation to the following chemists who collaborated in this work:

D. W. McLaren, Buffalo District—Food and Drug Administration.
Sylvia Shendleman, New York District—Food and Drug Administration.
Helen T. Hyde, San Francisco District—Food and Drug Administration.
Alin L. Suslam, Boston District—Food and Drug Administration.
Felix J. Sabatino, Philadelphia District—Food and Drug Administration.
J. L. Thomson, Food and Drug Division, Ottawa, Canada.

It is recommended† that the modified method as printed in *This Journal*, **34**, 76 (1951), be adopted as official.

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* For the report of Subcommittee C and action of the Association, see *This Journal*, **35**, 53 (1952).
† For report of Subcommittee C and action of the Association, see *This Journal*, **35**, 53 (1952).

REPORT ON ANTIOXIDANTS IN OILS, FATS, AND WAXES

PROPYL GALLATE

By Sidney Kahan (Food and Drug Administration, Federal Security Agency, New York 14, N. Y.), *Associate Referee*

The rapid increase in the use of antioxidants in edible fats and oils over the last decade has posed a difficult problem for the analytical chemist in devising methods for their detection and identification. A recent survey conducted by Porter* showed no fewer than twenty antioxidants have been added to edible fatty substances to retard development of rancidity. The problem has been particularly important with animal fats which contain only traces of natural antioxidant materials, and the greater proportion of the lard sold today has some synthetic antioxidant added to it.

To further complicate the issue for the chemist the most important antioxidants in use today are polyphenols, similar in chemical structure and in chemical properties. Mattill (1) has stated "that the only substances which have primary antioxygenic action on fatty acids are ortho and para di- and poly-phenolic compounds or substances with similar electronic configuration. All other inhibitors are merely synergists." Synergism means the property of two or more antioxidants to give a greater effect when used together than would be expected from the sum of the individual effects. Citric, malic, and ascorbic acids and similar materials are often used as synergists.

The antioxidants most commonly used today are propyl gallate (hereafter called PG), nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole (BHA) and tocopherols. As a start on the study, it was decided to develop a method for propyl gallate.

Mattil and Filer (2) have described a method for gallic acid in fats and oils, based on a color reaction between gallates and ferrous tartrate first described by Mitchell (3). This method has been adapted to the determination of propyl gallate, and it has been found that the intensity of the color is directly proportional to the amount of propyl gallate present. Terrier and Deshusses (4) have described a gravimetric method for the determination of propyl gallate which was felt to be unnecessarily time consuming for routine use.

Mahon and Chapman (5) have published a comprehensive method for the separation and determination of the four leading types of antioxidants, whether present singly or in combination. The following work is based almost entirely on their method for propyl gallate, with only such modifications as introduced by this author to make the method more convenient for routine use.

Of all the common antioxidants, propyl gallate is the only one to give

* W. L. Porter, Eastern Regional Research Laboratory, U. S. Department of Agriculture. Personal Communication.

a color with ferrous tartrate solution. Gallic acid, gallates and tannic acid give identical color reactions with ferrous tartrate solution, but are not commonly used as antioxidants in edible fats in this country or in Canada. According to Lundberg and Halvorson (6) most polyphenols will give a colored precipitate with ferrous tartrate, but since these can be removed by filtration or centrifugation they do not interfere with the colorimetric determination of PG. The only other common antioxidant which would be extracted under the conditions used for PG would be NDGA if present in large amounts, but this interference can be readily removed as indicated above. Lauryl gallate is not extracted by 1.67% ammonium acetate under the conditions used in this method.

Glasstone (7) has shown that the *p*H of the solution is an important factor in determining the intensity and character of the color developed in the ferrous tartrate-gallate reaction, and recommended the addition of ammonium acetate solution to bring the *p*H to the region 7.0–7.6 for maximum color intensity. The method proposed, by which the PG is extracted from the fat with 1.67% ammonium acetate solution, and the colors developed in 1% ammonium acetate solution seems to be able to hold all ordinary samples in the desired *p*H range. However, if the samples being analyzed are distinctly acid or alkaline, then it will be necessary to adjust the *p*H of the color solution before color development.

All previous work on this color reaction indicated that the color reagent should be freshly prepared prior to use. It was decided therefore to check the exact time limits controlling the use of the ferrous tartrate reagent, and experiments were performed using 2.5 mg of PG.

Length of time color reagent prepared before use	*Per cent recovery of PG*
15 min.	98.0
30 min.	97.8
1 hour	97.5
2 hours	97.0
3 hours	96.0
16 hours	82.4
1 week	74.0

The color reaction is a very rapid one, the color attaining maximum intensity within one minute and remaining substantially constant for several days, as shown by the following table:

Time of reading in spectrophotometer after color development	*Per cent recovery of PG*
1 min.	97.8
15 min.	97.5
30 min.	97.5
1 hour	97.0
3 hours	97.2
16 hours	95.0
40 hours	91.5

The solution of PG in aqueous ammonium acetate is not nearly so stable, however. Standing overnight at room temperatures will cause a breakdown of the PG as evidenced by the formation of yellow colorations, and the recovery will drop sharply.

Length of standing of extract before color development	*Per cent recovery of PG*
Immediately after extraction from fat	98.3
2 hours	97.5
6 hours	95.0
Overnight	73.0

The desirability of preparing a new standard curve for each series of determinations, or of using a single curve for a number of samples, was investigated next. A study of the reliability of the slope of the line can be used to determine whether new standards must be prepared. A statistical analysis of the data obtained using seven different sets of ferrous sulfate and sodium potassium tartrate reagents showed no significant difference at the 95 per cent level of probability from the data obtained by seven replications of a single set of reagents. It may therefore be concluded that a single standard curve may be used for a large number of determinations, but should be checked at one or two points with each series of analyses to be certain that conditions have not changed.

A number of different oils and fats not containing any added antioxidants were first investigated to determine whether any natural interferences existed. All gave negative results using the method outlined in this report. Oils investigated were: olive, peanut, cottonseed, soya, and wheat germ. Fats investigated were: lard, hydrogenated cottonseed oil, oleomargarine, and butter.

The maximum absorption of the gallate-ferrous tartrate complex is given in several papers (2, 3) as 540 mμ. This was checked using a recording spectrophotometer, and the absorption peak was determined as actually occurring at 536 mμ. It was decided to standardize the method at 540 mμ, however, since the differences in absorption are slight, and since some spectrophotometers may not have an exact setting at 536 mμ.

Both the Coleman Universal and the Beckman DU spectrophotometers were used and found to be satisfactory for this work, using approximately one cm. cuvettes. For concentrations of PG of the order of 0.01% and less, however, the colors are rather faint, and a thicker absorption cell gives better accuracy. It is therefore recommended that when available, 20 mm and even 40 mm absorption cells be used, particularly if the concentration of antioxidant is below 0.01 per cent.

Before starting the quantitative extraction of PG from a fat, it is advisable to determine qualitatively whether it is present or not. The following is a qualitative test useful for this purpose: Dissolve approximately 10 g of the fat in 50 ml of petroleum ether in a separatory funnel. Extract

the fat solution with 20 ml of 72% ethyl alcohol by shaking for about 3 minutes. To 5 ml of the alcoholic extract add 2 drops of concentrated ammonium hydroxide. The appearance of a rose color indicates the presence of PG. This is a very delicate test and will give a positive indication with as little as 1 gamma/ml of PG present. The test is also useful as an indication of completeness of extraction of PG from a fat in the quantitative method given. Sometimes, if large amounts of PG are present, 3 extractions may not give complete recovery of the PG.

METHOD

REAGENTS

(*1*) *Petroleum ether reagent.*—Mix one volume of good quality 30–60°C. petroleum ether (**13.67** or equivalent) with three volumes of 60–100°C. petroleum ether (Skellysolve B and H have been found satisfactory) and shake the mixture with one tenth its volume of concentrated sulfuric acid for five min. Discard the acid layer, wash several times with water, then once with 1% NaOH soln, and then again with water until washings are substantially neutral. Discard all washings and distill the petroleum ether in an all-glass apparatus, using suitable precautions.

(*2*) *Ammonium acetate solns.*—1.25%, 1.67% and 10% w/v aqueous solns. A soln containing 1.67% of ammonium acetate in 5% aqueous ethyl alcohol may also be required.

(*3*) *Ferrous tartrate reagent.*—Dissolve 0.100 g of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and 0.500 g of Rochelle salt ($NaKC_4H_4O_6 \cdot 4H_2O$) in distilled water and make up to 100 ml. The reagent should be freshly prepared (must be used within three hours of preparation).

PREPARATION OF STANDARD CURVES

Prepare a standard aqueous soln of propyl gallate containing 50 micrograms per ml (dissolve 50 mg PG in a liter of distilled water), and place suitable aliquots, covering the range from 50 micrograms to 1000 micrograms, in 50 ml glass stoppered Erlenmeyer flasks. At least seven points should be plotted to cover the range adequately. Add exactly 2.5 ml of 10% ammonium acetate to each flask, make up to exactly 24 ml with distilled water and then pipet into each flask 1 ml of ferrous tartrate reagent. Let solns stand at least 3 min, then measure the absorbancies at 540 mμ relative to a soln containing 20 ml of 1.25% ammonium acetate soln, 4 ml of water, and 1 ml of ferrous tartrate soln.

DETERMINATION

Dissolve 40 g of the fat or oil in the purified petroleum ether reagent and make up to 250 ml. (Gentle warming may be necessary to obtain complete soln.) Pipet 100 ml of the fat soln into a 250-ml separatory funnel. Extract the fat soln with 20 ml of the aqueous 1.67% ammonium acetate soln by continuously inverting the separatory funnel for 2.5 min. After the phases separate completely, withdraw the aqueous layer into a 100 ml volumetric flask being careful not to allow any oil droplets to fall into the flask.* Repeat the extraction twice more with 20 ml portions of 1.67% ammonium acetate soln, combining the aqueous layers in the volumetric flask. Finally, extract the fat soln with 15 ml of water for 30 seconds and combine the aqueous layer with the previous washings. (Time must be allowed after each

* In the case of some shortenings, a strong tendency to emulsify was noted during the aqueous extraction of propyl gallate. To prevent this emulsification, add 2 ml of *n*-octanol to the fat soln aliquot before beginning the extraction. A 1.67% ammonium acetate soln in 5% ethyl alcohol should be used for the extraction in place of the aqueous soln. This procedure need only be used when the normal method fails.

Collaborative results, propyl gallate determination

SAMPLE	ANTI-OXIDANTS ADDED	LABORATORY NO.									
		1	2	3	4	5	6	7	8	9	
A	PG	.0246	.0285	.0274	.0279	.0275	.0270	.0291	.0283	.0325	Highest av. = .0303
(oil)	(0.03%)	.0247	.0285	.0273	.0276	.0281	.0263	.0294	.0294	.0313	Lowest av. = .0247
			.0286		.0274	.0313	.0264	.0295	.0303	.0272	General av. = .0281
		av. = .0247	av. = .0285	av. = .0274	av. = .0277	av. = .0290	av. = .0266	av. = .0293	av. = .0293	av. = .0303	
B	NDGA (0.02%)	none	none	.0030	.0005	none	.0006	.0011	none	.0097	Highest av. = .0067
(oil)	BHA (0.02%)	found	found	.0027	.0016	found	.0005	.0013	found	.0065	Lowest av. = 0
	PG (none)				.0013		.0005	.0013		.0038	General av. = .0014
				av. = .0029	av. = .0012		av. = .0005	av. = .0012		av. = .0067	
C	PG	.0074	.0094	.0092	.0071	.0081	.0088	.0097	.0084	.0122	Highest av. = .0116
(lard)	(0.01%)	.0073	.0094	.0074	.0088	.0081	.0091	.0100	.0090	.0119	Lowest av. = .0074
			.0095		.0078	.0081	.0093	.0099	.0100	.0106	General av. = .0090
		av. = .0074	av. = .0094	av. = .0083	av. = .0079	av. = .0081	av. = .0091	av. = .0099	av. = .0091	av. = .0116	
D	None	none	none	.0002	none	none	.0005	none	none	.0038	Highest av. = .0024
(lard)		found	found	.0000	found	found	.0006	found	found	.0027	Lowest av. = 0
							.0007			.0008	General av. = .0003
				av. = .0001			av. = .0006			av. = .0024	
	.33%										
E	"Tenox II"	.0146	.0184	.0194	.0196	.0154	.0179	.0191	.0216	.0191	Highest av. = .0209
(short-ening)	equivalent to:	.0147	.0185	.0168	.0206	.0149	.0180	.0195	.0197	.0179	Lowest av. = .0147
	PG (0.02%)		.0181		.0200	.0150	.0179	.0194	.0213	.0159	General av. = .0180
	BHA (0.077%)	av. = .0147	av. = .0183	av. = .0181	av. = .0201	av. = .0151	av. = .0179	av. = .0193	av. = .0209	av. = .0176	

Laboratories:
(1) U. S. Food and Drug Administration, San Francisco, California.
(2) Department of National Health and Welfare, Ottawa, Canada.
(3) Tennessee Eastman Co., Kingsport, Tenn.
(4) U. S. Food and Drug Administration, Cincinnati, Ohio.
(5) Army Medical Dept. Research and Graduate School, Washington, D. C.
(6) General Foods Corporation, Hoboken, N. J.
(7) Swift and Company, Chicago, Ill.
(8) The Best Foods Company, Bayonne, N. J.
(9) U. S. Food and Drug Administration, Baltimore, Md.

washing for the layers to separate completely.) Add exactly 2.5 ml of 10% ammonium acetate soln to the combined extracts in the volumetric flask and make to volume with water. This soln now contains 1.25% ammonium acetate. Filter thru dry, rapid filter paper to remove any turbidity. (Colors must be developed on same day extract is prepared. If the combined extracts are allowed to stand for more than several hours, a yellow color may develop, and the solns must be discarded.)

Pipet aliquot of the extract, not exceeding 20 ml, into 50 ml glass stoppered Erlenmeyer flask. Dilute aliquot to 20 ml with 1.25% ammonium acetate soln. Add exactly 4 ml of distilled water and pipet 1 ml of ferrous tartrate reagent into the flask. Mix well, and measure the absorbancies at 540 mμ relative to a soln containing 20 ml of 1.25% ammonium acetate soln, 4 ml of water, and 1 ml of ferrous tartrate reagent.

COMMENTS OF COLLABORATORS

(2) A. If the amount of propyl gallate to be extracted is much in excess of 0.01%, it appears necessary to increase the number of extractions from 3 to 5, employing 15 ml of 1.67% ammonium acetate per extraction.

B. In order to analyze samples containing 0.003 to 0.010% PG, it is necessary to specify instruments in which wide (25 to 50 mm) absorption cells can be used.

(3) After the extracts are diluted to 100 ml, they should be mixed well and filtered through ordinary filter paper to remove any fat or oil droplets.

(4) A. Suggest swirling funnels in an inverted position, instead of the recommended repeated inversion, to prevent any loss of sample from the delivery tip of the funnel. This allows use of the stopcock to release pressure build-up safely.

B. Since not all the solutions were clear, they were all filtered through Whatman #42 before taking aliquots for color development. It is suggested as a possible improvement in the method.

C. The deviation from the blank on samples B and D was counted as due to the color from the fat itself.

(5) We had difficulty in analysis because a brown color developed in the aqueous acetate extracts of substances A, C and E when standing overnight. Ferrous tartrate failed to give the characteristic purple color obtained with the gallate. The analyses were repeated with no time lag between extraction and color development. The results obtained are the ones reported. We recommend that the method note that color development should be made soon after the extraction. We found that the color obtained was stable for at least 24 hours after it was developed.

(6) We are reporting all results to the fourth place, but in our opinion, the fourth decimal is not significant.

(7) In the case of extract prior to color development, we noted distinct yellow colors in samples A, B and E. Sample B showed no blue-violet color, only a yellowish color. Values were calculated, but we doubt there is any PG present.

DISCUSSION

The above method is a fairly rapid and direct determination of PG in fats and oils, suitable for routine use. NDGA, BHA, and tocopherols will not interfere, but gallic acid and tannic acid will. These latter substances are not in common use as antioxidants in this country or in Canada. Lauryl gallate, the only other commercial antioxidant of the gallate group, does not interfere.

Results on the three samples containing propyl gallate indicated an average of 90% recovery or better for 9 different laboratories. The two

samples which contained no added PG showed insignificant amounts of colored material reported as PG. Other common antioxidants present did not interfere with the method.

RECOMMENDATIONS*

It is recommended—

(1) That the above method for propyl gallate in fats and oils be adopted, first action.

(2) That further work be done on this method and on methods for butylated hydroxyanisole and nordihydroguaiaretic acid in fats and oils.

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No report was given on spectrophotometric methods or on peanut oil.

REPORT ON DAIRY PRODUCTS

BY WILLIAM HORWITZ (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

Soft Cheeses.—The Associate Referee has been successful in developing a procedure for sampling such a heterogenous product as creamed cottage cheese. Thorough stirring of commercial containers of creamed cottage cheese will give a uniform mixture which can be sampled from the top. The Waring Blendor is used for the preparation of homogenous samples for analysis. The possibility of moisture loss in the use of the Waring Blendor should be explored before adoption of this procedure.

Frozen Desserts.—The official method for fat in frozen desserts, apparently based upon the corresponding method for cheese, includes the unnecessary step of weighing the sample into a beaker prior to transferring to the extraction flask. A comparison of the present method with direct weighing into the Mojonnier tube showed an insignificant difference of 0.02% fat between the two methods. The change is merely one of technique, eliminating the possible source of error involved in a transfer of sample, and in view of the excellent collaborative results it should be adopted as official. An alternative procedure for the preparation of frozen desserts containing insoluble particles has also been used successfully by the Associate Referee and his collaborators. A method for titratable acidity for

* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 53 (1952).

frozen desserts is also recommended which uses the milk method **15.4,** together with a specification of the size of the sample. Experience with the present sucrose method for sweetened condensed milk as applied to frozen desserts shows that it is not entirely satisfactory for this purpose and the Associate Referee intends to submit a modified procedure for collaborative study.

Preparation of Butter Samples.—The Associate Referee has approached the problem in a novel way by preparing inhomogeneous samples for analysis. Hand shaking and the Meuron stirrer produced a relatively uniform sample but the mechanical shaking machine did not produce as uniform a sample. Since the stirrer method **15.106–15.107** is seldom used, it should be deleted.

Miscellaneous.—Methods which have been in first action status for two years should now be made official since no adverse comment has been received by the Referee. These methods are listed in the recommendations.

RECOMMENDATIONS*

It is recommended—

(1) That the stirring method for sampling commercial containers of creamed cottage cheese be adopted as a procedure.

(2) That the method for fat in frozen desserts, **15.153,** be modified to incorporate direct addition of the sample to the Mojonnier tube, and be adopted as official.

(3) That the stirrer procedure for the preparation of butter samples, **15.106–15.107,** be deleted.

(4) That sample container paragraph, **15.104(c),** and the shaking procedure for the preparation of butter samples, **15.104–15.105,** be rewritten as suggested by the Associate Referee and be adopted as official.

(5) That the following first action methods be made official:

(a) Casein in fluid milk, method II, **15.19–15.20.**

(b) Casein in malted milk and chocolate malted milk, **15.98**

(c) Fat in malted milk, **15.98.**

(d) Phosphatase test for pasteurization of all dairy products (other than milk, cream, cheddar type cheeses, and soft uncured cheeses which are already official): **15.47–15.49, 15.122, 15.144–15.147, 15.157.**

(6) That further work be performed on the following subjects:

(a) Preparation of soft cheese samples with the Waring Blendor.

(b) Preparation of samples of frozen desserts which contain insoluble material such as fruits and nuts.

(c) Determination of acidity of colored frozen desserts.

(d) Preparation of butter samples by mechanical shaking machine.

(e) Babcock method particularly as applied to homogenized milk.

(f) Phosphatase test.

* For report of Subcommittee C and action of the Association, see *This Journal,* 35, 50 (1952).

REPORT ON THE PREPARATION OF BUTTER SAMPLES

By Albert L. Weber (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*, and Howard Edelson, Washington, D. C.

This report is the result of recommendation (5), *This Journal*, **34**, 48 (1951), that various procedures for the preparation of butter samples be compared. Accordingly, further work was done and the following instructions were sent to the collaborators:

INSTRUCTIONS TO COLLABORATORS

Six (6) mason jars of butter will be furnished you. The jars will be labeled A, A_1, B, B_1, C and C_1. Use jars A and A_1 for Method I, jars B and B_1 for Method II, and jars C and C_1 for Method III.

Designation of Methods: I. Hand Shaking, II. Machine Shaking, III. Meuron Method (see below).

Description of Methods:

I.—Soften entire sample in the closed mason jar by warming in a water bath maintained at about 40°C., shaking intermittently to reincorporate any separated fat and when optimum fluidity is obtained, shake vigorously by hand at room temperature until homogenous semi-solid mass is obtained. Withdraw and weigh immediately nine portions for analysis.

(Method for withdrawing portions for analysis is described below.)

II.—Same as Method I, except for the words "shake vigorously by hand" substitute the words "shake jar in a bottle-shaking machine at moderate speed."

III.—The Meuron Method as described in the Book of Methods A.O.A.C., 7th Ed., 1950, **15.108** and **15.109.** When butter is mixed, use spatula to pack and level the butter in jar. This can be done with spatula while rotating jar.

Please analyze jars A, B and C for moisture and curd and salt, if possible on the first day. Jars A_1, B_1 and C_1 to be analyzed within two days of the first set of jars. If the curd and salt of jars A, B, and C cannot be done the same day as the moisture, do all 27 moistures on the same day, and leave the curd and salt determination until the next day, and treat jars A_1, B_1, and C_1 similarly.

Please give room temperatures at time of analysis. All comments and criticisms would be appreciated.

COLLECTION OF SAMPLES

To insure non-homogeniety of the butter in each jar, and yet to keep the composition the same in the different jars, the samples for collaborative analysis were made from six different lots of butter (four were salted and two unsalted or "sweet") in the following manner: enough pats were printed by a Chiplets machine to allow 6 pats from each lot to be placed in each of 36 jars, or a total of 216 pats from each lot; 72 of these pats weighed 1 lb. The pats were placed in the jars as follows: Pat 1, lot 1 in jar 1; pat 2, lot 1 in jar 2, etc. to pat 36, lot 1 in jar 36; pat 37, lot 1 in jar 2, etc. to pat 72, lot 1 in jar 1, and continuing until the 24 pats were distributed. The procedure was repeated with the other five lots, begin-

ning each lot with the jar following the jar containing the 216th pat of the previous lot. Each lot was analyzed separately to show that the collaborative samples were made from butter of different composition. The results are shown in Table 2.

The jars were sent to the different collaborators according to the following scheme:

Jars for Method	*I*	*II*	*III*	*I*	*II*	*III*
(Nos.)	2	9	15	23	25	32 to Collaborator #1
(Nos.)	3	10	17	22	27	33 to Collaborator #2
(Nos.)	5	12	13	21	30	31 to Collaborator #3
(Nos.)	1	11	16	19	26	34 to Collaborator #4
(Nos.)	4	8	14	24	28	35 to Collaborator #5
(Nos.)	6	7	18	20	29	36 to Collaborator #6

Table 1 (pp. 196–197) gives the results of the collaborators.

Once the jars of butter are prepared according to the above directions, do not stir the butter before withdrawing portions for analysis.

*Method of withdrawing analytical samples:**

All portions for analysis (weighing between 1.5 g and 2.5 g) should be withdrawn by the supplied syringe trier.

When the sample is properly prepared, the jar should be placed in one position and should not be moved while the analytical samples are being withdrawn. Above all, the contents should not be stirred between sample withdrawals.

The top, the middle, and the bottom levels should be sampled in that order to minimize evaporation losses and disturbances due to the use of the trier.

The top layer should be about ½ inch below the surface, and the bottom layer about ½ inch above the bottom of the jar; and the middle layer about half the distance between the top and bottom layers.

The syringe trier is used as follows:

With the pointed plunger down as far as possible, the proper location on the butter surface is selected and the trier is inserted *vertically*, to the proper depth. The plunger is withdrawn slowly for a distance of about 2 inches; it is then held securely with the finger and the trier is slowly withdrawn. The trier is wiped with a piece of paper towel, the open end struck off as squarely as possible and the charge extruded into the weighed dish, and weighed immediately.

The proper depths can be selected by holding the trier against the outside of the jar and marking its barrel with a marking pencil. The trier is then inserted to the mark. It is unnecessary to clean the interior of the barrel between samples from the same jar, but its outside and the point of the plunger should be wiped with a paper towel.

Of the nine analytical samples to be taken from each jar, three should be taken from each level. The three samples from each level shall be taken as follows:

One at the center of the jar, one at the edge, and one at any point about one inch from the center.

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* Note: In the Meuron method, the butter may not be fluid enough for the plunger of the syringe trier to bring enough butter into the syringe. In these cases completely withdraw plunger and use syringe as a pipet until two inches of butter is in the syringe. Replace the plunger and extrude the charge as in the other methods.

TABLE 1.—*The moisture, curd and salt, and butterfat (by difference) obtained by different methods of preparation of the butter*

	SUB		MOISTURE—PER CENT						CURD AND SALT—PER CENT						BUTTERFAT—PER CENT					
			H.M.B.[1] 25°C or 27°C.[2]	M.L.D. 27°C.– 29°C.	G.E.K. 25.6°C.– 25.8°C.	H.J.M. 23.5°C.– 24.5°C.	J.P.S. 25°C.– 27°C.	A.L.W. 20°C.– 22.°C.	H.M.B.	M.L.D.	G.E.K.	H.J.M.	J.P.S.	A.L.W.	H.M.B.	M.L.D.	G.E.K.	H.J.M.	J.P.S.	A.L.W.
Method I	A—Top	C[3]	16.61	16.18	16.13	16.36	16.16	16.25	lost	2.51	2.36	2.27	2.47	2.34	—	80.31	81.51	81.37	81.37	81.41
		E	16.63	16.18	16.16	16.38	16.15	16.23	2.33	2.46	2.40	2.21	2.33	2.39	81.04	80.36	81.44	81.41	81.52	81.38
		O	16.63	16.22	16.17	16.30	lost	16.25	2.36	2.47	lost	2.23	—	2.37	81.01	80.31	—	81.47	—	81.38
	A—Middle	C	16.66	16.22	16.16	16.36	16.17	16.20	2.35	2.42	2.38	2.37	2.39	2.31	80.99	80.36	81.46	81.27	81.44	81.49
		E	16.63	16.25	16.18	16.32	16.19	16.25	2.32	2.42	2.40	2.28	2.38	2.31	81.05	80.33	81.42	81.40	81.43	81.44
		O	16.63	16.22	16.18	16.35	16.16	16.24	lost	2.44	2.42	2.32	2.35	2.33	—	80.34	81.40	81.33	81.49	81.43
	A—Bottom	C	16.62	16.15	16.15	16.35	16.16	16.24	2.34	2.47	2.42	2.29	2.34	2.41	81.04	80.38	81.43	81.36	81.50	81.35
		E	16.65	16.14	16.20	16.35	16.17	16.24	2.34	2.44	2.38	2.32	2.35	2.37	81.01	80.42	81.42	81.33	81.48	81.39
		O	16.63	16.21	16.18	16.32	16.21	16.22	2.38	2.52	2.42	2.28	2.38	2.42	80.99	80.27	81.40	81.40	81.41	81.36
	A_1—Top	C	16.16	16.72	16.61	16.33	16.69	16.13	2.29	2.25	2.35	2.33	2.42	2.38	81.55	81.03	81.04	81.34	80.89	81.49
		E	16.17	16.66	16.54	16.32	16.70	16.15	2.33	2.30	2.37	2.36	2.38	2.41	81.50	81.04	81.09	81.32	80.92	81.44
		O	16.27	16 69	16.55	16.31	16.72	16.16	2.36	2.39	2.36	2.42	2.40	2.40	81.37	80.92	81.09	81.27	80.88	81.44
	A_1—Middle	C	16.19	16.66	16.60	16.33	16.70	16.20	2.32	2.38	2.39	2.31	2.37	2.33	81.49	80.96	81.01	81.36	80.92	81.47
		E	16.17	16.65	16.55	16.33	16.73	16.16	2.37	2.36	2.33	2.33	2.39	2.38	81.46	80.99	81.12	81.34	80.88	81.46
		O	16.18	16.67	16.55	16.33	16.70	16.11	2.32	2.38	2.38	2.36	2.38	2.36	81.50	80.95	81.07	81.31	80.92	81.53
	A_1—Bottom	C	16.19	16.64	16.52	16.33	16.71	16.14	2.30	2.33	2.38	2.39	2.41	2.38	81.51	81.03	81.10	81.28	80.88	81.48
		E	16.25	16.66	16.47	16.27	16.69	16.16	2.33	2.37	2.41	2.36	2.41	2.39	81.42	80.97	81.12	81.37	80.90	81.45
		O	16.22	16.68	16.52	16.31	16.68	16.14	2.24	2.36	2.41	2.39	2.41	2.41	81.54	80.96	81.07	81.30	80.91	81.45
Method II	B—Top	C	16.80	16.09	16.32	16.30	16.61	16.08	2.42	2.56	2.40	2.41	2.40	2.44	80.78	81.35	81.28	81.29	80.99	81.48
		E	16.85	16.17	16.29	16.36	16.58	16.06	2.45	2.52	2.41	2.44	2.41	2.37	80.70	81.31	81.30	81.20	81.01	81.57
		O	16.79	16.17	16.29	16.33	16.56	16.04	2.45	2.50	2.36	2.41	2.48	2.43	80.76	81.33	81.35	81.26	80.96	81.53
	B—Middle	C	16.56	16.06	16.30	16.37	16.55	16.03	2.57	2.54	2.39	2.43	2.43	2.42	80.87	81.40	81.31	81.20	81.02	81.55
		E	16.66	16.15	16.27	16.37	16.51	16.12	2.43	2.49	2.38	2.45	2.46	2.40	80.91	81.36	81.35	81.18	81.03	81.48
		O	16.71	16.16	16.30	16.31	16.50	16.06	2.43	2.52	2.39	2.40	2.51	2.43	80.86	81.32	81.31	81.29	80.99	81.51
	B—Bottom	C	16.49	16.10	16.30	16.32	16.51	16.00	2.39	2.48	2.41	2.42	2.39	2.43	81.12	81.42	81.29	81.26	81.10	81.57
		E	16.68	15.92	16.31	16.45	16.53	16.08	2.46	2.49	2.41	2.43	2.44	2.42	80.86	81.59	81.28	81.12	81.03	81.50
		O	16.53	15.86	16.28	16.55	16.55	16.01	2.35	2.54	2.42	2.45	2.44	2.45	81.12	81.60	81.30	81.00	81.00	81.54
	B_1—Top	C	16.53	16.25	16.04	16.41	16.38	16.59	2.23	2.30	2.49	2.34	2.33	2.39	81.24	81.45	81.47	81.25	81.29	81.02
		E	16.57	16.27	15.97	16.39	16.37	16.55	2.33	2.31	2.46	2.37	2.38	2.43	81.10	81.42	81.57	81.24	81.25	81.02
		O	16.54	16.27	16.06	16.42	16.31	16.60	2.35	2.24	2.43	2.34	2.38	2.36	81.11	81.49	81.51	81.24	81.31	81.04
	B_1—Middle	C	16.60	16.26	16.06	16.41	16.31	16.60	2.37	2.28	2.45	2.30	2.36	2.39	81.03	81.46	81.49	81.29	81.33	81.01
		E	16.58	16.22	16.03	16.41	16.29	16.56	2.30	2.35	2.44	2.32	2.34	2.37	81.12	81.43	81.53	81.27	81.37	81.07
		O	16.55	16.25	16.03	16.39	16.31	16.61	2.26	2.39	2.44	2.33	2.40	2.39	81.19	81.36	81.53	81.28	81.29	81.00
	B_1—Bottom	C	16.57	16.20	16.00	16.55	16.32	16.57	2.28	2.32	2.41	2.39	2.37	2.43	81.15	81.48	81.59	81.06	81.31	81.00
		E	16.62	16.20	15.97	16.48	16.29	16.60	2.31	2.25	2.46	2.34	2.33	2.39	81.07	81.55	81.57	81.18	81.38	81.01
		O	16.55	16.18	16.03	16.56	16.31	16.59	2.32	2.25	2.47	2.37	2.34	2.42	81.13	81.57	81.50	81.07	81.35	80.99

	SUB		MOISTURE—PER CENT						CURD AND SALT—PER CENT						BUTTERFAT—PER CENT					
			H.M.B.[1] 25°C. or 27°C.[2]	M.L.D. 27°C.– 29°C.	G.E.K. 25.6°C.– 25.8°C.	H.J.M. 23.5°C.– 24.5°C.	J.P.S. 25°C.– 27°C.	A.L.W. 20°C.– 22°C.	H.M.B.	M.L.D.	G.E.K.	H.J.M.	J.P.S.	A.L.W.	H.M.B.	M.L.D.	G.E.K.	H.J.M.	J.P.S.	A.L.W.
Method III	C—Top	C	16.39	16.73	16.31	16.36	16.33	16.13	2.38	2.43	2.44	2.32	2.24	2.32	81.23	80.84	81.25	81.32	81.43	81.55
		E	16.40	16.62	16.27	16.42	16.37	16.13	2.39	2.41	2.42	2.34	2.31	2.36	81.21	80.97	81.31	81.24	81.32	81.51
		O	16.43	16.71	16.30	16.41	16.37	16.14	2.34	2.37	2.43	2.32	2.29	2.33	81.23	80.92	81.27	81.27	81.34	81.53
	C—Middle	C	16.42	16.68	16.30	16.43	16.38	16.18	2.38	2.44	2.38	2.34	2.27	2.39	81.20	80.88	81.32	81.23	81.35	81.43
		E	16.42	16.54	16.33	16.43	16.37	16.16	2.41	2.50	2.40	2.36	2.29	2.37	81.17	80.96	81.27	81.21	81.34	81.47
		O	16.40	16.58	16.31	16.43	16.37	16.15	2.34	2.39	2.40	2.33	2.25	2.35	81.26	80.93	81.29	81.24	81.38	81.50
	C—Bottom	C	16.39	16.62	16.31	16.43	16.41	16.10	2.35	2.39	2.38	2.37	2.28	2.42	81.26	80.99	81.31	81.20	81.31	81.48
		E	16.37	16.73	16.29	16.42	16.38	16.15	2.40	2.50	2.42	2.42	2.25	2.44	81.23	80.97	81.29	81.16	81.37	81.41
		O	16.38	16.69	16.32	16.42	16.37	16.15	2.37	2.36	2.44	2.36	2.25	2.43	81.25	80.95	81.24	81.22	81.38	81.42
	C_1—Top	C	16.14	16.55	15.68	16.01	16.34	16.57	2.26	2.34	2.30	2.25	2.26	2.39	81.60	81.11	82.02	81.74	81.41	81.04
		E	16.03	16.61	15.61	16.02	16.27	16.59	2.30	2.28	2.31	2.25	2.38	2.35	81.67	81.11	82.08	81.73	81.35	81.06
		O	16.11	16.60	15.66	16.00	16.36	16.57	2.24	2.30	2.34	2.28	2.27	2.40	81.65	81.11	82.00	81.72	81.37	81.03
	C_1—Middle	C	16.09	16.63	15.65	16.00	16.37	16.55	2.24	2.28	2.37	2.31	2.42	2.48	81.67	81.09	81.98	81.69	81.21	80.97
		E	16.04	16.61	15.66	16.02	16.34	16.51	2.31	2.34	2.35	2.26	2.26	2.44	81.65	81.05	81.99	81.72	81.40	81.05
		O	16.12	16.59	15.68	15.96	16.38	16.51	2.26	2.33	2.34	2.30	2.29	2.39	81.67	81.08	81.98	81.74	81.33	81.10
	C_1—Bottom	C	16.03	16.66	15.67	16.01	16.36	16.54	2.29	2.34	2.33	2.30	2.39	2.38	81.68	81.00	82.00	81.69	81.25	81.08
		E	16.03	16.64	15.70	16.00	16.36	16.54	2.35	2.37	2.31	2.31	2.31	2.43	81.62	80.99	81.99	81.69	81.33	81.03
		O	16.03	16.61	15.69	15.99	16.38	16.54	2.18	2.40	2.33	2.34	2.39	2.45	81.79	80.99	81.98	81.67	81.23	81.01

[1] Collaborator.
[2] Room temperature when sample was prepared.
[3] C—Center; E—Edge; O—Any point about one inch from center.

This system should be used for the middle and bottom layers, except that the samples from the edges should not be directly beneath each other.

STATISTICAL ANALYSIS

The purpose of this investigation was to determine which of three methods of preparing butter samples is the most effective. In order to test the effectiveness of the methods, nonhomogenous jars of butter were mixed by the three different methods and samples were taken from nine

TABLE 2.—*Composition of the different lots of butter used to make the collaborative samples*

LOT NO.	SUB NO.	MOISTURE	CURD AND SALT	BUTTERFAT
		per cent	*per cent*	*per cent*
1	1	17.16	1.14	81.70
	2	17.20	1.16	81.64
2	1	17.80	0.80	81.40
		17.75	0.79	81.46
3	1	16.80	2.87	80.33
	2	16.79	2.87	80.34
4	1	15.15	3.15	81.70
	2	15.12	3.11	81.77
5	1	16.65	3.10	80.25
	2	16.67	3.08	80.25
6	1	15.47	3.21	81.32
	2	15.48	3.19	81.33

locations within each jar. The criterion for estimating the effectiveness of the mixing was the uniformity or lack of dispersion of the observations within a jar; the measure of dispersion was the standard deviation of results. The butter was analyzed for per cent of moisture and per cent of curd and salt; the per cent of butterfat was obtained by differences. For purposes of statistical control a design was used so that each of six analysts analyzed two different jars of butter by each method (each analyst analyzing six jars altogether), and took nine samples from specified positions in each jar.

Figures 1 and 2 show the results on butterfat and moisture, respectively. The values within a jar seem to be distributed randomly; that is, there is no tendency for any layer or position to deviate consistently from the others. The graphs show that Method II is not as effective as Methods I and III, since the points for each jar in Method II are, on the average, spread farther than those for Methods I and III. Table 3 shows the "within jar" standard deviations for each method. Tests of significance show

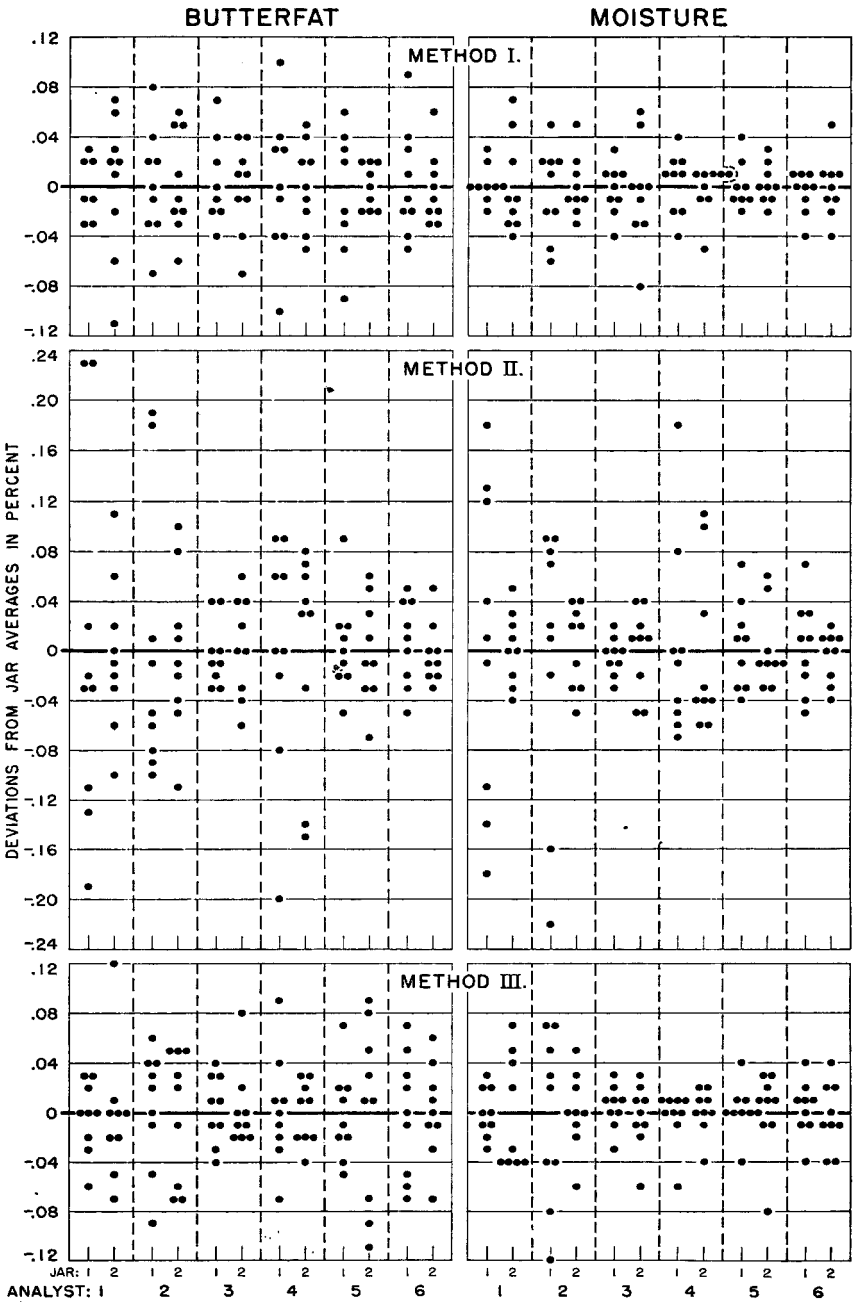
BUTTERFAT
MOISTURE
METHOD I.
METHOD II.
METHOD III.
DEVIATIONS FROM JAR AVERAGES IN PERCENT
.12
.08
.04
0
-.04
-.08
-.12
.24
.20
.16
-.16
-.20
-.24
JAR: 1 2
ANALYST: 1 2 3 4 5 6

FIG. 1 FIG. 2

Method I to be significantly more effective than Method III for moisture, but not for butterfat. The effectiveness of Method II is very significantly less than the other methods for both moisture and butterfat.

TABLE 3.—*Standard deviation "within jar"*

METHOD	BUTTERFAT	MOISTURE
I (hand shaking)	.041	.026
II (machine shaking)	.074	.063
III (Meuron mixer)	.045	.033

In addition, the data was analyzed for analyst differences for each method. This was to guard against the possibility that one method, perhaps because of greater complexity, might introduce a "between analyst" variation even though the "within jar" variation for that method was small. The data showed no significant difference between analysts for any of the three methods.

COMMENTS OF COLLABORATORS

Hugh M. Boggs: Subs A and B were liquid before shaking. Subs A_1 and B_1 were never liquid before shaking but handled under ideal conditions. Speed of shaking machine on Sub B was not as high as it should have been. Still prefer Method II.

Matthew L. Dow: The samples were examined during a spell of 90° weather which would approximate St. Louis weather conditions in the summertime. It was not possible to cool the butter by shaking in air to a consistency desirable for sampling. A small amount of separation may have taken place in some jars before sampling was completed. However there was no separation visible in any jars during sampling period. The machine shaking method appears to be the most practical method to use for routine work.

H. J. Meuron: When using the mechanical shaker, the butter whipped into a foam and I was not able to adjust the machine to prevent this. Last year I did not have this trouble and it may be in the butter itself. I believe foaming prevented proper mixing. Except for this difficulty I can see no objection to using a mechanical shaker.

J. Phyllis Skyrme: The Meuron stirrer is somewhat difficult to use when temp. is below 27°C.

Comments of Associate Referee. There is no doubt, as the statistical analysis of the results in Table 3 show, that hand shaking (Method I) and the Meuron stirrer (Method III) are good efficient methods. The mechanical shaker (Method II) is somewhat less efficient, but efficient enough for practical work. From comments of the collaborators and from personal observation I believe the mechanical shaker method can be made as efficient as hand shaking. I believe it has to do with the proper speed of the machine. An attempt will be made to find out if this is so, and what the speed should be.

In conjunction with this work a survey of many laboratories was made concerning the use of the mechanical stirrer as described in the 7th Ed. Book of Methods of the A.O.A.C., Paragraph **15.106.** Only three ever used this method and then but seldom. Only one at first objected to dropping this method; most would like to see it deleted.

REWORDING THE SHAKING METHOD

From a review of studies made by previous associate referees (Vorhes (1), Meuron (2), and the comments of Mathews (3) regarding the temperature of softening), together with previous reports of this writer (4) and the comments of the many collaborators who have participated in these studies, the following wording of the directions for choice of sample container and for the shaking method of sample preparation is recommended:

Paragraph **15.104(c):** Sample Containers: delete "preferably with glass tops."

Paragraph **15.105:** Soften entire sample in the sample container **15.104(c)** by warming in a water bath maintained at as low a temp. as practicable, not over 39°. Avoid overheating which results in visible separation of curd. Shake at frequent intervals during the softening process to reincorporate any separated fat and observe fluidity of sample. Optimum consistency is attained when the emulsion is still intact but fluid enough to reveal almost immediately the sample level. Remove from bath and shake vigorously at frequent intervals until sample cools to a thick creamy consistency and sample level can no longer readily be seen. Weigh portion for analysis promptly.

ACKNOWLEDGEMENT

The writer wishes to thank the following collaborators for their kind cooperation in the above work. Hugh M. Boggs, Mathew L. Dow, George E. Keppel, Herman J. Meuron, and J. Phyllis Skryme, all from the Food and Drug Administration. Also thanks to certain members of the Division of Food of the Food and Drug Administration, for their kind and helpful suggestions.

RECOMMENDATIONS*

It is recommended—

(1) That paragraphs **15.106** and **15.107** be deleted.

(2) That pargraphs **15.104(c)** and **15.105** be reworded as described above and made official.

(3) That further collaborative work be done, particularly with the mechanical shaker.

REFERENCES

(1) FRANK H. VORHES, JR., *This Journal*, **29**, 119 (1946).
(2) H. J. MEURON, *Ibid.*, **31**, 318 (1948).
(3) J. H. MATHEWS, *Ibid.*, **23**, 458 (1940).
(4) ALBERT L. WEBER, *Ibid.*, **33**, 544 (1950); **34**, 243 (1951).

* For report of Subcommittee C and action of the Association, see *This Journal*, **35**, 50 (1952).

REPORT ON FAT IN DAIRY PRODUCTS

METHODS FOR STANDARDIZING THE BABCOCK TEST

By E. O. Herreid, *Associate Referee*, L. H. Burgwald, B. L. Herrington, and E. L. Jack (Illinois, Ohio, New York, and California Agricultural Experiment Stations, respectively)

The Babcock fat test has been reported to yield results from 0.05 to 0.07 per cent higher for milk than does the ether extraction Mojonnier method. Investigators in experiment stations and technicians in commercial laboratories have recognized this fact for a long time. They have also known that the upper meniscus on the fat column is a variable factor in reading the Babcock test for fat in milk.

The authors compose a committee in the American Dairy Science Association* appointed to study methods for standardizing the Babcock test so as to obtain, as closely as possible, statistical agreement with the ether extraction method. It was agreed that a simple way to do this was to increase the volume of the milk sample and to eliminate the meniscus. Since the meniscus is included in the present Babcock test, it is necessary to increase the weight of the milk sample from 18.00 to 18.36 grams in order to obtain agreement with the ether extraction method. It was found that an 18.05 ml pipet delivered on the average 18.36 grams of milk of average specific gravity 1.025 at 35 to 36°C.

The modified Babcock test is conducted as follows:

(A) Heat milk samples to 35–38°C.; (B) mix thoroly by pouring four times from one container to another; (C) fill 18.05 ml pipet so that upper surface of the milk is at the graduated mark on the draw tube; (D) drain pipet into test bottle for 10–15 seconds after free outflow has ceased and blow out milk in the tip. Cool samples to 20–21°C. before adding acid; (E) have H_2SO_4 (sp. gr. 1.82–1.83 at 20°C.) at 20–22°C. The amount will vary from 15–17 ml depending on its strength; (F) proceed as in the regular Babcock method; (G) add 2 drops of colored mineral oil (glymol sp. gr. not to exceed 0.85 at 20°C.) as each bottle is taken from a water bath at 57.3–60°C. The oil must flow down the sides of the neck and must not be dropped onto the fat; (H) measure the fat from the bottom of the column to the fat-glymol line.

The Babcock test has been used for nearly 60 years without any significant refinements except for more accurate glassware and better centrifuges. For this reason it was thought advisable to submit this modified Babcock method to leaders in the dairy industry, to representatives in the colleges and experiment stations, and to various regulatory officials in order to obtain expressions of opinion regarding the desirability and practicability of changing this long established test. To obtain this information a letter was formulated, in which the modified test was described and from

* E. O. Herreid, L. H. Burgwald, B. L. Herrington, and E. L. Jack. "Standardizing the Babcock Test for Milk by Increasing the Volume of the Sample and Eliminating the Meniscus on the Fat Column." *J. Dairy Sci.*, 33 (10), 685–691. 1950.

which the following paragraph is quoted. "Do you approve of the principles involved: (1) changing the size of the pipet to secure better agreement between the Babcock test and the Mojonnier test; (2) using glymol to improve the precision of reading the tests; and (3) adopting a single temperature for pipetting all samples whether fresh or preserved? Would you advocate that these changes, if based on adequate experimental data, be incorporated into the Babcock regulations of your state?" This letter was sent to regulatory officials, experiment station and college officials and to representatives in industry in the 48 states and to the Canadian provinces. The replies are summarized in Table 1.

TABLE 1.—*Summary of replies to questions in regard to a proposed modification of the Babcock test for milk*

NUMBER:	REGULATORY AGENCIES[1]	COLLEGES AND EXPERIMENT STATIONS	DAIRY INDUSTRY		TOTAL
			U. S.	CANADA	
Replies	61	52	91	12	216
States or stations	36	42	26	5	
Favoring all or most of test	28	27	61	7	123
Opposing all or most of test	15	17	20	2	54
Noncommittal or ambiguous	18	8	10	3	39
Specific replies:					
Change of pipet					
Favor	15	18	52	3	88
Oppose	0	5	2	1	8
Glymol					
Favor	18	19	53	4	94
Oppose	1	7	3	1	12
Temperature of sampling					
Favor	16	20	52	3	91
Oppose	0	4	1	1	6

[1] Includes Departments of Public Health and Departments of Agriculture.

A total of 216 replies were obtained from 91 persons in the dairy industry in 26 States, from 61 in the regulatory agencies in 28 States, from 52 in the agricultural colleges and experiment stations in 42 States, and from 5 provinces in Canada. While it is extremely difficult to interpret all aspects of Table 1, it can be conservatively stated that there is much interest in the Babcock test for milk and that a considerable number of persons are interested in changing the present test methods.

The modified Babcock test has been published[1] with supporting data. It was compared with the Mojonnier ether extraction method by 14 collaborators on 232 samples of unpreserved milk. The data submitted by five experienced collaborators on 135 samples of unpreserved milk indicate that the modified method is accurate. They obtained mean dif-

[1] Chairman and members, respectively, of Subcommittee in the Manufacturing Section of the American Dairy Science Association to Standardize the Babcock Test for Milk Fat to agree with the Mojonnier ether extraction method.

ferences between the two methods of ±0.01 per cent. Another collaborator made 27 comparisons and the Mojonnier method yielded higher results by ±0.04 per cent. All the other collaborators did not present sufficient data from which to draw conclusions as to their ability to obtain accurate results with the modified method. Four collaborators mentioned their inexperience with the Babcock method as probably being responsible for the wide variations which they obtained between both methods.

Assuming that a modification of the Babcock test is found acceptable to the dairy industry and to the Association of Official Agricutural Chemists, the task of making it legal will not be easy. It will be necessary to change existing laws in at least 13 States in order to make a modified method legal. Obviously, any method would have to be accepted by all 48 States. A convenient arrangement would be for all States to have a simple law stating that the Babcock test shall be conducted according to the method prescribed by the Association of Official Agricultural Chemists; in fact, the laws in a few States follow this plan.

REPORT ON SAMPLING, AND PREPARATION OF SAMPLE, OF SOFT CHEESES

By Sam H. Perlmutter, *Associate Referee*, and William Horwitz
(Food and Drug Administration, Federal Security Agency, Minneapolis, Minn., and Washington, D. C.)

The heterogeneous nature of creamed cottage cheese makes the sampling of this product a rather difficult operation. On standing the cream tends to rise to the top and the curd absorbs cream in amounts which vary with the manufacturing process and the size of the curds. Since large curds are fragile, manipulation of the product during sampling must be kept to a minimum. The object of this study was to develop a practical method of sampling commercial containers of creamed cottage cheese. Since fat is the most valuable ingredient of this product, and since it would be most sensitive to changes in composition, the work was confined to this ingredient.

PREPARATION OF SAMPLE

Previous work by Steagall (1) showed that the Waring Blendor was suitable for the preparation of creamed cottage cheese. This was confirmed in the present study. Nine 12 ounce retail packages of creamed cottage cheese representing at least three different day's pack of a single manufacturer were prepared in the blendor and the fat was determined in quintuplicate by the official method (2). The results of this experiment are presented in Table 1. In addition, 15 different samples prepared in the same manner were analyzed for fat in duplicate with the results presented

in column 1 of Table 2. The excellent agreement within replicates in these two experiments demonstrates both the suitability of the method of preparing the sample and the method of analysis.

TABLE 1.—*Quintuplicate fat determination on nine different retail packages of creamed cottage cheese prepared in the Waring Blendor*

CARTON NO.								
1	2	3	4	5	6	7	8	9
per cent fat								
4.20	4.33	4.29	4.27	4.17	4.15	4.18	4.13	4.38
4.19	4.25	4.29	4.21	4.17	4.14	4.12	4.09	4.39
4.18	4.31	4.29	4.18	4.19	4.13	4.15	4.15	4.40
4.18	4.28	4.27	4.22	4.22	4.13	4.13	4.09	4.39
4.19	4.30	4.25	4.20	4.23	4.12	4.18	4.10	4.39

Because of the variation of the physical characteristics of the creamed cottage cheese, from sample to sample, the time required for the preparation will vary according to the characteristics of the product. With some samples the mixing and reduction in the size of the curds is almost immediate, while with others which are stiff, channeling occurs immediately. When this happens, the action of the blendor is stopped and the sample is spooned down into the blades and the process repeated until the blending action starts without channeling and a uniform, smooth product results.

METHOD OF ANALYSIS FOR FAT

During the preliminary stages of this investigation erratic results were obtained which were first ascribed to the method of preparation of the sample. Further work, however, demonstrated that the difficulty was caused by incompletely controlling the digestion time in the official Roese-Gottlieb method for fat in cheese (2). A full five minutes of actual boiling (not simmering) was necessary to completely liberate all of the fat. A few carborundum chips were used to control bumping. When precautions were taken to insure complete digestion, the very uniform replicate results in Tables 1 and 2 were obtained. Digestion of the samples in the Mojonnier tube as previously proposed for the harder cheeses (3) led to erratic results.

SAMPLING

Commercial cans of creamed cottage cheese ranging in net weight from 22 to 75 pounds were used in this study. In all cases the container and contents were allowed to stand undisturbed in the creameries' cooler for from 15 to 24 hours before sampling to insure a heterogeneous product.

Since it was thought that by proper choice and placement of a trier a representative sample of the creamed cottage cheese could be obtained, the cans were first sampled with triers. Three types of triers were used.

First, a grain trier with compartments, which was inserted into the can with the compartments open. These were closed after the trier was in the can for about 10 seconds. After the first trial, however, this trier was discarded because of the difficulty in cleaning it from sample to sample. Second, a quart size cream sediment tester barrel without the plunger was tried. This trier was rather heavy and it was too small in diameter. Finally, a frozen fruit trier, of about two inches inside diameter, was found to be light enough to handle easily and yield about a pint sample, and this trier was used in all subsequent experiments.

In general, all sampling experiments followed the same pattern. Samples were withdrawn with the trier or a spoon, placed in a pint jar, and assigned a number in accordance with its original position in the container. The following subdivision numbers were used to designate samples taken from specific positions of the containers:

Sub 1: A vertical core taken with a trier through the center of the can with or without preliminary mixing.

Subs 2, 3, and 4: Vertical cores taken with the trier near the outer edge of the container 120° apart.

Subs 5, 6, and 7: Diagonal cores taken with a trier from the outer edge to the opposite bottom rim at alternate spaces from subs 2, 3, and 4.

Subs 8 to 15 inclusive: The remainder of the can, after sampling with the trier, was transferred layer by layer with a large spoon into 8 separate containers. These layers, all of approximately the same size, were thoroughly mixed with a large spoon or dairy stirrer, and as they were returned in random order into the original container many small samples were removed with a small spoon and placed in a pint jar prior to sample preparation with the Waring Blendor. Sub 8 represents the top layer of the container and successive sub numbers represent the subsequent layers until sub 15, the bottom layer, was reached.

Subs 16, 17, 18, and 19: The creamed cottage cheese, after being sampled by layers and returned to the original container, was again thoroughly remixed with a large spoon or dairy stirrer and sampled by diagonal cores as in subs 5, 6 and 7.

Sub 20: When it became apparent that mixing with the dairy stirrer gave a relatively uniform product, this subdivision (replacing one of subs 16–19) was removed from the top of the can after remixing and before any of the final trier subdivisions were taken.

RESULTS

The first three cans were sampled as they were found in the cooler after standing overnight. The trier subdivisions 1 through 7 were obtained before any mixing was done. A different type of trier was used with each can and the results are given in Table 2.

TABLE 2.—*Fat determinations of various samples of creamed cottage cheese from a commercial container without initial mixing*

Creamery A—Per cent fat

EXPERIMENT NO.		1	2	3
SIZE OF CONTAINER, POUNDS		30	22	30
TYPE OF TRIER		GRAIN	CREAM SEDIMENT TESTER	FROZEN FRUIT
Sample	*Sub*			
Trier	1	4.54	3.71	4.81
		4.50		
	2	4.23	3.64	3.71
		4.25		
	3	5.14	3.68	4.39
		5.14		
	4	5.00	3.84	4.05
		4.98		
	5	5.10	3.74	4.25
		5.12		
	6	4.69	3.88	4.64
		4.66		
	7	4.84	3.69	4.85
		4.84		
Layers	8	4.80	3.89	4.28
		4.85		
	9	4.21	3.57	3.77
		4.24		
	10	4.22	3.43	3.91
		4.21		
	11	4.06	3.36	3.85
		4.06		
	12	4.04	3.29	3.67
		4.02		
	13	3.97	3.30	3.49
		3.99		
	14	3.95	3.19	3.47
		3.95		
	15	3.62	3.16	3.28
		3.63		
Trier	16	4.38 4.39	3.46	4.10
		4.40 4.39		
		4.39		
Average of all subdivisions		4.43	3.55	4.04
Average of initial triers (1–7)		4.79	3.74	4.39
Average of layers		4.11	3.43	3.73

The first conclusion from this set of experiments was that triers gave high fat results as compared with the average of the layers which had to be accepted as the best available estimate of the fat content of the container. This could be explained by assuming that the liquid cream flowed into the interstices and therefore gave a high fat sample. It should also be noted that there was a lowering of the fat content from layer to layer from top to bottom of the can, verifying by analysis the obvious appearance of a can of creamed cottage cheese which had stood overnight to allow the cream to rise to the top. The first 15 subs of the first experiment were

TABLE 3.—*Fat determination of various samples of creamed cottage cheese from a commercial container with initial mixing*

(Trier samples were removed with the frozen fruit trier)

Creamery A—Per cent fat

EXPERIMENT NO.		4	5	6	7	8
SIZE OF CONTAINER		30	30	30	75	75
MIXER:		SPOON	DAIRY STIRRER	DAIRY STIRRER	DAIRY STIRRER	DAIRY STIRRER
Sample	*Sub*					
Initial triers	1	4.15	4.23	3.48	4.61	4.71
	2	4.43	4.26	3.62	4.31	4.35
	3	4.38	4.44	3.46	4.82	4.66
	4	4.50	4.24	3.43	4.72	4.89
	5	4.50	4.64	3.66	4.56	4.48
	6	4.37	4.55	3.54	4.40	4.36
	7	4.50	4.44	3.50	4.40	4.35
Layers	8	4.56	4.33	3.45	4.48	4.46
	9	4.43	4.34	3.54	4.43	4.55
	10	4.37	4.37	3.51	4.30	4.55
	11	4.26	4.34	3.47	4.42	4.58
	12	4.12	4.19	3.59	4.35	4.49
	13	3.98	4.30	3.57	4.30	4.42
	14	3.96	4.29	3.50	4.01	4.41
	15	3.64	4.39	3.52	4.09	4.44
Triers after remixing	16	4.28	4.62	3.57	4.63	4.44
	17	4.18	4.49	3.63	4.38	4.44
	18	4.42	4.34	3.63	4.22	4.54
	19	4.31				
Top before 16–18	20					4.54
Average all subs		4.28	4.38	3.54	4.41	4.51
Average initial triers		4.40	4.40	3.53	4.54	4.54
Average layers (8–15)		4.17	4.32	3.52	4.30	4.54
Average final triers (16–20)		4.30	4.48	3.61	4.41	4.49

TABLE 4.—*Fat determinations from various samples of creamed cottage cheese from a commercial container. Initial mixing with dairy stirrer*

(Trier samples removed with the frozen fruit trier)

Per cent fat

EXPERIMENT NO.		9	10	11
CREAMERY		B	B	C
SIZE OF CONTAINER, POUNDS		50	50	50
Sample	*Sub*			
Initial triers	1	3.93	4.25	5.12
	2	3.89	4.43	5.20
	3	3.84	4.79	5.08
	4	3.82	4.85	5.15
	5	4.13	4.33	5.45
	6	4.40	4.40	5.45
	7	3.91	4.44	4.89
Layers	8	3.93	4.57	5.01
	9	4.04	4.49	4.93
	10	4.09	4.52	4.96
	11	3.90	4.43	4.87
	12	3.88	4.38	4.93
	13	3.92	4.40	4.99
	14	3.85	4.45	4.88
	15	3.83	4.35	4.85
Triers after mixing	16	4.19	4.55	5.24
	17	4.07	4.48	5.59
	18	4.02		
Top before 16–18	20	4.02	4.46	5.03
Average all subs		3.98	4.48	5.09
Average initial triers		3.99	4.50	5.19
Average layers (8–15)		3.93	4.45	4.93
Average final triers		4.09	4.52	5.42

run in duplicate and the last sub was run in quintuplicate. The excellent reproducibility as shown in this experiment as well as those listed in Table 1 led to the conclusion that the time spent in replications could better be assigned to additional sampling experiments.

The next five sampling experiments were performed in the same manner as the previous three but the can was stirred thoroughly for about 5 minutes with a large spoon in experiment 4 and with the conventional dairy stirrer (a $5\frac{1}{2}$ inch perforated concave metal disk attached to a 27 inch metal rod) in the other experiments before any samples were removed. The frozen fruit trier was used in these and all subsequent experiments. The results are given in Table 3.

The marked improvement shown with the introduction of the dairy stirrer is immediately apparent except in experiment 7 which was the first of the larger commercial sized containers. In the latter case the stirrer was not placed sufficiently deep in the can to reach the bottom layers, as shown by the sudden decrease in fat content in subs 14 and 15. It also becomes apparent at this point that if thorough mixing was achieved, a sample from the top layer should be just as good as attempting to obtain a satisfactory trier sampling position. Additional experiments with cottage cheese from two other creameries confirmed this observation as shown in Table 4.

In order to determine if commercial containers of creamed cottage cheese shipped by express could also be sampled after thorough mixing with the dairy stirrer, two cans of cheese were shipped, one from Chicago and one from St. Louis, to Minneapolis. The containers were wrapped with an insulating bat prior to shipment to keep them cool. Both cans arrived in good condition. The Chicago sample was relatively homogeneous but

TABLE 5.—*Fat determinations from various samples of creamed cottage cheese after mixing with dairy stirrer*

(Samples shipped by express overnight)

Per cent fat

EXPERIMENT NO.		12	13
CREAMERY		CHICAGO	ST. LOUIS
SIZE OF CONTAINER, POUNDS		30	32
Sample	*Sub*		
From top after mixing	8a	4.33	4.84
	8b	4.31	4.87
	8c	4.31	4.88
Layers	8	4.26	4.84
	9	4.23	4.82
	10	4.33	4.73
	11	4.25	4.74
	12	4.24	4.80
	13	4.27	4.66
	14	4.20	4.75
	15	4.30	4.86
From top after remixing	20a	4.26	4.79
	20b	4.28	4.81
	20c	4.27	4.76
Average all subs		4.27	4.80
Average layers (8–15)		4.26	4.78
Average top (orig.)		4.32	4.86
Average top (remixed)		4.27	4.79

the St. Louis sample had a relatively large amount of separated cream on top.

These two cans were not sampled by triers but were mixed with the dairy stirrer for five minutes and three separate samples (subs 8a, 8b, 8c) were removed with a spoon from the top of the container. The remainder of the cheese was layered as in the previous experiments (subs 8–15), the layers were returned to the can, and the contents were remixed with the dairy stirrer. The top layer was then resampled (subs 20a, 20b, 20c) as before. The results are shown in Table 5.

It is apparent that creamed cottage cheese shipped for a longer distance than customary can also be successfully sampled by the proposed procedure.

DISCUSSION

The most interesting conclusion of this study was the unsatisfactory nature of a sample taken with a trier either before or after mixing, both with respect to accurately representing the fat content of the can and with respect to reproducibility. It is interesting to compare the sample from the top layer (sub 8) after thorough mixing with the average of the layers (best value for the fat content of the can) and the sample from the top layers after thorough mixing, layering, and random replacement of the layers (sub 20). A summary of this data is given in Table 6.

TABLE 6.—*Fat content of the top layer of a can of creamed cottage cheese after mixing and remixing as compared with the "best" value for the can (average of the fat content of the layers)*

(Mixed with dairy stirrer)

EXPERIMENT	TOP LAYER AFTER MIXING (SUB 8)	TOP LAYER AFTER LAYERING AND REMIXING (SUB 20)	"BEST" VALUE FOR THE CAN (AVERAGE) SUBS 8–15)
5	4.33		4.32
6	3.45		3.52
7	4.48		4.30*
8	4.56	4.54	4.54
9	3.93	4.02	3.93
10	4.57	4.46	4.45
11	5.01	5.03	4.93
12	4.26 (4.33, 4.31, 4.31)	4.26, 4.28, 4.27	4.26
13	4.84 (4.84, 4.87, 4.88)	4.79, 4.81, 4.76	4.78

* Omitting the last two layers, 14 and 15, which were obviously not thoroughly mixed, the "best" value is 4.38.

METHOD

As a result of these experiments the following method for sampling creamed cottage cheese is recommended:

Stir the can thoroly for at least 5 min. with a dairy stirrer (a 5½ inch perforated concave metal disk attached to a 27 inch metal rod as a handle) so that all portions

of the container are reached. Remove portions from the top with a small spoon to fill a pint jar, and cover. Prepare sample for analysis by placing in the cup of the Waring Blendor and blend until a smooth homogeneous mixture is obtained. This may require stopping the blendor frequently after channeling and spooning the cheese back into the blades until the blending action starts.

SUMMARY

A study of the sampling of commercial containers of creamed cottage cheese showed that trier sampling before or after mixing gives results which are high in fat. A satisfactory procedure requires mixing the container with the dairy stirrer and removing a pint of the creamed cottage cheese from the top of the can. This sample is prepared in the Waring Blendor.

REFERENCES

(1) E. F. Steagall, Food and Drug Administration. Unpublished report on the preparation of samples of creamed cottage cheese.
(2) *Methods of Analysis, A.O.A.C.*, Seventh Edition, 1950, **15.131.**
(3) W. Horwitz and L. F. Knudsen, *This Journal*, **31**, 300 (1948).

RECOMMENDATIONS*

It is recommended—

(1) That the described method for the sampling and preparation of creamed cottage be adopted as a procedure.

(2) That further work on sampling and preparation of sample of other types of soft cheese be continued.

REPORT ON FROZEN DESSERTS

By Hugh M. Boggs (U. S. Food and Drug Administration, Federal Security Agency, Philadelphia, Pa.), *Associate Referee*

At the suggestion of the Referee on Dairy Products a comparison was made between weighing samples for fat according to *Methods of Analysis* **15.153,** that is, into a beaker and then transferring to a Mojonnier flask, or weighing directly into the Mojonnier flask. This comparison was carried out by four collaborators in three laboratories on thirty-seven samples, and showed an average difference of only 0.018 per cent. Results are given in accompanying table.

The work done by the various Districts and the Washington laboratories of the U. S. Food and Drug Administration on frozen desserts has demonstrated the desirability of the inclusion in the frozen desserts section of the *Methods of Analysis* methods for sucrose, by polarization at 20°C. before and after inversion, *p*H, and titratable acidity. A questionnaire submitted to the sixteen Districts showed that the methods used on the whole were satisfactory. The method for sucrose, however, while rated as

* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 50 (1952).

Fat in ice cream by method **15.153** *and by direct weighing into Mojonnier flask*

FLAVOR	FAT MOJONNIER	(15.153) FAT BEAKER	DIFFERENCE
	per cent	*per cent*	*per cent*
Chocolate Chip	12.35	12.20	−0.15
Vanilla	12.94	12.96	+0.02
French Vanilla	12.79	12.89	+0.10
Maple Nut	12.87	12.87	0.00
Vanilla	12.55	12.62	+0.07
Peppermint Candy	12.32	12.30	−0.02
Chocolate	12.15	12.27	+0.12
Vanilla-Cherry	11.81	11.84	+0.03
Vanilla	12.05	11.91	−0.14
Vanilla	11.62	11.39	−0.23
Vanilla	12.31	12.25	−0.06
Vanilla	12.07	11.93	−0.14
Vanilla	13.19	13.16	−0.03
Vanilla Ice Cream	12.54	12.56	+0.02
Butter Pecan Ice Cream	17.31	17.20	−0.11
Butter Pecan Ice Cream	14.87	14.85	−0.02
Fudgsicle Pop	0.70	0.71	+0.01
Strawberry Ice Cream	13.73	13.74	+0.01
Chocolate Ice Cream	20.18	20.08	−0.10
Vanilla	13.95	13.88	−0.07
Vanilla	11.83	12.03	+0.20
	11.75	11.91	+0.16
Banana	13.60	13.60	0.00
Vanilla	12.33	12.33	0.00
Vanilla	11.83	11.95	+0.12
Vanilla	10.74	10.63	−0.11
	10.62	10.56	−0.08
Peach	15.15	15.20	+0.05
Vanilla	10.88	10.89	+0.01
Vanilla	10.50	10.43	−0.07
Chocolate	10.64	10.62	−0.02
Vanilla	12.54	12.62	+0.06
Chocolate	15.58	15.44	−0.14
Chocolate	14.48	14.43	−0.05
Vanilla	12.51	12.40	−0.11
Vanilla	12.17	12.18	−0.01
Average			−0.018

fairly satisfactory by many analysts, has been objected to by others, and other additional objections have been discovered by the Associate Referee in the literature and in his subsequent experimental work.

SUGARS

The method for sucrose used in this work was **15.90,** which appears in the section on sweetened condensed milk. The literature reference (1)

indicates that this method was taken from the literature in 1902, and no indication can be found that any collaborative study was ever made of it. It was adopted in 1916, being listed as Patrick's Method and using Patien and Dufaru's (1) reagent.

Knight and Formanek (2) in 1916 proposed a method for sucrose in sweetened condensed milk based on polarization before and after inversion, after clarification with phosphotungstic acid and neutral lead acetate. These authors rejected mercuric nitrate as a clearing agent, basing their rejection on the work of Richmond (3). The reagent in question was acid mercuric nitrate rather than the neutral solution used in **15.90.** However, most of the same objections can be urged against the neutral reagent, particularly incomplete precipitation of the milk proteins and also the fact that the solutions contain Hg^{++} ions. The above mentioned paper (2) forms the basis for the U. S. Customs present tentative methods for confectionery, condensed milk products, milk powders, etc.(4).

In 1930 a committee of the British Society of Public Analysts reported on the determination of sucrose in sweetened condensed milk (5). They recommended determination of sucrose by polarization, before and after inversion. As clearing agents they recommended zinc acetate and potassium ferrocyanide, but also found phosphotungstic acid quite satisfactory. Collaborative results are given for both methods.

The Referee has found neutral lead acetate to be a satisfactory clearing agent.

Any of the above clearing agents are preferable to the neutral mercuric nitrate used in **15.89,** which is hard to prepare properly, difficult to keep, and difficult to use (the proper neutralization point with 0.5 *N* NaOH is hard to determine), and gives solutions that become cloudy on standing, that cannot be inverted with invertase, and that contains mercuric ions (Hg^{++}).

The Associate Referee's experimental work has convinced him that polarimetric methods are superior to chemical methods for sucrose in ice cream. This conclusion is also borne out in the British report (5).

A method for sucrose in ice cream has been developed by the Associate Referee, using larger samples (52 g/200 ml) so as to get larger readings on the saccharimeter, after clearing solutions with neutral lead acetate and deleading with potassium oxalate.

It is intended, after a little additional experimental work, to submit samples to collaborators so that this method may be made official.

OTHER DETERMINATIONS

Of the other determinations made for the use of the food standards committee the determination of solids by paragraph **15.151** was routine, and any trouble encountered was undoubtedly due to failure in keeping samples properly mixed while weighing. This point cannot be over-

emphasized, and some limited re-mixing should be done before the weighing out of every sample for a particular determination.

The *p*H and titratable acidity determination should be performed immediately after the sample is prepared, and the prepared sample should be returned to the deep freeze in a closed container as soon as samples for these and other determinations are taken. The weighing of further samples necessitates a careful re-melting and thorough re-mixing. The making up of a sample solution as in **15.82(b)** was found to be an unsatisfactory procedure by most analysts.

A procedure for the preparation of samples was tried which provided that with fruit, nut, and candy ice creams the solid matter should be removed on a 30 mesh sieve, and analysis be made on the fluid portion with due allowance for the solids. This gave the composition of the original mix as well as the finished ice cream.

RECOMMENDATIONS*

It is recommended—

(1) That under **15.150(b)** "Frozen desserts containing insoluble particles," the present paragraphs shall be designated **(b1)** and an alternative paragraph added: "Or **(b2)** put the melted sample through a 30 mesh screen to remove particles. The weight of the particles removed should be reported as the per cent of the sample. Description of material separated should be reported."

(2) That a method for total acidity based on **15.4** be inserted in the frozen dessert section; by reference to **15.4** followed by the following statement: "Using a 20 gm sample, each ml of 0.1 *N* alkali is equivalent to 0.045% acidity expressed as lactic acid."

(3) That **15.153** "Fat" be changed by substitution for the first sentence in the method the following: "Weigh accurately 4–5 g of thoroughly mixed sample directly into a Mojonnier fat-extn. flask, using a short, bent, free flowing pipet; dilute with H_2O to ca 10 ml, working the charge into the lower chamber and mixing by shaking."

(4) That further work be done on a method for sucrose in ice cream and that this method be submitted to collaborative work.

SELECTED REFERENCES

(1) (*J. Pharm. Chem.*, **15**, 221 (1902); (*Z. Unt. Nahr. Genussm.* **5**, 726 (1902).
(2) *J. Ind. Eng. Chem.*, **8**, 28 (1916).
(3) *Analyst*, **35**, 516 (1910).
(4) U. S. Customs Tentative Method, **506.1 to 506.47.**
(5) *Analyst*, **55**, 111 (1930).

No reports were given on tests for reconstituted milk; phosphatase test in dairy products; or sampling, fat, and moisture in hard cheeses.

* For report of Subcommittee C and action of the Association, see *This Journal*, **35**, 50 (1952).

The contributed paper entitled "Water-Insoluble Fatty Acids and Butyric Acid in Butter Manufactured by the 'Continuous' Process," by Fred Hillig and S. W. Ahlmann, and the paper entitled "Effect of Excess Alkali in the Determination of Water-Insoluble Fatty Acids in Butter," by Fred Hillig, were both published in *This Journal*, November, 1951, pages 777 and 782, respectively.

REPORT ON TOTAL SOLIDS IN FISH AND OTHER MARINE PRODUCTS

By MENNO D. VOTH (Food and Drug Administration, Federal Security Agency, Boston, Mass.), *Referee*

The determination of moisture in fish and other marine products has been the subject of considerable study.[1] Because of the characteristics of the flesh of most marine products the usual methods of drying result in a hornlike crust which traps residual moisture, making a longer heating time necessary. Various procedures for determining total solids in marine products have been investigated by the Referee. Definite conclusions regarding the most suitable method were reached.

The constant temperature oven at 100°C. was considered superior to the vacuum oven.

TABLE 1.—*Results obtained in vacuum oven and constant temperature oven*

(Average percentage, total solids)

PRODUCT	VACUUM OVEN 4–8 HRS @ 70°C.	AIR OVEN 4–6 HRS @ 100°C.
Fresh Haddock	19.00	18.88
Fresh Cod	19.83	19.64
Canned Mackerel	33.88	33.72
Canned Mackerel	34.13	33.96
Canned Mackerel	34.13	34.05

There was no excessive charring. A forced draft oven was not available but would undoubtedly give good results and be a time-saver. Because of the nature of the marine products it is advisable to mix asbestos, sand, pumice, etc., with them to facilitate removal of the moisture. The Referee considers the use of asbestos superior to sand or pumice. Standard asbestos fibers prepared for Gooch crucibles are suitable.

The heating time necessary to obtain reasonably constant weight was established. It was found that a period of four hours in a convection type constant temperature oven dried most marine products to a point where the weight change upon further heating was only about .05 per cent. Some

[1] Manuel Tubis, *This Journal*, **24**, 706 (1941).

oily fish, such as canned mackerel, have a tendency to increase in weight after prolonged heating, due to oxidation. Certain other fish reach a constant weight in three hours. Other products, such as scallops, apparently need four hours. Oysters must be considered separately and are being made the subject of a special investigation, as will be mentioned later.

TABLE 2.—*Effect of heating time at 100° in constant temperature oven on per cent total solids*

(Sample mixed with asbestos)

PRODUCT	TIME IN HOURS								
	1	2	3	4	5	6	7	10	20
Fresh Carp (Average 6 subs)	20.87	20.68	20.65	20.66	20.64	20.65	20.64		
Fresh Scallops (Average 3 subs)	25.20	23.24	22.72	22.66	22.64	22.60	22.61		
Fresh Haddock				19.13			19.07	19.07	19.02
Fresh Cod				24.82			24.82	24.82	24.76
Fresh Mackerel				33.82			33.73		33.63
Canned Mackerel				33.80			33.89		
Canned Mackerel				34.14			34.22		
Canned Mackerel				33.65				34.31	
Fresh Haddock				18.79				18.80	

TOTAL SOLIDS IN FISH AND OTHER MARINE PRODUCTS (EXCEPT OYSTERS)

Prepare a 9 cm flat-bottomed covered weighing dish in the following manner Cut into short lengths and add ca 2 g asbestos fibers of the type used in preparing Gooch crucibles and an 8 mm stirring rod with flattened end. Dry dish, asbestos, and rod in air oven one hour and tare. Weigh to the nearest mg 9–10 g of prepared sample. Add 20 ml distilled water and mix sample intimately with asbestos. Support end of stirring rod on edge of dish and evaporate just to dryness on steam bath, stirring once while still moist. Drop rod into dish and heat 4 hrs in air oven at 100°C. Cover dish, cool in desiccator, and weigh promptly.

DISCUSSION

The addition of water to the weighed product and asbestos is essential because it gives the mixture a smooth consistency. The final product is light and fluffy, entirely lacking in any hornlike substance.

During these investigations it was found that with proper manipulation the use of asbestos could be omitted with equally good results obtained. This manipulation consisted of carefully and slowly mixing water with the product until a smooth paste was obtained, then heating on the steam bath with frequent stirring till almost dry. This handling eliminates the formation of the hornlike crust. While this method would obviate the use of asbestos, it was felt that it would be very difficult to

write directions which would assure proper manipulation. The use of asbestos provides a simpler method and eliminates individual interpretation of complicated directions.

As mentioned before, the determination of solids in oysters offers a special problem. Regulatory work on oysters demands the results of the analysis at the earliest possible moment. Any saving in time during analysis is most desirable. Work at the Baltimore District of the Food and Drug Administration had shown that the use of the Waring Blendor and a reduction in the heating time was possible without any sacrifice in accuracy. Consequently an Associate Referee on the determination of total solids in oysters was appointed at the Baltimore District and a report was submitted for this year. The Associate Referee in his report suggests that collaborative work be done on this subject during the coming oyster season. The Referee heartily agrees with this suggestion.

RECOMMENDATIONS*

It is recommended—

(1) That the above described method for determining total solids in fish and other marine products (except oysters) be subjected to collaborative study.

(2) That work on the determination of total solids in oysters by the Associate Referee be continued.

REPORT ON SALT AND SOLIDS IN OYSTERS

By D. D. PRICE (Food and Drug Administration, Federal Security Agency, Baltimore, Md.), *Associate Referee*

The intent of this paper is to show that in preparing fresh oysters for salt and solids determination, the Waring Blendor may be used without sacrifice of accuracy of results and with a considerable saving in time and effort, and that abbreviation of drying time has a like effect.

The Baltimore District laboratory of the Food and Drug Administration in 1950 examined some 200 samples of fresh shucked oysters. By a conservative estimate, 30 minutes could have been saved in the preparation of each sample and at least 1 hour in the drying of each sample.

Baltimore District laboratory has been doing work on this problem during the seasons of 1949 and 1950, as has Analyst W. C. Woodfin of Pittsburgh Food and Drug Administration subdistrict. Analysts involved in the work at Baltimore were Hugh I. Macomber, Shirley M. Walden, Garland L. Reed, Herbert E. Gakenheimer, John P. Traynor, and the author.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 51 (1952).

METHOD

PREPARATION OF SAMPLE

After two min. skim on regulation skimmer, weigh solids, reconstitute liquid and take a representative 1 qt. sample. (This sample is best taken by stirring the gallon, immersing a ladle in the liquid oyster mixture, allowing a few seconds for liquid to fill the oyster interstices, then ladling out sample.) Transfer 1 qt. sample to Waring Blendor and blend for 3 min. after checking for pieces of shell. Blend 1 pt. at a time, mixed together, and take a 1 pt. sample.

PROCEDURE

Total Solids.—Weigh quickly 10 g of the emulsion into a dried, tared, flat-bottomed metal dish ca 9 cm in diameter. Evaporate just to dryness on a steam bath, dry in oven at 98–100° for 3 hrs. Cool in dessicator and weigh promptly.

Salt.—Use open Carius method, **18.6**, with a 10 g sample of the emulsion.

DISCUSSION

Approximately $\frac{1}{3}$ of the determinations in Table 1 were made on samples with drained liquid in excess of 10 per cent. These samples examined by the food-chopper method of preparation took about double that time which the Waring Blendor method required, since in the former method liquid and meats were examined separately involving double the number of weighings and annoying calculations to reduce the liquid and meat solid figures to a single figure, expressing per cent of solids in the original sample.

Also eliminated by using the blendor was the necessity of hand chopping the several grams of meats which wrap around the grinder axle and do not become ground. The grinder must be disassembled and these meats manually removed and chopped, if the present method is to be followed to the letter. All the while these operations are being carried out, there is evaporation which tends to raise the per cent of solids.

As to accuracy of results, samples prepared by the Waring Blendor method are probably more to be trusted than otherwise, since evaporation is limited, and the emulsion is much more uniform in composition.

The emulsion is stable for many hours, a fact of academic interest only, since it tends to prove the high quality of the emulsion. It is not recommended that prepared samples be examined for solids later than a day after their preparation; samples held in our cold room at 10°C. for longer than 2 days began to show gaseous decomposition with rapidly decreasing solids content.

The blendor method of preparation has the same advantages for salt analysis in oysters as it did in the solids analysis, namely more speed, greater accuracy of results, elimination of calculations, and extra work involved in separate liquid and meat analysis when the drained liquid exceeds 10 per cent.

Table 2 shows work done on 6 samples of oysters to determine their solids content. From these results and similar results not tabulated, 3

TABLE 1.—*Comparison of results using the two methods of sample preparation*

ANALYST	TOTAL SOLIDS—PER CENT							
	FOOD CHOPPER METHOD				WARING BLENDOR METHOD			
	1	2	AVE.	DEV.	1	2	AVE.	DEV.
HIM	12.81	12.83	12.82	.02	13.28	13.32	13.30	.04
HIM	13.01	13.04	13.03	.03	12.58	12.70	12.64	.12
HIM	9.36	9.38	9.37	.02	9.40	9.53	9.47	.13
HIM	12.75	12.78	12.77	.03	12.72	12.70	12.71	.02
HIM	13.57	13.53	13.55	.04	13.41	13.38	13.40	.03
HIM	12.37	12.32	12.35	.05	12.31	12.19	12.25	.12
GLR	11.03	11.09	11.06	.06	11.25	11.29	11.27	.04
SMW	13.72	13.66	13.69	.06	13.41	13.54	13.48	.13
SMW	11.47	11.55	11.51	.08	11.34	11.30	11.32	.04
HIM	13.09	13.00	13.05	.09	12.92	12.91	12.92	.01
HIM	14.10	14.01	14.06	.09	14.12	14.10	14.11	.02
HIM	13.37	13.27	13.32	.10	13.39	13.39	13.39	.00
SMW	12.00	12.14	12.07	.14	12.36	12.38	12.37	.02
HIM	13.28	13.14	13.21	.14	12.68	12.70	12.69	.02
HIM	13.89	13.75	13.82	.14	13.32	13.34	13.32	.02
HIM	13.43	13.28	13.36	.15	12.90	13.03	12.96	.13
HIM	12.39	12.23	12.31	.16	12.64	12.62	12.63	.02
HIM	10.61	10.79	10.70	.18	11.14	11.07	11.11	.07
GLR	11.75	11.57	11.66	.18	11.78	11.83	11.81	.05
HIM	13.31	13.11	13.21	.20	13.20	13.16	13.18	.04
HIM	12.79	12.59	12.69	.20	12.78	12.79	12.79	.01
HIM	10.90	11.11	11.01	.21	10.69	10.83	10.75	.14
HIM	12.50	12.74	12.62	.24	12.35	12.30	12.33	.05
SMW	12.66	12.90	12.78	.24	12.36	12.36	12.36	.00
HIM	12.22	11.97	12.10	.25	12.11	12.10	12.11	.01
HIM	13.85	14.11	13.98	.26	13.84	13.86	13.85	.02
HIM	11.74	11.45	11.60	.29	11.65	11.59	11.62	.06
GLR	11.02	11.31	11.17	.29	11.84	11.71	11.78	.13
HIM	11.39	11.71	11.55	.32	12.23	12.11	12.17	.12
HIM	10.73	11.14	10.94	.41	11.65	11.71	11.68	.06
SMW	12.17	12.63	12.40	.46	12.74	12.75	12.75	.01
HIM	12.99	13.53	13.26	.54	12.68	12.73	12.70	.05
HIM	11.43	11.98	11.71	.55	11.53	11.51	11.52	.02
HIM	13.16	12.71	12.94	.45	12.74	12.77	12.76	.03
HIM	11.35	11.91	11.63	.56	12.12	12.14	12.13	.02
SMW	11.30	11.91	11.61	.61	12.22	12.24	12.23	.02
GLR					11.20	11.25	11.23	.05
GLR					11.01	11.01	11.01	.00
SMW					11.93	11.97	11.95	.04
	Average			0.22				0.05

hours should be the maximum drying time for solids. The same dishes were weighed after 2, 3, and 4 hours drying. In no case did the percentage loss of liquid between the 3rd and 4th hour exceed a few hundredths. In several cases there was no decrease.

In this connection, more work is indicated on drying techniques. Possibly the use of asbestos and a forced draft oven will lesssen the time necessary to dry to constant weight. It is intended to arrange for collaborative work with other laboratories during the next oyster season, provided transportation problems of this perishable item can be overcome. If the probability of the product's not reaching all collaborators in a uniformly fresh condition seems poor, perhaps it will be sufficient for the recipient to examine the product, as received, by the two methods of preparation, and to dry the same weighed samples for different periods.

TABLE 2.—*Comparison of variations in solids using different drying times*

DRYING TIME	PER CENT		
	I	II	III
2 hrs.	11.45, 11.45	11.80, 11.78	11.33, 11.41
3 hrs.	11.31, 11.31	11.77, 11.76	11.23, 11.24
4 hrs.	11.29, 11.28	11.69, 11.73	11.23, 11.24
	IV	V	VI
2 hrs.	11.92, 11.87	10.31, 10.35	11.44, 11.42
3 hrs.	11.78, 11.76	10.27, 10.29	11.39, 11.43
4 hrs.	11.77, 11.76	10.27, 10.27	11.39, 11.42

It is recommended* that work on the method for determination of total solids in oysters, as described by the Associate Referee, be continued, with investigation of possible loss of moisture in the high speed mixer.

REPORT ON SPICES AND OTHER CONDIMENTS

By E. C. DEAL (Food and Drug Administration, Federal Security Agency, New Orleans, La.), *Referee*

Last year ten topics were recommended by Subcommittee C for study in this field. All of these had been suggested earlier by the Referee on spices and other condiments. No new topics were suggested by the Referee, since it was felt that considerable work remained to be done on some of the problems, while others had not been assigned for study. Five Associate Referees were appointed. The Referee received reports from three of the Associate Referees.

VINEGAR

The Associate Referee undertook additional collaborative work designed to test the reproducibility of the permanganate oxidation number. He concluded that the method is reproducible within narrow limits and should therefore be retained as an official method. The Referee concurs.

* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 51 (1952).

Continuing a program outlined in last year's report, the Associate Referee presents tables showing the results of analysis of twenty samples of fresh juice pressed from apples obtained from the 1950 crop. He proposes to analyze the vinegars produced from this crop of apples as the next step in the program.

MUSTARD

After analyzing twelve commercial samples of prepared mustard and similar products for ether extract by first action method **(28.31)** and ascertaining that the method gave results which were reproducible, the Associate Referee submitted four samples of prepared mustard to eight collaborators, in addition to three analysts in the Denver District. The results show conclusively that the method is satisfactory for the determination of ether extract in prepared mustard products.

The Associate Referee recommends that method **28.31** be made official, with a change in wording, and that no more work be done on this problem. The Referee agrees in principle with the recommendations but recommends that the change in wording should read "with Whatman single thickness thimble or other close-textured thimble" (or other similar wording), which will permit an alternative in case Whatman thimbles are not obtainable.

The Associate Referee has done some preliminary work on ash in prepared mustard with the view of developing a satisfactory method for this determination. He plans to continue the work next year.

FRENCH DRESSING

The standards for french dressing recognize two types of products: the separable liquid food and the emulsified viscous product. After determining that the procedure for preparation of samples of mayonnaise and salad dressing is not applicable to french dressing of the separable type, the Associate Referee experimented with various substances which might prove satisfactory in emulsifying the dressing and permit uniform sampling. Egg albumin powder was chosen as the most satisfactory substance to use for this purpose.

Two samples of french dressing of the separable type were prepared in the laboratory according to a representative formula which was worked out, and samples of the dressings were subjected to collaborative study. The results obtained were in excellent agreement, indicating that the proposed procedure is satisfactory for preparation of samples of french dressing of the separable type. The Associate Referee recommends that the procedure be adopted as first action and that no further work be done on the problem. The Referee concurs in this recommendation.

Limited collaborative study was also carried out on french dressing of the emulsified type. Results of this study indicate that the procedure for preparation of samples of mayonnaise and salad dressing, **28.38,** is

applicable to french dressing of the emulsified type. The Associate Referee recommends that french dressing of the emulsified type be included by name in the procedure. The Referee endorses the recommendation.

PREPARATION OF SAMPLE, AND FAT IN MAYONNAISE AND SALAD DRESSING

Through an oversight, no topic was assigned to the Associate Referee on this subject. Last year, work was reported (1) covering a study of the effect of treatment of samples of mayonnaise and salad dressing with chloroform before the removal of fat with petroleum ether prior to acid digestion for nitrogen determination. The preliminary treatment with chloroform was found to be of no benefit, but it was determined that by increasing the quantity of sulfuric acid used in the determination, the samples would be readily digested. This change in the method was approved (2). With the increase in the quantity of acid the method appears to be satisfactory. The Referee feels that no further work is necessary on this method.

In 1932, Lepper and Vorhes reported (3) the results of a collaborative study on laboratory prepared samples of mayonnaise and salad dressings. The results for fat were somewhat higher than the theoretical. They recommended that the method for total fat be adopted as tentative.

In 1939, the Association recommended (4) that the tentative methods for preparation of sample, and for determination of fat be studied collaboratively. The following year Associate Referee Ryan prepared and sent out for collaborative study a sample of normal mayonnaise and a sample similar to commercial salad dressing. Certain modifications were made in the tentative methods, providing for mixing of the samples with a spatula and reducing the size of the fat sample to 1 gram. Results obtained by the collaborators were gratifying, and the Associate Referee recommended that the methods be adopted as official (first action), but the Referee, although approving the changes in the methods, recommended that further work be done.

At the 1943 meeting of the Association, the Referee again recommended (5) that the tentative method for the preparation of samples of mayonnaise and salad dressing, and the tentative method for the determination of total fat in mayonnaise and salad dressing be further studied. In 1947, the recommendation as to the study of the tentative method for fat in salad dressings was repeated (6), with emphasis on those dressings containing less than 50 per cent fat.

In 1949, Associate Referee Juanita E. Breit made an extensive study of the method for fat in salad dressings. She modified the method slightly and submitted it for collaborative study. On the basis of the satisfactory collaborative results, the method, as modified by the Associate Referee, was adopted (7) as first action. The method has now been in use for two

years, and no complaints have been received concerning it. The Referee believes that the method should now be made official. Likewise, the procedure for preparation of samples of mayonnaise and salad dressing would appear to be satisfactory. The Referee believes that no further work is necessary in this field and recommends that the subject of preparation of sample, and fat in mayonnaise and salad dressing be dropped.

No work was done on volatile oils in spices or on seeds and stems in ground chili. The studies should be continued on these subjects.

RECOMMENDATIONS*

It is recommended—

(1) That the method for ether extract in prepared mustard, **28.31,** be made official and that no further work be done.

(2) That the procedure outlined by the Associate Referee for preparation of sample of the separable type french dressing be adopted, and that no further work be done.

(3) That french dressing of the emulsified type be included in the procedure for the preparation of mayonnaise and salad dressing, **28.38.**

(4) That the method for total fat in mayonnaise and salad dressing, **28.46,** be made official.

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REPORT ON ETHER EXTRACT IN PREPARED MUSTARD

By J. E. ROE (Food and Drug Administration, Federal Security Agency, Denver, Colorado), *Associate Referee*

INTRODUCTION

The Referee for Spices and Condiments recommended, *This Journal,* **33,** 574 (1950), that the method for ether extract in prepared mustard, **28.31,** be made first action, and the action be confirmed by collaborative studies.

The method was devised by Winton and Andrews in 1905 and later adopted as tentative. No collaborative study of the method had been made.

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* For report of Subcommittee C and action of the Association, see *This Journal,* **35,** 53 (1952).

PRELIMINARY WORK

Twelve commercial samples of prepared mustards and prepared mustard products (4 prepared yellow mustards, 4 prepared brown mustards, 3 prepared yellow mustards with horseradish, and 1 prepared yellow mustard with sweet pickle relish containing chopped cucumbers and cauliflower) were run in duplicate by Method **28.31** for ether extract. Soxhlet extractors with Whatman Double Thickness thimbles were used.

The results of these determinations, shown in Table 1, are satisfactory. During this work it was observed that the ether in some of the extraction flasks was cloudy with a fine, white particulate material. After the ether was evaporated, varying amounts of the particulate material was observed in the extracted material. However, the greatest range in duplicate determinations was 0.16 per cent.

TABLE 1.—*Determination of ether extract in prepared mustard and prepared mustard products*

SAMPLE NO.	PRODUCT	PER CENT ETHER EXTRACT			RANGE
		DUPLICATE 1	DUPLICATE 2	AVERAGE	
1	Yellow Mustard	4.65	4.57	4.61	0.08
2	Yellow Mustard	4.65	4.64	4.65	0.01
3	Yellow Mustard	3.49	3.49	3.49	0.0
4	Yellow Mustard	3.81	3.86	3.84	0.05
5	Brown Mustard	7.15	7.26	7.21	0.11
6	Brown Mustard	8.29	8.26	8.28	0.03
7	Brown Mustard	7.72	7.88	7.80	0.16
8	Brown Mustard	5.19	5.22	5.21	0.03
9	Mustard Pickle Spread	1.76	1.64	1.70	0.12
10	Mustard with Horseradish	6.54	6.54	6.54	0.0
11	Mustard with Horseradish	6.67	6.72	6.70	0.05
12	Mustard with Horseradish	5.46	5.46	5.46	0.0

PREPARATION OF COLLABORATIVE SAMPLES

All samples were commercially prepared mustards of unknown composition. Sample 1 was prepared brown mustard, samples 2 and 3 were duplicate samples of prepared yellow mustard. Sample 4 was prepared yellow mustard from a manufacturer different from samples 2 and 3.

After thoroughly mixing the sample, about 100 grams were added to each of twelve jars in succession. This process was repeated twice more and the lids screwed on tightly.

Samples were sent to eight collaborators with a request to run each sample in duplicate by method **28.31** and to report presence of particulate material. Samples were also run by three analysts in Denver District.

RESULTS OF COLLABORATORS

Results were received from ten collaborators. Collaborator No. 10 was given permission to use a Goldfisch Extractor rather than a Soxhlet. Although his results are tabulated in Table 2, they are not included in Table 3, which shows a comparison of collaborator results.

TABLE 2.—*Determination of ether extract in prepared mustard*

COLLABORATOR	PER CENT ETHER EXTRACT			
	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
1	4.89	4.04	4.01	4.47
	4.89	4.05	4.03	4.44
2	4.89	4.00	4.03	4.45
	4.89	3.99	3.93	4.46
3	4.88	3.94	(4.38)[1]	(4.34)[1]
	4.89	4.01	4.16	4.46
	4.99			
4[2]	4.89	4.01		4.44
	4.98	4.04		4.48
5	5.00	4.01	4.05	4.43
	5.00	(3.49)[1]	(3.35)[1]	4.49
6	4.91	4.02	4.00	4.44
	4.90	4.02	4.01	4.46
7	4.87	4.02	4.08	4.49
	4.88	4.03	4.06	4.47
8	4.83	4.07	3.97	4.46
	4.92	3.98	4.02	4.41
9	4.86	4.02	4.03	4.46
	4.89	4.06	4.03	4.45
10[3]	4.92	4.24	4.00	4.52
	5.08	4.42	4.08	4.50

[1] Results rejected according to Pierce criterion.
[2] Sample No. 3 broken in transit.
[3] Analyst 10 used Goldfisch Extractor and results are not included in Table 3.

A compilation of the data is shown in Table 2. Analyses of the data are shown in Table 3. The results in parenthesis marks in Table 2 were not included in the analyses in Table 3 because they showed a significant deviation from the mean and consequently were rejected according to the Pierce* criterion. No result was rejected unless it proved to be too far outside the permitted range as determined for the data as a whole.

* Pierce and Haenisch, "Quantitative Analysis," 3d Ed.

Table 3 shows the standard deviation to be quite small for all samples.

TABLE 3.—*Comparison of results*

ITEM	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
Arithmetic Mean	4.91	4.02	4.03	4.46
Median	4.89	4.02	4.03	4.46
Mean Deviation	0.038	0.022	0.034	0.015
Standard Deviation	0.05	0.03	0.05	0.02
Probable error single Determination	0.03	0.02	0.04	0.01
Probable error of Arithmetic Mean	0.007	0.005	0.010	0.002
Coefficient of Variation	1.02	0.75	1.24	0.45
Range Maximum	5.00	4.07	4.16	4.49
Minimum	4.83	3.94	3.93	4.41
Range Permitted Maximum	5.03	4.10	4.19	4.50
Range Permitted Minimum	4.77	3.94	3.87	4.42

In the duplicate samples 2 and 3 the means are 4.02% and 4.03%, but the standard deviations are 0.03% and 0.05%, respectively. This is due to the wider range and greater dispersion of results from the mean in the frequency distribution. Consequently, the probable error of the mean for sample 3 is twice that for sample 2. Also, the coefficient of variation for sample 3 is greater than for sample 2.

The true means of all samples representative of the method have a 50% chance of falling within $\pm 0.01\%$ of the calculated means.

From the results obtained it can be concluded that the method is reliable.

COMMENTS OF COLLABORATORS

Douglas D. Price: "During extraction all extracts except duplicate 1 of sample 4 were cloudy because of solid matter which apparently passed through the thimbles. Duplicate 1 of sample 4 was extracted in a different thimble because this Soxhlet extractor was different size than the others; this extract was clear throughout the extraction, and the dried extract was clearer than the others. The other extracts varied in the amounts of particulate matter, but they all had some present.

"It is my belief that the difference in weight of the duplicate is caused by the presence of varying amounts of particulate matter.

"The five thimbles used were made by W. & R. Balston Ltd. The one thimble was made by J. Green, labelled: 'Green's 703 Hand Made Extraction Thimbles.' Perhaps the proposed method could specify a thimble, which due to its limited porosity, would give an extract free of particles."

Other collaborators reported noting no particulate material, some particulate matter, or small amount of particulate matter, with no correlation between the presence of particulate matter and sample number.

After the question of extraction thimbles was raised by Douglas D. Price, new Whatman Double Thickness and Whatman Single Thickness Extraction Thimbles

were obtained. D. W. Johnson, Denver District, was requested to run each of the four samples in duplicate using the two kinds of Whatman Thimbles.

His results from the duplicate samples using the Single Thickness Thimble checked better in three of the four samples than his results from the duplicate samples using the double thickness thimble.

D. W. Johnson commented: "Considerable particulate matter was noted in the extract from the double thickness thimbles. None or a very small amount of particulate matter was noted in the extract from single thickness thimbles. It may be concluded that the weight of particulate matter in the latter instance would be negligible."

T. J. Klayder (using Whatman Single Thickness Thimbles) commented: "No particulate matter was noted in any of the ether extracts but after drying some solid material appeared."

DISCUSSION OF THIMBLES

From the above comments, it appears that single thickness extraction thimbles are less porous than double thickness thimbles. However, the actual amounts of particulate material passing through the more porous thimble and the amounts passing through the less porous thimbles are well within the range of this method, as shown by the results of Douglas D. Price. Nevertheless, for the sake of uniformity, since the method is empirical, the thimble to be used should be specified.

ACKNOWLEDGEMENTS

The Associate Referee wishes to thank the following collaborators for assistance in this problem: Andrew G. Buell, Janice C. Bloomingdale, A. Kleinman, Robert E. Canny, Douglas D. Price, Hymen D. Silverberg, J. P. Aumer, T. J. Klayder, D. W. Johnson, all from the Food and Drug Administration.

RECOMMENDATIONS*

It is recommended—

(1) That method **28.31** be made official with the following change: Delete all of the last sentence of **28.31** beginning with "as directed under, etc." and substitute "by extracting for 16 hours with anhydrous ether in a Soxhlet Extractor with Whatman single thickness thimble. Dry extract as directed under **22.25.**"

(2) That no more work be done on this problem.

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* For report of Subcommittee C and action of the Association, see *This Journal*, **35**, 53 (1952).

REPORT ON VINEGAR

By GEORGE A. MICHAEL (*Associate Referee*), and RUTH D. WILLIAMS, Chemist (Food and Drug Division, Mass. Dept. of Public Health, Boston, Mass.)

In order to obtain further proof of the reproducibility of the permanganate oxidation test, Methods of Analysis, 7th Ed., **28.76,** the following procedure was employed.

A gallon of cider vinegar and a gallon of white distilled vinegar were bought on the open market. Two sets of four samples each were made up as follows:

	Vinegar 4% *per cent*	*Acetic Acid 4%* *per cent*
Original	100	0
No. 1	75	25
No. 2	50	50
No. 3	25	75

The above samples were submitted to three collaborators who were requested to apply the permanganate oxidation test as set forth in the method. The following results were obtained.

TABLE 1.—*Permanganate oxidation number*

SAMPLE	COLLABORATOR			AVERAGE	THEORETICAL
	A	B	C		
Cider Vinegar					
Original	—	3.41	3.62	3.52	3.52
No. 1	2.91	2.72	2.54	2.72	2.64
No. 2	1.92	1.82	1.69	1.81	1.76
No. 3	0.98	0.93	0.82	0.91	0.88
White Distilled Vinegar					
Original	3.95	—	3.78	3.87	3.87
No. 1	3.11	2.85	2.79	2.92	2.90
No. 2	1.95	1.97	2.01	1.98	1.94
No. 3	1.09	1.05	1.08	1.07	0.97

These results, and the work of previous Associate Referees[1] who have applied other phases of testing this method, show that the method is reproducible within narrow limitations of error.

The above facts are conclusive proof that this method should be maintained as an official method.

Appreciation is extended to M. J. H. Loughrey and J. Phyllis Skyrme of the U. S. Food and Drug Administration, and Ruth Williams and

[1] *This Journal*, 31, 341 (1948).

George Edwards of the Food and Drug Division of the Massachusetts Department of Public Health, for their collaborative efforts.

In the fall of 1950, a start was made on the program outlined by the Associate Referee in *This Journal*, **34**, 266 (1951).

Sixteen known varieties of apples were obtained by the laboratory. These were ground in a meat chopper. The juice was pressed out and filtered through four layers of gauze. The total solids and sugars were determined. From the sugars the theoretical yields of alcohol and acetic acid were calculated. Analyses were also made on the juice pressed from four unknown varieties. The results are tabulated in Tables 2 and 3.

TABLE 2.—*Juice of known varieties*

VARIETY	TOTAL SOLIDS	TOTAL SUGARS AFTER INVERSION	THEORETICAL YIELD OF C_2H_5OH	THEORETICAL YIELD OF CH_3COOH
Golden Delicious	12.77	10.70	5.47	7.13
Cortland	13.39	12.42	6.35	8.28
Wealthy	11.16	9.50	4.86	6.34
Delicious	14.56	12.12	6.20	8.08
Red Spy	14.60	11.51	5.89	7.68
MacIntosh	13.58	10.68	5.46	7.11
MacIntosh	13.89	10.59	5.42	7.07
Macoun	15.24	11.84	6.06	7.90
Tompkins King	13.91	11.22	5.74	7.48
Gravenstein	13.75	11.14	5.70	7.43
Baldwin	12.33	10.20	5.22	6.80
Baldwin	14.62	12.15	6.21	8.10
Wagner	12.36	10.59	5.42	7.07
Northern Spy	13.99	11.41	5.84	7.61
Russet	18.99	15.10	7.72	10.06
Talman Sweet	14.94	12.93	6.61	8.62
Average	14.01	11.51	5.89	7.67

TABLE 3.—*Juice of unknown varieties*

VARIETY	TOTAL SOLIDS	TOTAL SUGARS AFTER INVERSION	THEORETICAL YIELD OF C_2H_5OH	THEORETICAL YIELD OF CH_3COOH
J582	11.85	10.37	5.30	6.91
J585	13.03	9.23	4.72	6.16
J602	13.59	11.72	5.99	7.81
E904	12.17	10.62	5.43	7.08
Average	12.66	10.49	5.36	6.99

There is variation among samples of the same variety which come from different locations, as shown by the Baldwins. The Russets and Talman Sweets had a dry instead of a juicy flesh, resulting in high total solid and sugar content. Table 4 gives the range of the theoretical yields of acetic acid.

TABLE 4.—*Range of theoretical yields of acetic acid*

	MINIMUM	MAXIMUM	AVERAGE
Known Varieties	6.34	10.06	7.67
Unknown Varieties	6.16	7.81	6.99

The above figures for the total sugars may be accepted as typical for the apples grown in this section of Massachusetts under normal conditions. The rainfall was lighter than usual in the summer of 1950. However, there was no drought as in 1949, causing the low solids in vinegar mentioned by the Associate Referee in his last report.

During the coming year it is planned to analyze the vinegars coming from this crop of apples.

REPORT ON PREPARATION OF SAMPLE AND SAMPLING OF FRENCH DRESSING

By ALDRICH F. RATAY (Food and Drug Administration, Federal Security Agency, Cincinnati 2, Ohio), *Associate Referee*

The study involving the preparation of sample and sampling of french dressing was undertaken as directed by the Referee for Spices and Condiments.

The problem involved two types of french dressing: separable and emulsified.

SEPARABLE TYPE

Experimental work disclosed that the procedure for preparation of samples of mayonnaise and salad dressing **(28.38)** is not applicable to french dressing of the separable type.

Three samples were procured which were representative of the french dressings now on the market and their physical properties were studied. On account of their instability, a study was made to determine what emulsifier could be used to stablize them. The following emulsifiers were experimented with: gum arabic, algin powder, dreft, liquid egg yolk, liquid egg albumin, and albumin egg powder. Albumin egg powder produced the best results when 0.20 gram or more per 100 grams of sample was added to the sample and stirred in a Waring Blendor.

After a suitable emulsifier was determined, a representative formula for separable french dressing was worked out as follows:

Vegetable Oil	90.0 g
Catsup	70.0 g
Cider Vinegar	60.0 g
Salt	4.0 g
Sugar	2.0 g
Paprika	2.0 g

Collaborative samples were prepared in the laboratory and the following procedure was worked out:

FRENCH DRESSING—(SEPARABLE)

PREPARATION OF SAMPLE—PROCEDURE

Weigh bottle containing sample. Shake bottle for one minute and empty contents into Waring Blendor. Let bottle drain for one minute. Weigh empty bottle to determine weight of sample. Add 0.20 g of albumin egg powder per 100 g of sample and place cover on blendor. Stir for 5 min.; then transfer to suitable container of larger capacity than volume of sample. Shake sample ca 20 times, and stir with spatula or spoon ca 20 times, before each portion is removed for analysis. Make all weighings immediately after preparation of sample.

Correct analytical results for added emulsifier.

Two duplicate samples of the laboratory-prepared french dressing were sent to each of nine collaborators with a request that each sample be prepared as indicated in the above procedure and each sample then be analyzed for total solids (in quadruplicate) using method **28.39;** and for total fat (in duplicate) using method **28.46.** It was also requested that a spoon be used to transfer each portion for the total solids determination and that a pipet with a large delivery hole (ca 5 mm) be used to transfer each portion for the total fat determination.

In correcting for the added emulsifier the per cent total fat was multiplied by 1.002, and for total solids the per cent moisture was subtracted from 100.00, and then the result was divided by 1.002.

Results are shown in Tables 1, 2, 3 and 4, pp. 233–34.

EMULSIFIED TYPE

Two samples of emulsified french dressing now on the market were procured and prepared as indicated in the procedure for mayonnaise and salad dressing **28.38.** They were then analyzed for total solids and for total fat using methods **28.39** and **28.46,** respectively, by two different analysts in the same laboratory. The results obtained are shown in Tables 5 and 6, page 235.

DISCUSSION OF RESULTS

Separable type.—The crux of the whole problem is to determine whether or not the above method has promoted uniform sampling of the prepared french dressing. To find the answer, an analysis of the collaborative results on total solids and fat must be made. The total solids determinations are listed by each chemist in Table 1 and fat analyses in Table 3. The total solids method **(28.39)** involves fewer manipulations and chances for errors than the fat method, and hence the total solids method will be discussed first.

In examining Table 1 it is noted that the arithmetic means of the duplicate subs by each chemist vary, at the most, 0.07% total solids, except by Analyst E, who varied 0.33% on his duplicates (50.47 and

RESULTS

TABLE 1.—*Per cent total solids*

ANALYST	SAMPLE NO.	RESULTS	ARITHMETIC MEAN	ARITHMETIC DEVIATION
A	1	50.76	50.89	−.13
		50.79		−.10
		51.01		+.12
		50.98		+.09
	2	50.86	50.88	−.02
		50.86		−.02
		50.85		−.03
		50.95		+.07
B	3	50.84	50.73	+.11
		50.74		+.01
		50.64		−.09
		50.70		−.03
	4	50.68	50.66	+.02
		50.59		−.07
		50.62		−.04
		50.73		+.07
C	5	50.55	50.51	+.04
		50.47		−.04
		50.52		+.01
		50.48		−.03
	6	50.56	50.53	+.03
		50.51		−.02
		50.50		−.03
		50.53		.00
D	7	50.75	50.70	+.05
		50.68		−.02
		50.70		.00
		50.66		−.04
	8	50.64	50.65	−.01
		50.65		.00
		50.66		+.01
		50.65		.00
E	9	50.83	05.80	+.03
		50.82		+.02
		50.82		+.02
		50.73		−.07
	10	50.46	50.47	−.01
		50.56		+.09
		50.32		−.15
		50.52		+.05
F	11	50.57	50.47	+.10
		50.44		−.03
		50.40		−.07
	12	50.47	50.41	+.06
		50.37		−.04
		50.58		+.17
		50.21		−.20
G	13	50.69	50.61	+.08
		50.56		−.05
		50.58		−.03
		50.59		−.02
	14	50.52	50.60	−.08
		50.63		+.03
		50.64		+.04
		50.60		.00
H	15	50.56	50.57	−.01
		50.58		+.01
		50.53		−.04
		50.59		+.02
	16	50.52	50.54	−.02
		50.54		.00
		50.54		.00
		50.55		+.01
I	17	50.51	50.55	−.03
		50.48		−.07
		50.61		+.06
		50.60		+.05
	18	50.51	50.55	−.04
		50.55		.00
		50.54		−.01
		50.59		+.04

TABLE 2.—*Statistical analysis of the results on total solids in Table 1*

Arithmetic Mean	50.61 %
Standard Deviation	0.146%
Probable Error, Single Determination	0.099%
Probable Error, Arithmetic Mean	0.012%
Coefficient of Variation	0.288
Actual Range	50.21 –51.01%
Range Permitted	50.21 –51.01%

TABLE 3.—*Per cent total fat*

ANALYST	SAMPLE NO.	RESULTS	ARITHMETIC MEAN	RANGE	ANALYST	SAMPLE NO.	RESULTS	ARITHMETIC MEAN	RANGE
A	1	41.02 40.86	40.94	0.16		10	40.82 40.84	40.83	0.02
	2	40.84 40.81	40.83	0.03	F	11	40.41 40.42	40.42	0.01
B	3	40.44 40.27	40.36	0.17		12	40.42 40.32	40.37	0.10
	4	40.66 40.42	40.54	0.24	G	13	40.30 40.31	40.31	0.01
C	5	41.12 41.04	41.08	0.08		14	40.32 40.35	40.34	0.03
	6	40.92 40.95	40.94	0.03	H	15	40.52 40.73	40.63	0.21
D	7	40.70 40.88	40.79	0.18		16	40.79 40.59	40.69	0.20
	8	40.73 40.75	40.74	0.02	I	17	40.30 40.43	40.37	0.13
E	9	40.77 40.74	40.76	0.03		18	40.36 40.32	40.34	0.04

TABLE 4.—*Statistical analysis of the results on fat in Table 3*

Arithmetic Mean	40.62 %
Standard Deviation	0.255%
Probable Error, Single Determination	0.172%
Probable Error, Arithmetic Mean	0.028%
Coefficient of Variation	0.627
Actual Range	40.27 –41.12%
Range Permitted	39.93 –41.31%

TABLE 5.—*Per cent total solids*

ANALYST	SAMPLE NO.	RESULTS	ARITHMETIC MEAN	RANGE	ANALYST	SAMPLE NO.	RESULTS	ARITHMETIC MEAN	RANGE
J	19	57.74 57.66 57.74 57.75	57.73	0.09	J	20	59.87 59.86 59.94 59.93	59.90	0.08
K	19	57.66 57.59 57.58 57.67	57.63	0.09	K	20	59.85 60.02 59.96 59.90	59.93	0.17

TABLE 6.—*Per cent total fat*

ANALYST	SAMPLE NO.	RESULTS	ARITHMETIC MEAN	RANGE	ANALYST	SAMPLE NO.	RESULTS	ARITHMETIC MEAN	RANGE
J	19	37.43 37.34	37.39	0.09	J	20	41.82 41.90	41.86	0.08
K	19	37.54 37.60	37.57	0.06	K	20	41.90 41.96	41.93	0.06

50.80%). The low average for all analysts was 50.41%, as compared to a high of 50.89%. This is important in that Analyst E approached the high and low means of all analysts as determined by his results, which were fairly consistent by subs, and there is a significant difference between E's duplicate subs based on total solids. This scattering of the means possibly reflects to some extent the way the ingredients were added one at a time to the bottles to set gross weights on a large balance calibrated to 0.5 gram. Each sample was thus individually prepared, but this proved to be the only feasible method.

All analysts were fairly consistent in obtaining small variation in their results for each duplicate sub except Analysts A and F. Analyst A was quite consistent on one sub but variable on the other, indicating that the one sub was not properly emulsified at time of sampling. Analyst F was quite variable on total solids for both subs, which may be the result of improper emulsification at time of sampling or poor technique. Since Analysts A and F have uniform results for fat, they may have sampled for fat first and permitted the subs to stand awhile before running them for moisture. Standing eventually results in partial separation of the emulsified separable french dressings. Incidentally, Analyst H had the most variability in fat analysis, and he evidently weighed moisture subs first and may have had partial separation before sampling for fat.

Table 2 is an analysis of the results on total solids in Table 1. Employing

the range determined arithmetically from distribution within which 99.3% of the results should fall, no determinations were rejected by being outside the permitted range of 50.21 to 51.01% total solids. The arithmetic mean is 50.61%, exactly midway between the actual range of 50.21 to 51.01% (identical with the permitted range). The probable error for single determinations based on the standard deviation of 0.146% is 0.099%, and the band $50.61 \pm 0.10\%$ which should include 50% or more determinations actually includes 65%. The probable error of the arithmetic mean is 0.01%, which gives the true mean a 50% chance of falling within 50.60 to 50.62%. The coefficient of variation is small, being only 0.288.

The variations contributed by preparation of the subs, differences in analysts, and other physical factors were small enough that a normal balanced distribution of values for total solids is obtained with a high concentration about the mean. It can be concluded that the method for emulsifying separable french dressing to obtain uniform and proper sampling is completely satisfactory based on these solids determinations.

In discussing the results for fat in Table 3, it should be noted there are only 36 determinations (four for each chemist), as compared to 71 for total solids. On each sub determinations in general were quite consistent. The arithmetic means by subs ranged from 40.31 to 41.08%. Table 4 gives the measures of variation for the distribution of values given in Table 3, and here also a greater variation is noted in the results for fat than for total solids. This is to be expected, as the fat method involves more manipulations and analyst's techniques assume more significance. The latter is indicated in Table 3 by each analyst by general agreement between his duplicates, although differing from those of the others.

The arithmetic mean for fat is 40.62%, and the actual range is 40.27 to 41.12%, as compared to 39.93 to 41.31% which can be permitted. Hence, no results were ignored. Although the measures of variation are greater for the fat analysis, they are still relatively small. A normal distribution of values is obtained. The fat method also indicates that the emulsification of separable french dressings as described promotes proper sampling.

Emulsified type.—While extensive collaborative work was not done on this phase of the problem, several samples prepared as directed in the procedure for mayonnaise and salad dressing, **28.38,** were examined for total solids and total fat by two analysts in the same laboratory. Table 5 shows that for total solids the variation between individual analyst's subs was 0.08 to 0.17% and between collaborators subs 0.01 to 0.17%. Table 6 shows that for total fat the variation between individual analyst's subs was 0.06 to 0.09%, and between collaborator's subs 0.06 to 0.09%.

These results indicate that the procedure for preparation of samples of mayonnaise and salad dressing, **28.38,** is applicable to french dressing of the emulsified type.

RECOMMENDATIONS*

It is recommended—

(1) That the procedure for french dressing of the separable type as outlined by the Associate Referee be accepted as first action, and that no further work be done on this phase of the problem.

(2) That french dressing of the emulsified type be included in the procedure for the preparation of mayonnaise and salad dressing, **28.38.**

ACKNOWLEDGEMENT

The Associate Referee wishes to thank the following collaborators for their prompt assistance: J. P. Aumer, William S. Cox, Halver C. Van Dame, Luther G. Ensminger, Alfred W. Hanson, Richard F. Heuermann, W. H. Munday, D. W. Johnson, and Arthur C. Thomson, all of the U. S. Food and Drug Administration. The Associate Referee further wishes to acknowledge the assistance of Luther G. Ensminger in connection with the statistical analysis of the results.

No report was given on preparation of sample and fat in mayonnaise and salad dressings; seeds and items in ground chili; or on sugar, ash, and pungent principles in mustards.

REPORT ON ALCOHOLIC BEVERAGES

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

Mr. Rankine of the Commonwealth of Australia called our attention to errata in the alcohol table **41.23** of the *Book of Methods*, page 838. Mr. Howard Bean of the National Bureau of Standards recalculated the values for us from the basic data used for the other values in the table, and the new correct figures follow:

Apparent specific gravity	*Percentages by volume at 60°F. of ethyl alcohol at 22/22*
0.9808	0.69
07	.77
06	.86

The erroneous values were 0.79, 0.67, and 0.76, respectively.

The Referee's recommendations, which include those submitted by the Associate Referees, for the work next year follow:

* For report of Subcommittee C and action of the Association, see *This Journal*, **35**, 53 (1952).

RECOMMENDATIONS*

MALT BEVERAGES, BREWING MATERIALS AND ALLIED PRODUCTS

It is recommended—

(1) That the study of methods for the determination of essential oils and resins in hops be continued.

(2) That the first action methods for the spectrophotometric and photometric determination of color in beer[1] be made official.

(3) That study of beer turbidity methods be continued.

(4) That methods either of removing or compensating for turbidity in samples, for color measurement, and for color in samples for turbidity measurement be studied.

(5) That methods for the degassing of beer in such a manner as to result in no change in either color or turbidity be studied.

(6) That the direct, non-ash orthophenanthroline method for iron in beer, described in this year's report of the Associate Referee, be adopted, first action.

(7) That the direct, non-ash orthophenanthroline procedure be further studied.

(8) That the wet-ash orthophenanthroline method be further studied and modified by the Associate Referee before submitting for collaborative study.

(9) That the first action thiocyanate procedure, **10.30, 10.31,** and **10.32,** be dropped.

(10) That the Associate Referee correlate further work on copper in beer with the work of the Subcommittee on Copper in Beer of the American Society of Brewing Chemists.

(11) That work on tin be postponed until the work on iron and copper has been completed.

WINES

It is recommended—

(1) That collaborative work be continued on the method for the determination of non-volatile acids in wines by paper chromatography, using the procedure described in this year's report of the Associate Referee.

(2) That the method for the determination of alcohol in wines and liqueurs using the Etienne tube (*This Journal,* **33,** 1016 (1950)), as modified in this year's report of the Associate Referee, be adopted as a procedure.

(3) That the spectrophotometric method for determining phosphates in wines and spirits be adopted, first action, and that collaborative work on it be continued.

(4) That the method for spectrophotometric determination of tannin in wines and whiskies be studied collaboratively.

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* For report of Subcommittee D and action of the Association, see *This Journal,* **35,** 54 (1952).

[1] Proc. Am. Soc. Brewing Chemists, 1950, p. 193.

DISTILLED LIQUORS

It is recommended—

(1) That the study of colorimetric methods for fusel oil be discontinued.

(2) That the official method, **9.29,** for methanol by the immersion refractometer method, be studied in the light of the findings of Beyer and Reeves, *This Journal,* **28,** 800 (1945).

(3) That study be continued on the methods for methanol in distilled liqueurs and drugs with a view to their correlation.

(4) That the investigation of methods for the determination of higher alcohols in distilled spirits by chromatography be continued.

(5) That the Williams method (*Ind. Eng. Chem.,* **18,** 841 (1926)) for determination of alcohol in distilled spirits as modified in this year's report of the Associate Referee, be adopted as a procedure.

CORDIALS AND LIQUEURS

It is recommended—

(1) That the method for citric acid in fruits and fruit products (*This Journal,* **34,** 74 (1951)) be studied collaboratively on samples of cordials and liqueurs.

REPORT ON DISTILLED SPIRITS

THE EFFECT OF TEMPERATURE ON ALCOHOL DETERMINATION WITH THE WILLIAMS TEST METHOD

By A. D. ETIENNE, Alcohol and Tobacco Tax Division, Laboratory Branch, Bureau of Internal Revenue, Washington, D. C.

A study has been made of the effect of temperature on the determination of the percentage of ethyl alcohol in whiskey by means of the Williams test.[1] It has been reported that the test gives slightly high results on blended whiskey. This investigation not only shows the effect of temperature on the determination but the difference between 86 proof whiskey and 100 proof whiskey at the same temperature.

A number of determinations were made on a straight whiskey, 100 proof (50.0% alcohol by volume) and on a popular brand of blended whiskey, 86.8 proof (43.4% alcohol by volume, 336 grams solids per 100 liters), at temperatures ranging from 60°F. to 95°F.

In Table 1, the results of these determinations are listed for the various temperatures as per cent alcohol and also the differences between the actual percentages present and the percentages found. These differences are shown graphically on Fig. 1. The lines for 43%, 45%, and 47.5% were constructed by interpolation.

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[1] *Ind. Eng. Chem.,* **18,** 841 (1926). The apparatus is available from Fisher Scientific Company, 711 Forbes St., Pittsburgh, Pa. Details of the method are published in *This Journal,* **35,** 66 (1952).

TABLE 1.—*Tabulated results*

TEMP. °F.	100 PROOF WHISKEY		86.6 RPOOF WHISKEY	
	% ALCOHOL	% DIFFERENCE	% ALCOHOL	% DIFFERENCE
60	48.8(3)	−1.2(3)	42.8, 42.9 43.0(2)	−0.6, −0.5 −0.4
63	49.1	−0.9	43.1	−0.3
68	49.2	−0.8	43.5	+0.1
70	49.3	−0.7	—	—
70.5	49.5(2)	−0.5(2)	43.5, 43.6	+0.1, +0.2
71.3	49.5(2)	−0.5	43.6(2)	+0.2(2)
77	50.0(2)	0.0(2)	44.0(2)	+0.6(2)
77.5	50.1(2)	+0.1(2)	44.1(2)	+0.7(2)
79	50.2	+0.2	44.1	+0.7
79.4	50.1(2)	+0.1(2)	44.2(2)	+0.8
80			44.4	+1.0
80.8	50.3		44.3	+0.9
81.5	50.3(2), 50.2	+0.3(2), +0.2	44.3	+0.9
82	50.3	+0.3	—	—
84	50.3	+0.3	—	—
86	50.6	+0.6	—	—
88	50.8	+0.8	—	—
89.5	50.8	+0.8	45.0	+1.6
90	51.0	+1.0	45.0	+1.6
90.25	51.0	+1.0	45.0	+1.6
90.5	50.9(2)	+0.9(2)	—	—
95	51.2(2)	+1.2(2)	45.3(3), 45.4	+1.9(3), +2.0
100	51.5	+1.5	—	—

NOTE.—Figures in parenthesis indicate the number of determinations.

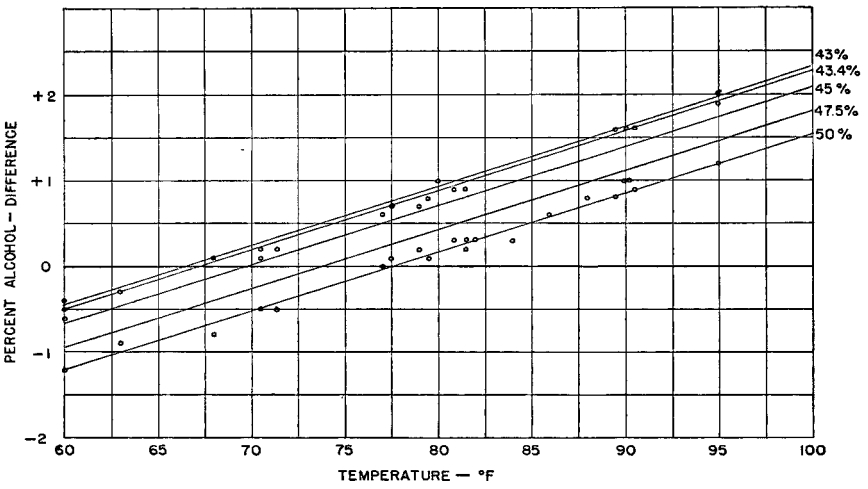

FIG. 1.—Temperature effect on whiskeys of various proof.

From Figure 1, it will be noted that the temperature at which correct percentage readings are obtained (the zero line or critical temperature), for 86.8 proof (43.4%) whiskey is 67.5°F. and for 100 proof (50.0%) whiskey is 77.5°F. The critical temperature for each percentage from 43% to 50% is given in Table 2.

TABLE 2.—*Critical temperatures*

PER CENT	TEMP.	PER CENT	TEMP.
43.0	66.5	47.0	72.8
43.4	67.2	47.5	73.6
44.0	68.1	48.0	74.3
45.0	69.6	49.0	75.9
46.0	71.2	50.0	77.5

For an accuracy of ±0.5% the determination on an 86.8 proof whiskey should be conducted between 60°F. and 75°F., while on a 100 proof whiskey the determination should be conducted between 70°F. and 85°F. As the temperature rises above the critical point the readings become high.

Figure 1 also indicates that an 86.8 proof whiskey at 67.5°F. will show the correct percentage but a 100 proof whiskey at the same temperature will read 0.7%. It also indicates that a 100 proof whiskey at 77.5°F. will show the correct percentage but an 86.8 proof whiskey at the same temperature will read 0.7% high.

Table 3 shows the percentages or correction factors which should be added to or subtracted from any reading for various temperatures to correct for temperature and alcoholic content. For all practical purposes they may be rounded to the first decimal (*This Journal,* **35,** 66 (1952)).

Examples:

A whiskey tests 48% at 66°F. The correct percentage would be 48.0% + 0.6% = 48.6%. A whiskey tests 48% at 92°F. The correct percentage would be 48.0% − 1.2% = 46.8%.

A fairly accurate rule-of-thumb correction is as follows: Multiply the difference between the critical temperature for the indicated percentage (Table 2) and the actual temperature, by 0.07. Subtract or add this figure to the indicated percentage to get the correct percentage.

Examples:

Indicated per cent, 48%; Temperature 66°F. Critical Temperature for 48% = 74.3°. 74.3° − 66° = 8.3° Difference. 8.3 × 0.07 = 0.58% + 48% = 48.6%.

Indicated per cent, 48%; Temperature 92°F. Critical Temperature for 48% = 74.3°. 92° − 74.3° = 17.7°. 17.7 × 0.07 = 1.23%. 48% − 1.2% = 46.8%.

Table 4 shows the indicated percentage readings of the usual packaged whiskeys that should be obtained at various temperatures.

It was found that when a 100 proof whiskey was reduced to 86.8 proof

TABLE 3.—*Correction factors*

PER CENT	60°	62°	64°	66°	68°	70°	72°	74°	76°	78°
43	+ .45	+ .32	+ .18	+ .03	− .10	− .23	− .38	− .51	− .66	− .79
43.4	+ .52	+ .37	+ .23	+ .09	− .05	− .18	− .32	− .46	− .60	− .74
44	+ .58	+ .43	+ .29	+ .15	+ .01	− .12	− .26	− .40	− .54	− .68
45	+ .68	+ .54	+ .40	+ .26	+ .12	− .01	− .15	− .29	− .43	− .57
46	+ .79	+ .65	+ .51	+ .37	+ .23	+ .10	− .04	− .18	− .32	− .46
47	+ .89	+ .75	+ .61	+ .47	+ .34	+ .20	+ .06	− .07	− .21	− .35
47.5	+ .95	+ .81	+ .67	+ .53	+ .40	+ .26	+ .12	− .02	− .16	− .30
48	+1.0	+ .86	+ .72	+ .58	+ .44	+ .31	+ .17	+ .03	− .11	− .25
49	+1.1	+ .96	+ .82	+ .69	+ .55	+ .41	+ .27	+ .14	+ .00	− .14
50	+1.2	+1.07	+ .93	+ .80	+ .66	+ .52	+ .38	+ .25	+ .11	− .03
PER CENT	**80°**	**82°**	**84°**	**86°**	**88°**	**90°**	**92°**	**94°**	**96°**	**98°**
43	− .94	−1.07	−1.21	−1.35	−1.48	−1.62	−1.76	−1.90	−2.04	−2.17
43.4	− .88	−1.02	−1.15	−1.29	−1.43	−1.58	−1.72	−1.85	−1.98	−2.13
44	− .82	− .96	−1.09	−1.23	−1.37	−1.51	−1.64	−1.78	−1.92	−2.06
45	− .71	− .85	− .98	−1.12	−1.26	−1.40	−1.53	−1.67	−1.81	−1.95
46	− .60	− .74	− .87	−1.01	−1.15	−1.29	−1.42	−1.56	−1.70	−1.84
47	− .49	− .63	− .77	− .90	−1.04	−1.18	−1.31	−1.45	−1.59	−1.73
47.5	− .44	− .58	− .72	− .85	− .97	−1.12	−1.27	−1.40	−1.54	−1.68
48	− .38	− .52	− .66	− .80	− .94	−1.08	−1.21	−1.34	−1.48	−1.62
49	− .28	− .42	− .56	− .69	− .83	− .97	−1.10	−1.23	−1.37	−1.51
50	− .17	− .31	− .45	− .58	− .72	− .86	− .99	−1.12	−1.26	−1.40

TABLE 4.—*Indicated percentage readings for correctly labeled proof whiskeys at various temperatures*

PROOF	60°	62°	64°	66°	68°	70°	72°	74°	76°	78°
86.0	42.6	42.7	42.8	43.0	43.1	43.2	43.4	43.5	43.7	43.8
86.8	42.9	43.0	43.2	43.3	43.4	43.6	43.7	43.9	44.0	44.1
90.0	44.3	44.5	44.6	44.7	44.9	45.0	45.2	45.3	45.4	45.6
100.0	48.8	48.9	49.1	49.2	49.3	49.5	49.6	49.8	49.9	50.0
PROOF	80°	82°	84°	86°	88°	90°	92°	94°	96°	98°
86.0	43.9	44.1	44.2	44.4	44.5	44.6	44.8	44.9	45.0	45.2
86.8	44.3	44.4	44.6	44.7	44.8	45.0	45.1	45.3	45.4	45.5
90.0	45.7	45.8	46.0	46.1	46.3	46.4	46.5	46.7	46.8	47.0
100.0	50.1	50.3	50.5	50.6	50.7	50.9	51.0	51.1	51.3	51.4

with distilled water, the alcohol determinations at varying temperatures conformed to those made on blended whiskey of the same proof. This indicates that the solids in blended whiskey have no material effect on the Williams test for alcohol.

REPORT ON TRACE ELEMENTS IN BEER

By A. L. BRANDON (Anheuser-Busch, Inc., St. Louis, Mo.),
Associate Referee

IRON

During the past ten years there has been a trend toward the use of direct, non-ashing procedures for the determination of iron in beer. In 1940, Harrison (9) reported a collaborative study of a wet-ashing, methyl cellosolve-thiocyanate method, which was further studied by Clifcorn (6) in 1941. On Clifcorn's recommendation, the Association adopted this method as tentative. In reviewing the previous work, Bendix (3), in 1944 noticed a trend away from the use of thiocyanate and toward the use of α,α'-dipyridyl or orthophenanthroline in direct, non-ash methods. He (4) later reported on a comparative study conducted by Nissen (10) in which the direct orthophenanthroline method was compared to the thiocyanate method. The first collaborative study of direct methods in the A.O.A.C. was made in 1948 by Bendix (5), who recommended that the direct orthophenanthroline method be further studied to eliminate the reducing agent, if possible, and also to ascertain if crystalline ferrous ammonium sulfate could be used as standard material in lieu of pure iron wire. A comprehensive study of the direct method, using either α,α'-dipyridyl or orthophenanthroline, was made by the Subcommittee on Iron in Beer of the American Society of Brewing Chemists (14, 15) in 1948 and 1949 and was adopted by the Society in 1950 for inclusion as an

official method in the "Book of Methods" of the A.S.B.C. This method eliminates the use of the reducing agent (except for high-iron beers or highly oxidized beers; in which cases ascorbic acid is used as the reducing agent) and uses ferrous ammonium sulfate in the calibration of the photometer.

The last report made to the A.O.A.C. was that of Stammer (12) in 1949. He reported the collaborative study of two direct methods, one similar to the method used by Bendix in 1948 and another in which the reducing agent was incorporated with the color-forming agent (orthophenanthroline). Stammer concluded from this study that direct, non-ashing procedures were not adequate as an A.O.A.C. official method and recommended that the General Referee on alcoholic beverages consider the adoption of a wet-ash orthophenanthroline method as official, and consideration of a direct, non-ashing procedure (with appropriate standardization) as an alternate method for the determination of iron in beer.

The A.O.A.C. work completed in the past year was designed to follow these recommendations. Inasmuch as the last Associate Referee did not specify any particular wet-ash procedure and since the Associate Referee's laboratory had not previously used a wet-ash procedure for determining iron in beer, it was necessary to develop such a procedure. Also, since time was not available to evaluate critically all the wet-ash procedures described in the literature, the one reported by Clifcorn (6) was adapted to the orthophenanthroline method and submitted to collaborative study. The direct, non-ash method submitted to collaborative study was similar to that used by Bendix (5) and Stammer (12), except that iron in beer was used for photometric calibration.

Samples

Five samples, containing 0.0, 0.1, 0.5, and 2.0 p.p.m. of added iron, were submitted for analysis. The samples were prepared by adding the calculated amount of iron standard (ferric) to a stainless steel half-barrel ($15\frac{1}{2}$ gallons $\pm\frac{1}{2}$ pint), filling with a low-iron beer, bottling with a head pressure of CO_2, crowning the bottles with aluminum spot crowns, and pasteurizing. This procedure was used to ensure a known amount of added iron and the same concentration of total iron in all of the samples. The samples were then distributed so that one sample containing 0.0 and 2.0 p.p.m. added iron was sent to all the collaborators, two samples containing 0.1 p.p.m. and one sample containing 0.5 p.p.m. added iron was sent to some of the collaborators, and one sample containing 0.1 p.p.m. and two samples containing 0.5 p.p.m. added iron was sent to the rest of the collaborators. A sufficient volume of the sample containing no added iron (low-iron beer) was sent to the collaborators for photometric calibration in Method No. 2. Each collaborator also received a bottle of standard iron solution.

METHODS

PHOTOMETER

Any of the commercial types of photometers will be suitable for this determination. Use a filter or wave length peak in the blue-green region (500 to 550 mμ.) or more specifically, 505 to 520 mμ.

REAGENTS

(1) *Standard iron solution.*—1 ml = 0.1 mg Fe. (1 ml soln diluted to 100 ml = 1.00 p.p.m. Fe.)

(a) *Iron Wire.*—Dissolve 0.500 g of reagent grade iron wire, wiped free of oxide, in 5 ml. of 20% HCl plus 1 ml. of conc. HNO_3. Cover with a watch glass, heat and evaporate to dryness; add distilled water and evaporate to dryness again. Take up the residue with 3–5 ml of conc. HCl, cool, and transfer quantitatively to a 500 ml volumetric flask. Add 2 drops of bromine water and dilute to volume and mix. This is soln A. Transfer 50 ml of soln A and 2 drops of bromine water to a 500 ml volumetric flask and dilute to volume with distilled water. This is soln B.

(b) *Ferrous ammonium sulfate hexahydrate* ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$).—Dissolve 3.512 g of the above salt in distilled water, add 5 ml conc. hydrocyloric acid, and dilute to 500 ml. This is soln A. Transfer 50 ml of soln A to a 500 ml volumetric flask and dilute to volume with distilled water. This is soln B.

METHOD NO. 1

Wet-ashing O-phenanthroline Procedure

REAGENTS

(1) *Nitric acid.*—Redistill thru Pyrex and store in Pyrex bottles.

(2) *Perchloric acid—70–72%.*—Double vacuum-distilled 70–72% $HClO_4$. Store in Pyrex bottle. (May be obtained from G. Frederick Smith Chemical Co., 867 McKinley Ave., Columbus, Ohio).

(3) *Sodium acetate soln (2 M.).*—Dissolve 272 g $NaC_2H_3O_2 \cdot 3H_2O$ in distilled water and dilute to 1 liter.

(4) *Hydroxylamine hydrochloride (10%).*—Dissolve 100 g of hydroxylamine hydrochloride in water and dilute to 1 liter.

(5) *O-phenanthroline (0.3%).*—Dissolve 1.5 g of *o*-phenanthroline monohydrate[1] in 500 ml of water heated to not more than 80°C. (to aid solution).

CALIBRATION OF PHOTOMETER

Depending on the size of photometer cell to be used, prepare a series of 25 or 50 ml *water standards* containing 0, 0.25, 0.5, 1.0, 1.5, 2.0 and 3.0 p.p.m. iron, 2 ml 70–72% $HClO_4$, and 10 ml of the sodium acetate soln. Develop the color in these standards according to the procedure outlined below. Transmission values may be plotted against p.p.m. iron on semi-log paper or converted to corresponding optical density values, or optical density values may be read directly and the data used to calculate a suitable factor for converting densities to p.p.m. Fe.

PROCEDURE

Depending on size of photometer cell to be used, pipet two aliquots of 25 or 50 ml of thoroly degassed beer to 100 ml extraction flasks (Pyrex No. 5160) and boil down to a syrupy consistency. Add 25 ml of the HNO_3 and 2 ml of the $HClO_4$ to each. Cover the flasks with watch glasses and heat gently until the initial reaction

[1] May be obtained from G. Frederick Smith Chemical Co.

begins, at which stage a fairly vigorous boiling with the evolution of brown oxides of nitrogen takes place. After the reaction has subsided, again heat the contents of the flasks to a slow boil and continue the boiling until all the HNO_3 is driven off. (If the boiling is too rapid, the oxidation may not be complete.) Evidence that all HNO_3 has been expelled is the appearance of copious fumes of $HClO_4$. On cooling, the residues should be colorless. If this is not the case, further heating with small additions of HNO_3 should be used.

Cool the digests, add 5 ml of distilled water to each and warm the mixtures until the precipitated salts are in solution. Cool the solns to room temp. and add 10 ml of the sodium acetate soln to each. Dilute to 25 or 50 ml (depending on the size of photometer cell to be used), and mix.

Add 1 ml of the hydroxylamine hydrochloride reagent to each soln and allow to stand for 30 min.; then to one add 2 ml of the *o*-phenanthroline reagent, and to the other 2 ml of distilled water. After 30 min., compare the colored solns in the photometer against distilled water at a wave length of 505–520 mμ (or in the blue-green region 500 to 550 mμ).

At the same time read the colored solns of the sample, with the blank, instead of distilled water, set at 100% transmission.

METHOD NO. 2

Non-ashing O-phenanthroline Procedure

REAGENTS

(1) *Hydroxylamine hydrochloride (10%).*—Dissolve 100 g of hydroxylamine hydrochloride in 1 liter of distilled water.

(2) *O-phenanthroline (0.3%).*—Dissolve 1.5 g of *o*-phenanthroline monohydrate in 500 ml distilled water heated to not more than 80°C.

Calibration of Photometer

Depending upon the size of the photometer cell to be used, prepare a series of 25 or 50 ml *beer standards* containing 0, 0.25, 0.5, 1.0, 1.5, 2.0, and 3.0 p.p.m. iron. Develop the color in these standards according to the procedure outlined below. Transmission values may be read, and plotted against p.p.m. Fe on semi-log paper or they may be converted to corresponding optical density values, or optical densities may be read directly and the data used to calculate a suitable factor converting densities to p.p.m. Fe.

PROCEDURE

Depending on the size of photometer cell to be used, pipet two aliquots of 25 or 50 ml of degassed beer to 125 ml Erlenmeyer flasks or 150 ml beakers. Add 1 ml of the hydroxylamine hydrochloride reagent to each, cover, and allow to stand for 30 min. Then to one add 2 ml of the *o*-phenanthroline reagent and to the other 2 ml of distilled water. After 30 min. compare the solns in the photometer against distilled water at a wave length of 505 to 520 mμ (or in the blue-green region, 500 to 550 mμ).

At the same time read the colored solution of the sample with the blank, instead of distilled water, set at 100% transmission.

CALCULATIONS

(1) If transmission values are read, and
   (a) Distilled water is used as the reference, the iron values of the sample and the blank are read directly from the standard curve and the difference is the p.p.m. of iron in the beer. (P.p.m. Fe in sample) − (p.p.m. Fe in blank) = p.p.m. Fe in beer, or
   (b) The blank is used as the reference, the iron value of the sample is read

directly from the standard curve and is the p.p.m. of iron in the beer (p.p.m. Fe in sample = p.p.m. Fe in beer).

(2) If optical density values are read or converted from transmission values, and

(a) Distilled water is used as the reference, the difference between the optical density values multiplied by a calculated factor will give p.p.m. of iron in the beer. (O.D. Sample − O.D. blank) × factor = p.p.m. Fe in the beer, or

(b) The blank is used as the reference, the optical density of the sample multiplied by a calculated factor will give p.p.m. of iron in the beer.

TABLE 1.—*Instruments and wavelengths used in this study*

COLLABORATOR	INSTRUMENT	CELL SIZE mm.	WAVE LENGTH mμ.
No. 1	Coleman Spectrophotometer, Model 14	15 ml.	508
No. 2	Beckman Spectrophotometer, Model DU	10	520
No. 3	Bausch and Lomb, Monochromatic Colorimeter	½ in.	509
No. 3	Coleman Spectrophotometer, Model 11	½ in.	515
No. 4	Klett-Summerson Colorimeter	20	540
No. 5	Lumetron Colorimeter, Model 402-E	10[1] 50[2]	515
No. 6	Coleman Spectrophotometer, Model 11	19	520
No. 7	Coleman Spectrophotometer, Model 14	40	505

[1] Method No. 1.
[2] Method No. 2.

DISCUSSION OF RESULTS

The instruments, cell size, and wave lengths used in this study are listed in Table 1. The results by Method No. 1 are tabulated in Table 2. Only two of the seven collaborators were able to get consistent values, and these values were obtained by modifying the method. All collaborators encountered difficulty in buffering the perchloric acid with sodium acetate. One collaborator titrated the digests to *p*H 3.5 with NH_4OH and did not encounter any further difficulty. It appears from the collaborators' comments that the following changes in Method No. 1 should be studied: (1) the effect of removing the excess perchloric acid, or substitution of another wet ash procedure, (2) the effect of filtering the insoluble residue from the acid digest, and, (3) the effect of titrating the acid digest to *p*H 3.5 with ammonium hydroxide instead of buffering with sodium acetate.

The values obtained by Method No. 2 are tabulated in Table 3. In appraising Method No. 2, it was desired to learn if there was any difference between results when distilled water was used as the reference (adjustment of the photometer to 100% transmission against distilled water) and when the beer blank was used. Reference to Table 3 indicates that there is practically no difference. Furthermore, use of the beer blank as the reference eliminates the necessity of subtracting the reading of the blank from the reading of the sample.

TABLE 2.—*Results of collaborative study—Method No. 1*

COLLABORATOR	DISTILLED WATER AS REFERENCE—P.P.M. IRON								REAGENT BLANK AS REFERENCE—P.P.M. IRON							
	IRON ADDED—P.P.M.								IRON ADDED—P.P.M.							
	NONE		0.1		0.5		2.0		NONE		0.1		0.5		2.0	
	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.
No. 3[1]	0.18		0.23 0.23		0.57		2.08		0.21		0.25 0.28		0.60		2.00	
	0.13	0.16	0.23 0.23	0.24	0.57	0.57	2.02	2.04	0.21	0.21	0.25 0.28	0.26	0.60	0.61	1.95	1.97
	0.18		0.26 0.23		0.57		2.02		0.21		0.24 0.28		0.63		1.95	
	0.20		0.26 0.28		0.60		2.14		0.30		0.37 0.38		0.65		2.19	
No. 3[2]	0.15	0.18	0.24 0.28	0.26	0.60	0.61	2.14	2.14	0.25	0.27	0.38 0.38	0.38	0.65	0.65	2.08	2.12
	0.20		0.24 0.28		0.63		2.14		0.25		0.37 0.38		0.65		2.08	
No. 7[3]	0.18	0.18	0.26	0.26	0.71 0.73	0.72	2.22	2.22	0.18	0.18	0.27	0.27	0.72 0.73	0.73	2.26	2.26
Average		0.17		0.25		0.62		2.11		0.23		0.32		0.65		2.07
Std. Dev.		0.03		0.02		0.06		0.07		0.04		0.06		0.05		0.12

[1] Bausch and Lomb Colorimeter.
[2] Coleman Spectrophotometer—Model 11.
[3] Single Determinations.

TABLE 3.—*Results of collaborative study—Method No. 2*

COLLABORATOR	DISTILLED WATER AS REFERENCE—P.P.M. IRON								BEER BLANK AS REFERENCE—P.P.M. IRON							
	IRON ADDED—P.P.M.								IRON ADDED—P.P.M.							
	NONE		0.1		0.5		2.0		NONE		0.1		0.5		2.0	
	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.	TRIP.	AVE.
No. 1	0.12 0.09 0.09	0.10	0.20 0.19 0.14 0.14 0.16 0.16	0.17	0.46 0.56 0.61	0.54	1.89 1.98 1.73	1.87	0.14 0.12 0.11	0.12	0.18 0.18 0.18 0.16 0.15 0.18	0.17	0.64 0.67 0.67	0.66	2.15 1.93 1.73	1.94
No. 2[3]	0.04	0.04	0.10	0.10	0.42 0.45	0.44	1.79	1.79	0.03	0.03	0.11	0.11	0.42 0.45	0.44	1.78	1.78
No. 3[1]	0.09 0.06 0.09	0.08	0.15 0.15 0.12 0.15 0.12 0.15	0.14	0.55 0.55 0.58	0.56	2.13 2.10 2.03	2.09	0.10 0.10 0.10	0.10	0.18 0.18 0.15 0.18 0.15 0.18	0.17	0.58 0.58 0.58	0.58	2.08 2.13 2.13	2.11
No. 3[2]	0.09 0.12 0.06	0.09	0.22 0.16 0.22 0.16 0.22 0.16	0.19	0.61 0.59 0.57	0.59	1.94 2.01 1.98	1.98	0.13 0.13 0.10	0.12	0.18 0.18 0.18 0.18 0.18 0.18	0.18	0.59 0.59 0.62	0.60	1.99 2.05 2.05	2.03
No. 4	0.00 0.00 0.00	0.00	0.09 0.11 0.10 0.11 0.10 0.12	0.11	0.48 0.46 0.48	0.47	1.83 1.83 1.82	1.83	0.00 0.00 0.00	0.00	0.09 0.12 0.10 0.12 0.10 0.12	0.11	0.48 0.46 0.48	0.47	1.79 1.79 1.78	1.79
No. 5	0.10 0.14 0.09	0.11	0.14 0.13 0.13	0.13	0.49 0.50 0.48 0.49 0.49 0.50	0.49	1.96 1.90 2.06	1.97	0.13 0.12 0.12	0.12	0.13 0.13 0.13	0.13	0.45 0.47 0.45 0.45 0.47 0.46	0.46	1.88 1.86 1.92	1.89
No. 6	0.08 0.09 0.07	0.02[4] 0.08	0.13 0.12 0.13	0.11[4] 0.13	0.48 0.43 0.47 0.47 0.47 0.49	0.47[4] 0.47	1.92 1.92 1.94	1.81[4] 1.93	0.07 0.10 0.05	0.07	0.12 0.12 0.14	0.13	0.49 0.42 0.47 0.46 0.48 0.49	0.47	1.91 1.92 1.95	1.93
No. 7[3]	0.03	0.03	0.14	0.14	0.57 0.58	0.58	2.11	2.11	0.05	0.05	0.15	0.15	0.57 0.60	0.59	2.16	2.16
Average		0.07		0.14		0.51		1.94		0.09		0.15		0.52		1.95
Std. Dev.		0.04		0.04		0.06		0.11		0.05		0.03		0.08		0.14

[1] Bausch and Lomb Colorimeter.
[2] Coleman Spectrophotometer—Model 11.
[3] Single determinations.
[4] Determined by A.S.B.C. Method.

TABLE 4.—*Summary of iron recoveries—Method No. 1 and Method No. 2*

COLLABORATOR	DISTILLED WATER AS REFERENCE—P.P.M. IRON												BLANK AS REFERENCE—P.P.M. IRON											
	IRON ADDED—P.P.M.												IRON ADDED—P.P.M.											
	METHOD #1						METHOD #2						METHOD #1						METHOD #2					
	0.1		0.5		2.0		0.1		0.5		2.0		0.1		0.5		2.0		0.1		0.5		2.0	
	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.	AVE.	% REC.
No. 1	—	—	—	—	—	—	0.07	70	0.44	88	1.77	88.5	—	—	—	—	—	—	0.05	50	0.54	108	1.82	91.0
No. 2[3]	—	—	—	—	—	—	0.06	60	0.40	80	1.75	87.5	—	—	—	—	—	—	0.08	80	0.41	82	1.75	87.5
No. 3[1]	0.08	80	0.41	82	1.88	94	0.06	60	0.48	96	2.01	100.5	0.05	50	0.40	80	1.76	88	0.07	70	0.48	96	2.01	100.5
No. 3[2]	0.08	80	0.43	86	1.96	98	0.10	100	0.50	100	1.91	95.5	0.11	110	0.38	76	1.85	92.5	0.06	60	0.48	96	1.91	95.5
No. 4	—	—	—	—	—	—	0.10	100	0.47	94	1.83	91.5	—	—	—	—	—	—	0.11	110	0.47	94	1.79	89.5
No. 5	—	—	—	—	—	—	0.02	20	0.39	78	1.86	93	—	—	—	—	—	—	0.01	10	0.34	68	1.77	88.5
No. 6	—	—	—	—	—	—	0.05	50	0.39	78	1.85	92.5	—	—	—	—	—	—	0.06	60	0.40	80	1.86	93.0
No. 7[3]	0.08	80	0.54	108	2.04	108	0.11	110	0.55	110	2.08	104.0	0.09	90	0.55	110	2.08	104	0.10	100	0.54	108	2.11	105.5
Average	0.08	80	0.46	92	1.96	98	0.08	80	0.44	88	1.88	94	0.08	80	0.44	88	1.90	95	0.07	70	0.44	88	1.88	94.0
Std. Dev.	—	—	—	—	—	—	0.03	—	0.06	—	0.11	—	—	—	—	—	—	—	0.03	—	0.07	—	0.13	—

[1] Bausch and Lomb Colorimeter.
[2] Coleman Spectrophotometer.
[3] Single determinations.

The standard deviations for the total iron values (from Table 3) and for the iron recoveries (from Table 4) as well as the percentage recovery of added iron from Table 4, compare favorably with the data reported by Stammer (12) and the Subcommittee on Iron in Beer of the A.S.B.C. (15). However, there are points in the method that need further study. One factor which especially concerned the Associate Referee was that most of the standard curves were linear in only part of the range, although an average factor was used. The non-linear portions of the calibrations were not consistently at the same level of iron concentration; some were at lower levels and some were at higher levels of concentration. This is a disturbing factor and it may account for some of the variations. Another factor that should be studied further is the feasibility of diluting a high-iron beer with iron-free distilled water to a level below 1.0 p.p.m. or to that level which will give an instrument response in the linear portion of the calibration.

The Subcommittee on Iron in Beer of the A.S.B.C. has concluded that the direct, non-ashing procedure is capable of producing values sufficiently consistent to be used as a routine method; that either α,α'-dipyridyl or orthophenanthroline can be used as the color forming reagent, and that a reducing agent is unnecessary unless high-iron beers or highly oxidized beers are analyzed. In the latter case, 25 mg. of ascorbic acid should be used. It is therefore recommended that the present direct, non-ash procedure, as modified by the findings of the Subcommittee on Iron in Beer of the A.S.B.C., be considered for adoption, first action; and that work be continued by the Associate Referee on Method No. 1 and Method No. 2 before further collaborative study is attempted.

COLLABORATORS' COMMENTS

Collaborator 1 experienced difficulty with a siliceous residue which appeared after the wet-digestion in Method 1. If this was filtered off, subsequent results for Fe were very low. With Method 1, Collaborator 2 did not get a linear standard curve with the spectrophotometer and concluded from auxiliary observations that the final pH(<2.0) is apt to be too low for full development of the *o*-phenanthroline color. He recommends a reduction in the specified amount of $HClO_4$ of from 2.0 to 1.5 ml. Collaborator 3 also spoke of difficulties with a digestion residue in Method 1 and preferred the direct, non-ashing procedure. Collaborator 4 found the wet-ashing method to be "tedious . . . and unreliable." Collaborator 6 did not get consistent standard curves with Method 1 and encountered turbidity troubles. Collaborator 7, with Method 1, actually titrated the digested samples with NH_4OH "to the proper pH for good color development."

COLLABORATORS

1. P. E. Dakin, Miller Brewing Co., Milwaukee, Wisconsin.
2. Jack Marder, Federal Security Agency, Food and Drug Administration, Washington, D. C.
3. Tod J. Stewart, Wahl-Henius Institute, Chicago, Illinois.
4. Albert E. Boyer, Blatz Brewing Co., Milwaukee, Wisconsin.

5. Eugene Heger, Anheuser-Busch, Inc., St. Louis, Missouri.
6. Thaine A. Johnson, Falstaff Brewing Co., St. Louis, Missouri.
7. R. E. Henry, Continental Can Co., Inc., Chicago, Illinois.

COPPER

Several methods have been proposed for the determination of small amounts of copper. In the selection of a method, the analyst must consider sensitivity, reproducibility, simplicity and the effect of interferences.

In 1939, Parker and Griffin (11) proposed the use of sodium diethyldithiocarbamate as a reagent for the determination of copper in biological materials. In their procedure, iron is the only interference which they considered. Iron is isolated from copper by shaking out with isoamyl acetate after the addition of α,α'-dipyridyl and sodium diethyldithiocarbamate; the copper-carbamate complex goes into the iso-amylacetate phase and the ferrous-α,α'-dipyridyl complex remains in the aqueous phase. This procedure formed the basis of a method proposed by Stone (13) for the determination of copper in beer and wort. Last year the Stone method was studied collaboratively by the Subcommittee on Copper in Beer of the American Society of Brewing Chemists (16). This study is being continued with substitution of a wet-ashing procedure for the dry-ashing used in Stone's method. Other methods that have been proposed for the determination of copper in beer are the combined dithizone-diethyldithiocarbamate method of Greenleaf (7), the dithizone single-color method of Bendix and Grabenstetter (2), and the dithizone two-color method of Greenleaf (8).

Inasmuch as iron is the main interference in the determination of copper in beer, it does not appear, in the opinion of the Associate Referee, that dithizone methods are necessary for the determination of copper. The method now being studied by the Subcommittee on Copper in Beer of the A.S.B.C. will probably be better adapted to this determination.

(No collaborative work on copper was done during the past year and none is contemplated for the coming year).

TIN

No work has been done nor is any work contemplated on tin until the work on iron and copper is completed. The only promising methods that have been proposed for tin are the spectrographic method of Alexander (1), and the Henry-Strodtz polarographic method mentioned by Stammer (12) in his 1949 report. There is a need for a chemical method, if such a method can be worked out, that will be capable of determining from 0.01 to 0.1 p.p.m. of tin.

The Associate Referee is indebted to Eugene Heger for assistance in the preparation of samples, and for his many helpful suggestions, and to all of the collaborators who participated in this collaborative study.

RECOMMENDATIONS*

It is recommended—

(1) That the direct, non-ash orthophenanthroline procedure as outlined herein be adopted, first action and that it be further studied by the Associate Referee.

(2) That the wet-ash orthophenanthroline method be further studied by the Associate Referee before submitting it to collaborative study.

(3) That the NH_4SCN procedure **10.30, 10.31,** and **10.32,** previously adopted as first action, be deleted from *Methods of Analysis.*

(4) That any collaborative work on copper be temporarily postponed.

(5) That collaborative work on tin be postponed until work on iron and copper has been completed.

(6) That the Associate Referee investigate chemical methods for tin.

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REPORT ON CHROMATOGRAPHIC ADSORPTION OF WINES AND DISTILLED SPIRITS

By ALEX P. MATHERS (Alcohol and Tobacco Tax Division, Laboratory Branch, Bureau of Internal Revenue, Washington 25, D. C.), *Associate Referee*

Preliminary studies and investigations have been made on the chromatographic separation of anthocyanins, alcohols, fluorescent materials, and non-volatile acids occurring in wines and distilled spirits. The separation of the higher alcohols found in fusel oil by this technique appears promising, but a suitable procedure for general laboratory use has not

* For report of Subcommittee D and action of the Association, see *This Journal*, **35,** 55 (1952).

been devised. The separation and identification of fruit acids by paper chromatography has advanced to the point that it provides a useful tool in the analysis of wine. Only this phase of the work is presented at this time.

The paper entitled "Paper Chromatography of Organic Acids" by Stark, Goodban, and Owens (1) deals particularly with the separation and identification of organic acids occurring in sugar-beet processing liquors. The analytical procedure outlined is almost perfectly adaptable for the separation and identification of the non-volatile acids in wines. Their method may be exactly followed for analysis of several samples simultaneously. The method presented below differs only in the size of the filter paper and amount of sample employed, as it was considered desirable to conduct the preliminary collaborative work on only a few wine samples initially.

TABLE 1.—*$R_f \times 100$ for non-volatile acids in wines*

	MUSCATEL	CONCORD	VERMOUTH (SWEET)	APPLE	CHERRY	STANDARD ACID SOLN.
Tartaric	19	20	18	—	—	19
Citric	25	27	25	26	25	26
Malic	42	44	40	43	42	42

Unknown faint spots from 60–90 in the wine chromatograms indicate traces of other acids.
Developing solvent—Phenol, 3 grams; water, 1 ml. 90% formic acid, 1%.

METHOD

Glass jars with glass covers are used to contain the solvent and paper. Whatman No. 1 paper (strips 10 inches by 3 inches) is spotted with wine sample and standard acid solution (2 g each of tartaric, citric, and malic acids per liter). The spots are placed 3 cm. from bottom of paper and 1 inch apart. Three droplets of about 0.01 ml. each of the wine and acid solution are applied to the respective spots on the paper, drying between applications. The paper is suspended in the jar from a glass rod with the lower tip immersed in the developing solution, and development is allowed to proceed for three hours. The papers are then removed from the glass jar and dried 45 minutes in a circulating air oven at 100°C. The position of acids on the chromatogram is shown by spraying the dried paper with bromophenol blue, 400 mg. per liter in 95% alcohol. The indicator solution is made slightly alkaline with sodium hydroxide. The acid spots are yellow against a blue background. The spots are circled, the centers marked and the R_f values calculated for the individual spots.

The results shown in Table 1 are in close agreement with the values reported by Stark, Goodban, and Owens (1). Slight variations may be due to high sugar content in some of the wines.

DISCUSSION

The paper by Stark, Goodban, and Owens (1) describes seven developing solvents and shows the R_f values of 18 organic acids. The method of Lugg and Overell (2, 3) is very similar to the above, though higher acid content in the developer was used.

Further studies should be made of developing solvents, the effect of sugar and solids in the wines, methods for concentrating the acids occurring in trace quantities, and removal of some interfering substances. For the detection and identification of tartaric, citric, and malic acids no prior treatment of the wine appears necessary.

Preliminary work on quantitative estimation of the non-volatile acids has been initiated but much remains to be done on this phase of the work.

The value of paper chromatography in this field lies in its simplicity and specificity.

RECOMMENDATIONS*

It is recommended that collaborative work for the detection and determination of non-volatile acids in wine by paper chromatography be continued.

It is also recommended that further studies be made upon the separation, identification, and determination of the higher alcohols in distilled spirits by chromatographic means.

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REPORT ON SPECTROPHOTOMETRIC DETERMINATION OF TANNIN IN WINES AND WHISKIES

By Maynard J. Pro, Alcohol and Tobacco Tax Division, Laboratory Branch, Bureau of Internal Revenue, Washington, D. C.),
Associate Referee

The method is based on the fact that phosphotungstomolybdic acid is reduced by tannin-like compounds in alkaline solution, producing a highly colored blue solution, the intensity of which is measured spectrophotometrically.

REAGENTS

Folin-Denis Reagent.—100 g of sodium tungstate, 20 g of phosphomolybdic acid, 50 ml phosphoric acid, 750 ml water, refluxed for two hours and made to one liter with water.

* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 55 (1952).

Sodium carbonate soln.—350 g sodium carbonate dissolved in one liter of water at 70–80°C. Cooled, seeded, and filtered thru glass wool.

Tannic acid soln.—100 mg tannic acid (U.S.P.) in one liter of water.

Spectral Transmittance Curve:

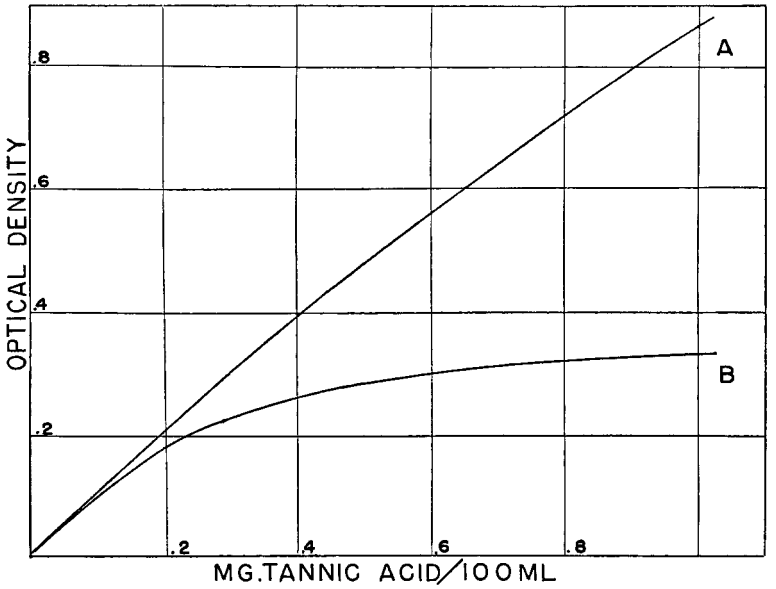
The point of minimum transmittance was determined with the Beckman spectrophotometer for concentrations of 0.1, 0.5, and 1.0 mg of tannic acid per 100 ml, and was found to be 760 mμ.

Reagent Concentrations:

The method outlined by the A.O.A.C.* produces a blue color which deviates considerably from Beer's law for tannic acid concentrations in excess of 0.35 mg. Adjustment of the phosphotungstomolybdic acid and sodium carbonate concentrations produced a line that showed only a slight deviation for tannic acid concentrations up to 1.0 mg per 100 ml. Tannic acid purer than the U.S.P. grade might produce a straight line. (See calibration curves.)

Stability of Color:

The intensity of color reached its maximum after 25 minutes at room



Calibration Curves:—**A,** 5 ml Folin-Denis reagent, 10 ml sodium carbonate solution; **B,** 1 ml Folin-Denis reagent, 5 ml sodium carbonate solution.

* *Methods of Analysis, A.O.A.C.*, 7 Ed., p. 134.

temperature, and was stable for at least three hours. This fact holds for both the color produced by the A.O.A.C. method and this modification.

METHOD

Pipet 1 ml of wine or whisky into a 100 ml volumetric flask containing 75 ml of water. Add 5 ml of Folin-Denis reagent, 10 ml sodium carbonate soln, and dilute to 100 ml with water. Shake well, and determine the optical density after 30 min. If the optical density for a 1 cm cell is greater than 0.9, make a 5:1 dilution of the sample before taking a 1 ml aliquot, and proceed as indicated above.

Calculations:

Optical Density $= x$ mg Tannic Acid/100 ml (from curve).

$$\text{Grams Tannic Acid/100 ml Spirits} = \frac{\text{mg tannic acid/100 ml}}{1000} \times \text{Reciprocal of dilutions}$$

DISCUSSION

This method has the advantage of speed and greater accuracy over a much wider range than the present tannin method of the A.O.A.C. The method determines tannin-like compounds of wines and whiskies, calculated as tannic acid. Many hundred samples of various types of wines, vermouth, brandies, and whiskies have been examined, and the results found to be in line with previous data.

It is recommended* that a spectrophotometric method for the determination of tannin in wines and whiskies be studied collaboratively.

REPORT ON SPECTROPHOTOMETRIC DETERMINATION OF PHOSPHATES IN WINES

By JOHN E. BECK and MAYNARD J. PRO, *Associate Referee*
(Alcohol and Tobacco Tax Division, Laboratory Branch, Bureau of Internal Revenue, Washington 25, D. C.)

The following method is based on the fact that phosphorus will combine with ammonium molybdate to form a reducible complex. When reduced, this complex gives a highly colored blue solution, the intensity of which is determined spectrophotometrically.

REAGENTS

Sulfuric acid.—10 *N*.

Sulfuric acid.—0.1 *N*.

Molybdate reagent.—25 grams of ammonium molybdate and 500 ml of 10 normal sulfuric acid made to 1 liter.

Bisulfite soln.—150 g of sodium hydrogen sulfite per liter of soln. Let stand two days and filter. Keep well stoppered.

Sulfite soln.—200 g of anhydrous sodium sulfite per liter of soln. Filter if necessary and keep well stoppered.

Sulfonic acid reagent.—1.25 g of pulverized 1,2,4-aminonaphtholsulfonic acid

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* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 55 (1952).

to 490 ml bisulfite soln. After shaking, sulfite soln is added in 5 ml portions until a clear soln results, normally requiring 25 ml sulfite. This reagent should be checked against known phosphate samples at intervals of about one week.

Standard phosphate.—0.4389 g of reagent grade anhydrous potassium dihydrogen phosphate per liter of soln. (1 ml contains 0.1 mg P or 0.229 mg P_2O_5.)

Spectral Transmittance Curve:

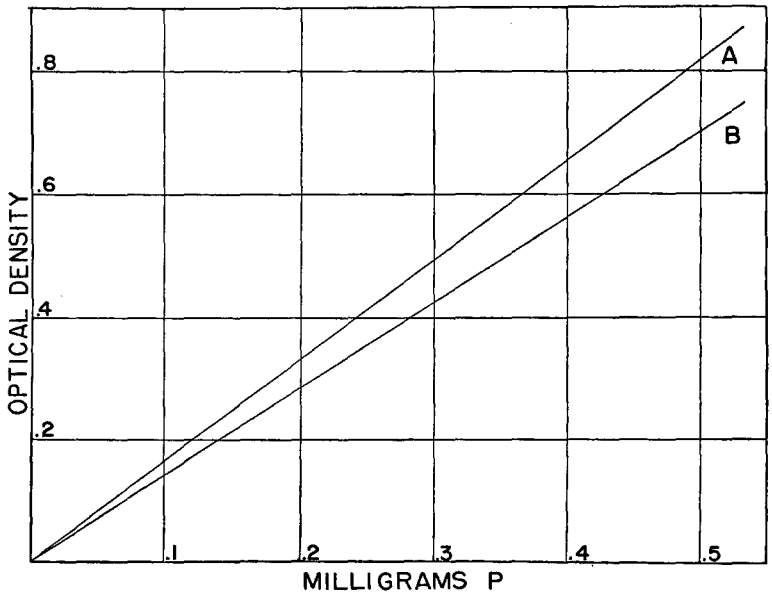
The point of minimum transmittance was determined for concentrations of 0.1, 0.2 and 0.3 mg of phosphorus, and was found to be 830 mμ. (Coleman 10S and Beckman DU Spectrophotometers.)

Reagent Concentrations:

A $\pm$20 per cent variation in reagent concentrations has no effect on the procedure, and Beer's law was obeyed for phosphorus concentrations to 0.5 mg. (See calibration curves for Coleman 10S and Beckman DU Spectrophotometers.)

Stability of Color:

The intensity of color increases with time and does not reach a maximum. It was necessary to use a specific time at which readings were to be observed. For convenience, a time interval of 10 minutes $\pm$15 seconds was found to be practical.



Calibration Curves:—A, Coleman 10S spectrophotometer, cell length ca 13 mm, wavelength 830 mμ, slit 30 mμ. Slope = 0.61. B, Beckman DU spectrophotometer, cell length = 10 mm, wavelength 830 mμ, red-sensitive phototube. Slope = 0.71.

Temperature Effect:

Increasing temperature affects the reaction by increasing the color development. However, operation at room temperature was found to be feasible because a spread of 6 or 7 degrees centigrade introduces an almost negligible error. If temperature changes are extreme, calibration curves may be determined at these temperatures.

DETERMINATION

Pipet 10 ml of wine or spirits into a platinum dish and evaporate to dryness in a 100°C. oven. Carefully char over a low flame to avoid decrepitation and ash in a muffle furnace at a temp. not to exceed 550°. Dissolve the ash with 10 ml of 0.1 *N* sulfuric acid, transfer quantitatively to a 100 ml volumetric flask, and dilute to mark.

Pipet 25 ml of the above soln into a 100 ml volumetric flask, add 50 ml of water, 10 ml of molybdate reagent, and 4 ml of sulfonic acid reagent, shaking after each addition, and dilute to mark. Determine the optical density exactly ten min. after the addition of the sulfonic acid reagent. The temp. of the sample may be ±3°C. from the temp. at which the calibration curve was determined without introducing appreciable error.

Calculations:

Coleman 10S Spectrophotometer

$$\text{Mg } P_2O_5/100 \text{ ml Wine} = \text{O.D.} \times 0.61 \times 2.29 \times \frac{100}{10} \times \frac{100}{25}$$

$$\text{(or) Mg } P_2O_5/100 \text{ ml Wine} = 56 \times \text{O.D.} \qquad (56)$$

Beckman DU Spectrophotometer

$$\text{Mg } P_2O_5/100 \text{ ml Wine} = \text{O.D.} \times 0.71 \times 2.29 \times \frac{100}{10} \times \frac{100}{25}$$

$$\text{(or) Mg } P_2O_5 /100 \text{ ml Wine} = 65 \times \text{O.D.} \qquad (65)$$

COMPARISON TO A.O.A.C. METHOD

Phosphates were determined on nine different standard wines made in the Alcohol Tax Unit Laboratory by Peter Valaer. The results of the spectrophotometric method were checked against the official A.O.A.C. titration method. Wines used included three boysenberry, four cherry, one blackberry, and one elderberry. Results obtained are tabulated below:

WINE NO.	A.O.A.C. METHOD MG. P_2O_5/100 ML	SPECTROPHOTOMETRIC MG P_2O_5/100 ML	DEVIATION MG P_2O_5
1	17.5	17.6	−0.1
2	29.4	29.2	−0.2
3	18.7	18.9	+0.2
4	16.9	16.5	−0.4
5	37.4	37.0	−0.4
6	20.7	20.7	±0.0
7	26.8	26.9	+0.1
8	20.9	21.1	+0.2
9	28.5	28.6	+0.1

The maximum deviation was found to be 0.4 mg P_2O_5/100 ml wine, which is equivalent to 0.006 optical density, or almost the sensitivity of the instrument.

DISCUSSION

This method has the advantage of speed and ease of manipulation over the official method. Calcium, magnesium, iron, and manganese were found to have no effect on the color development until the usual concentration in wine was far exceeded.

It is recommended* that the spectrophotometric method for determining phosphates in wines and spirits, as recommended by the Associate Referee, be adopted, first action, and that collaborative work be continued.

No reports were received on malt beverages, sirups, extracts, and brewing materials; hops; color and turbidity in beer; caramel in wines; cordials and liqueurs; or on methanol.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

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

RECOMMENDATIONS†

It is recommended—

(1) That the changes recommended by the Associate Referee be made in the directions for the photometric method for vanillin as given in *This Journal*, **34**, 72 (1951), and that the revised method be adopted as first action.

(2) That the changes recommended by the Associate Referee be made in the directions for the photometric method for coumarin as given in *This Journal* **34**, 73 (1951) and that the revised method be adopted as first action.

(3) That the method for the determination of isopropyl alcohol in lemon and orange flavors as given in this year's report of the Associate Referee on organic solvents in flavors be adopted as first action.

(4) That the method for the determination of Essential Oil in Emulsions, as given in this year's report of the Associate Referee on this subject, be adopted as first action.

(5) That the method for the determination of essential oil in citrus

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* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 55 (1952).
† For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 58 (1952).

juices and other beverages, as given in this year's report of the Associate Referee on this subject, be adopted as first action.

(6) That collaborative studies be continued on the following:

(a) Photometric method for vanillin.
(b) Photometric method for coumarin.
(c) Method for essential oil in emulsions.
(d) Reflex method for essential oil in citrus fruit juice and other beverages.
(e) Beta-ionone where small amounts are present.
(f) Propylene glycol in vanilla extracts, *This Journal*, **33**, 103 (1950).
(g) Gravimetric method for vanillin and coumarin, **19.4** and **19.5**, as applied to imitation vanilla flavors and vanilla extracts.

(7) That methods **19.6** and **19.7** be deleted, first action.

REPORT ON ESSENTIAL OILS IN EMULSION FLAVORS

By John B. Wilson (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee.*

This year, instead of preparing the emulsion flavors by diluting a primary emulsion to the required volume with water, each sample was prepared separately by mixing the requisite quantities of gum arabic, essential oil, and water with a soda fountain mixer for several minutes and then passing the mixture three times through the same homogenizer as was used previously.[1]

In analyzing the samples the same procedure was used as before, except that a tightly fitting finger condenser was substituted for the Liebig condenser. Collaborators were requested to measure the 5–10 ml of emulsion in a tared glass-stoppered graduated cylinder, and also to find the weight of the sample. The oil content is reported as ml of oil found per 100 g of sample, and the oil content of the emulsions is calculated to the same basis by weighing the mixture of ingredients before subjecting them to the mixing process. This procedure was adopted because it was found to be somewhat difficult to estimate the true volume of the emulsions, because of the formation of bubbles and froth which rose to the top when the emulsions were allowed to stand after mixing.

The data are given in Table 1.

No correction was made on the results given in Table 1. The recoveries are somewhat higher than those reported previously. This is believed to be due to the use of the tightly fitting finger condenser which does not retain oil as is sometimes the case when the Liebig condenser is used. Details of the method have been published in *This Journal*, **35**, 78 (1952).

The Associate Referee recommends* the adoption of the method as first action, and that the collaborative study be continued.

[1] *This Journal*, 31, 200 (1948).
* For report of Subcommittee D and action of the Association, see *This Journal*, 35, 58 (1952).

TABLE 1.—*Determination of essential oil in emulsion flavors*

NUMBER	LEMON				ORANGE			
	1	2	3	4	5	6	7	8
Oil, ml	5	7	4	20	20	4	8	5
Gum Arabic, g	16	14	17	17	17.5	12.5	15	14
Water, ml	81	80	90	75	64	75	88	82
Total Weight, g	102	100	107.5	108.5	99	90.5	110	100
Oil present: ml/100 ml	4.90	7.00	3.72	18.43	20.20	4.42	7.27	5.00
Oil found: ml/100 ml								
J.B.W.	4.60	6.60	3.73	17.7	18.10	4.38	6.86	4.68
C.J.H.	4.80	6.80	3.62	17.6	18.00	4.25	6.83	4.59
Recovery:								
J.B.W.	94	94	100	96	90	99	94	94
C.J.H. %	98	97	97	96	89	96	94	92
Average	96.5 (Lemon)				93.5 (Orange)			

REPORT ON PEEL OILS IN CITRUS JUICES AND OTHER BEVERAGES

By John B. Wilson (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee.*

The reflux method for peel oils in citrus juices, using the modified oil separatory trap,[1] has been in use for some time. It is applicable to the fresh, canned, or frozen juices, to citrus ades, bottled sodas, and other food products containing essential oil.

While only three collaborators were used this year, a considerable number and variety of samples were analyzed. For next year's work, it is planned to increase the number of collaborators and to decrease the number of samples.

The finger condensers used had projections at the bottom to facilitate the return of the distillate to the modified oil separatory trap, which we

Table 1.—*Collaborative results on essential oil in citrus juices and other beverages*

Collaborator	Essential oil in %		
	H.	M.	W.
Canned Orange Juice:			
Brand A	0.005	0.008	0.008
A	0.005		0.005
B	0.007		0.009
C	0.006		0.008
Frozen Orange Juice:			
Brand D (1 can used)	0.001	0.005	0.004
D (3 cans used)	0.012		
E	0.007		0.008
F	0.014		0.014
G	0.005	0.004	0.006
Canned Grapefruit Juice:			
Brand H	0.002	0.001	0.001
I	0.007		0.008
Canned Blended Orange and Grapefruit Juice:			
Brand J	0.015		0.015
K	0.004	0.004	0.003
Frozen Blended Orange and Grapefruit Juice:			
Brand L (2 cans used)			0.008
Canned Orangeade:			
Brand M	0.010		0.010

[1] *This Journal*, **27**, 201 (1944).

believe increases the accuracy and reproducibility of the method. One liter samples were used in most cases, and any departure from this volume is noted in Table 1, which contains the data. Except for the substitution of a 2 liter boiling flask the method is the same as that for Essential Oil in Emulsions.

In view of the agreement between analysts, shown in Table 1, the Associate Referee recommends adoption of the method as first action, and that the collaborative study of the method be continued. The method is given in detail in *This Journal*, **35**, 79 (1952).

RECOMMENDATION*

It is recommended that the method for peel oil in citrus juices and other beverages as given in this report be adopted as first action.

REPORT ON VANILLA EXTRACTS AND IMITATIONS

COLORIMETRIC DETERMINATION OF VANILLIN AND COUMARIN

By Luther G. Ensminger (Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

INTRODUCTION

The first report on this subject (*This Journal*, **34**, 330, 1951) gave the development of photometric methods for the determination of vanillin and coumarin in imitation vanilla flavors and included the collaborative analyses which led to the adoption of these methods as first action at the 1950 meeting. The methods are detailed in *This Journal*, **34**, 72 (1951).

During this year the Associate Referee has carried out the recommendation of the previous report that studies be continued on vanilla extracts with the above methods.

PREPARATION OF COLLABORATIVE SAMPLES

An authentic vanilla extract was obtained from a local processing firm. Tahiti and Bourbon beans were blended to get an extract to which alcohol, water, and sugar were added. The finished extract was put into one ounce bottles and designated "Sample I." Sample II was prepared by enriching the authentic extract with 0.3000 g of vanillin and 0.1400 g of coumarin per 100 ml of the extract and was also put into one ounce bottles. Each collaborator received one bottle of each sample and a copy of the photometric methods previously published.

PRELIMINARY STUDIES

Preliminary analyses were made on the two collaborative samples em-

* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 58 (1952).

ploying the gravimetric method **19.4–19.5** to determine the vanillin and coumarin, in order to evaluate the collaborative results. Low recoveries of the added vanillin and coumarin in Sample II were obtained and are given in Table 1 below.

TABLE 1.—*Recovery of vanillin and coumarin in samples I and II*

SAMPLE NO.	VANILLIN		COUMARIN	
	G/100 ML	PER CENT RECOVERY OF ADDED AMOUNT	G/100 ML	PER CENT RECOVERY OF ADDED AMOUNT
I	0.1984	—	0.0240	—
II	0.4832	94.9	0.1328	70.6

These low recoveries prompted the Associate Referee to make up an alcohol-water solution (35% alcohol) of known amounts of vanillin and coumarin, and to analyze it by the same official method. The results of this sample, designated "Sample IV," are listed in Table 2.

TABLE 2.—*Recovery of vanillin and coumarin from sample IV*

INGREDIENT	ACTUAL AMOUNT (G/100 ML)	RECOVERY (G/100 ML)	PER CENT RECOVERY
Vanillin	0.3000	0.2662	88.7
		0.2896	96.5
Coumarin	0.1400	0.1164	83.1
		0.1188	84.9

As a result of the above recoveries by methods **19.4–19.5** it was considered impracticable to calculate the per cent recoveries obtained by the photometric methods based on the gravimetric methods, assuming the latter gave the correct amounts.

A photometric analysis was made on a pure Bourbon vanilla extract (Sample III), and the content of coumarin was identical with that in Sample I. Qualitative and organoleptic tests proved the pure extracts tested contained coumarin, but in small amounts.

Low recoveries were also obtained by the Associate Referee using the colorimetric methods **19.6** and **19.7.** Recoveries as low as 60 per cent were obtained on samples with concentrations of vanillin exceeding 0.6 g/100 ml. R. M. Way and W. R. Gailey have reported (*This Journal,* **34,** 726, 1951) low recoveries by the same colorimetric methods.

Vanilla extracts enriched with vanillin at the rate of about one to three ounces per gallon were analyzed by the collaborative procedures, except that a $\frac{1}{2}$ inch cell was used, and standards were prepared to include the range required for the samples. See Table 3 below for recoveries. Sample

TABLE 3.—*Recovery of vanillin from enriched extracts*

SAMPLE NO.	ACTUAL CONTENT*	FOUND	RECOVERY
	(g/100 ml)	*(g/100 ml)*	*per cent*
V	—	0.260	—
	—	0.259	—
VI	1.260	1.256	99.7
		1.219	96.7
VII	1.690	1.600	94.7
		1.600	94.7
VIII	2.550	2.619	102.7
		2.625	102.9

* Actual content is calculated by adding the amount of enrichment to the content of the original extract (Sample V), as determined by the colorimetric procedure.

V is the original extract, and Samples VI, VII, and VIII represent various stages of enrichment.

The average per cent recovery for the vanillin-enriched extracts was 98.6. In constructing the standard curve for vanillin concentrations above 1.00 g/100 ml of extract, it should be noted that the slope of the curve increases at a faster rate which would naturally magnify analytical variations. Hence, should any enriched extracts contain more than 1.00 g vanillin per 100 ml, they should be diluted with water to bring the concentration of the portion to be analyzed below 1.00 g/100 ml.

COLORIMETRIC DETERMINATION OF VANILLIN IN VANILLA EXTRACTS

Collaborative Procedure—Details of the method, as sent to the collaborators, are published in *This Journal*, **34**, 72 (1951).

Results of Collaborators—Sixteen collaborators analyzed Samples I and II, reporting results in duplicate in terms of g/100 ml except chemists 3 and 13 who made single determinations. Chemist 2 evidently lost one determination on Sample I.

Chemist 5 made duplicate determinations on one day and single determinations on another, and the duplicates only were retained for two reasons—the single determinations agreed well with the duplicates, and one set of results should not be weighted statistically more than the others. This could not be avoided in cases of chemists 3 and 13, whose single determinations carry less weight in the statistical analysis, but which are reported in order to represent the work of those analysts.

Table 4 gives the collaborative results on vanillin. These results are listed to the second decimal point only, because some analysts cannot compare color intensities and read results closely enough on the neutral wedge photometer to make the third decimal point significant.

DISCUSSION OF RESULTS ON VANILLIN

An inspection of Table 4 shows the ability of various chemists to obtain

TABLE 4.—*Determination of vanillin in collaborative samples*

CHEMIST	VANILLIN (G/100 ML)		CHEMIST	VANILLIN (G/100 ML)	
	SAMPLE I	SAMPLE II		SAMPLE I	SAMPLE II
1	0.24	0.58	9	0.26	0.56
	0.24	0.57		0.26	0.56
2	0.28	0.56	10	0.26	0.57
	—	0.56		0.26	0.56
3	0.25	0.53	11	0.28	0.56
				0.27	0.56
4	0.24	0.55			
	0.24	0.55	12	0.26	0.57
				0.26	0.57
5	0.25	0.54			
	0.24	0.54	13	0.26	0.55
6	0.26	0.53	14	0.26	0.56
	0.26	0.56		0.26	0.56
7	0.25	0.53	15	0.25	0.56
	0.25	0.58		0.26	0.56
8	0.23	0.54	16	0.27	0.53
	0.23	0.54		0.29	0.59

relatively close checks with the colorimetric method on duplicates. Sample I gives less variation on duplicates than does II, but both give the same range of 0.06 g/100 ml. Chemist 16 did not have the precision of duplicates equal to that of the other analysts, but his average recovery of added vanillin was good. This indicates he apparently was more careless with the method.

Table 5 is an arithmetic statistical analysis of the data given in Table 4 on vanillin. The arithmetic means for Samples I and II are 0.256 and 0.556 g/100 ml, and the difference of 0.300 g is identical with the 0.3000 g added, giving a 100.0% recovery. The calculated meaures of variation—standard deviation, probable error of a single determination, and probable error of the arithmetic mean—are small and disclose high consistency between the two concentrations of vanillin for the method. On repeat determinations a single assay has a 50% chance of falling within the interval 0.247 to 0.265 g/100 ml for Sample I, and 0.545 to 0.567 g for Sample II. The slightly greater actual variability of Sample II is to be expected because of the higher concentration. For both samples the 50% confidence interval for the arithmetic mean is 0.004 g/100 ml (the arithmetic mean for each sample plus or minus 0.002 g). The coefficient of variations, 5.47 and 2.88 for Samples I and II respectively, are small, the first being higher because of a lower vanillin concentration.

TABLE 5.—*Analysis of collaborative results, vanillin (Table 4)*[a]

(1) SAMPLE NO.	(2) ARITHMETIC MEAN	(3) AVERAGE RECOVERY ADDED VANILLIN (%)	(4) STANDARD DEVIATION	(5) PROBABLE ERROR, SINGLE DETERMINATION
I	0.256	—	0.014	0.009
II	0.556	100.0	0.016	0.011
	(6) PROBABLE ERROR OF ARITHMETIC MEAN	(7) COEFFICIENT OF VARIATION	(8) RANGE	(9)[b] RANGE PERMITTED
I	0.002	5.47	0.23–0.29	0.22–0.29
II	0.002	2.88	0.53–0.59	0.51–0.60

[a] Data in columns 2, 4, 5, 6, 8, and 9 are in terms of grams per 100 ml of sample.
[b] Calculated on basis of arithmetic mean plus or minus 4 times the probable error of a single determination.

All these measures of variation show high consistencies between the two frequency distribution curves, and a high centering of determinations about the means. This is further borne out by 17 values out of 29 for Sample I and 15 out of 30 for Sample II falling within the range arithmetic mean plus or minus 0.006 g/100 ml.

In Table 5, column 8 gives the actual ranges of the two distributions and column 9 the permitted ranges. It is seen that the former falls within the latter in each case. Any determination that would fall without the permitted range would represent a significant error. Since none were outside, all results were kept and analyzed.

It should be added that the above results are less variable for vanillin in vanilla extracts this year than were the assays for vanillin in imitation vanilla flavors as determined and reported in 1950. This appears to be due to the changes in the collaborative procedure used on imitations that were incorporated into the revised method as published.

COLORIMETRIC DETERMINATION OF COUMARIN IN VANILLA EXTRACTS

Collaborative Procedure. The method as published in *This Journal*, **34**, 73 (1951) was sent to the collaborators.

Results of Collaborators.—Sixteen collaborators submitted results in duplicate, except for chemists 3, 13, and 16, on the same two samples analyzed for vanillin. Chemist 10 repeated the coumarin analysis with the Beckman spectrophotometer, but since duplicates were at most only 0.002 g/100 ml from those determined by the neutral wedge photometer and the per cent recovery was identical for the added coumarin, the Beckman photometer results are not reported. Chemist 5 determined cou-

marin only with Coleman spectrophotometer. All other results were obtained on the Clifford neutral wedge photometer as far as is known. All the results are tabulated in Table 6 for coumarin and are rounded off to the second decimal point.

TABLE 6.—*Determination of coumarin in collaborative samples*

CHEMIST	COUMARIN (GRAMS/100 ML)		CHEMIST	COUMARIN (GRAMS/100 ML)	
	SAMPLE I	SAMPLE II		SAMPLE I	SAMPLE II
1	0.03	0.16	9	0.04	0.18
	0.03	0.17		0.04	0.18
2	0.04	0.17	10	0.03	0.16
	0.04	0.17		0.03	0.16
3	0.04	0.17	11	0.03	0.16
				0.03	0.16
4	0.03	0.16			
	0.03	0.16	12	0.04	0.17
				0.04	0.17
5	0.04	0.18			
	0.04	0.18	13	0.04	0.17
6	0.04	(0.36)	14	0.04	0.17
	0.04	(0.36)		0.04	0.17
7	0.04	0.17	15	0.04	0.18
	0.04	0.17		0.04	0.18
8	0.03	0.17	16	0.04	0.17
	0.03	0.17		0.04	—

DISCUSSION OF RESULTS ON COUMARIN

According to Table 6, all collaborators obtained very good checks on duplicates for coumarin. Chemist 6 had duplicates of 0.36 g/100 ml on sample II which are quite out of line with all others, and since they are without the permitted range and represent a significant mistake, they are not used in the derivation of a statistical analysis of the results. (See Table 7.) Apparently a mistake was made in the transfer of volumes by chemist 6.

In Table 7 the arithmetic means for coumarin are shown to be 0.037 and 0.170 g/100 ml for Samples I and II, respectively, giving an average recovery of 95.0%. Three measures of variation—standard deviation, probable error of a single determination, and probable error of the arithmetic mean—compare in magnitude to those obtained last year for collaborative results on imitation vanilla flavors, and are relatively small.

The coefficient of variation 12.98 for Sample I may seem large by com-

TABLE 7.—*Analysis of collaborative results, coumarin (Table 6)*[a]

(1) SAMPLE NO.	(2) ARITHMETIC MEAN	(3) AVERAGE RECOVERY (%)	(4) STANDARD DEVIATION	(5) PROBABLE ERROR, SINGLE DETERMINATION
I	0.037	—	0.005	0.003
II	0.170	95.0	0.007	0.005
	(6) PROBABLE ERROR OF ARITHMETIC MEAN	(7) COEFFICIENT OF VARIATION	(8) RANGE	(9)[b] RANGE PERMITTED
I	0.001	12.98	0.03–0.04	0.03–0.05
II	0.001	4.12	0.16–0.18	0.15–0.19

[a] Data in columns 2, 4, 5, 6, 8, and 9 are in terms of grams per 100 ml.
[b] Calculated on basis of arithmetic mean plus or minus 4 times the probable error of a single determination.

parison to Sample II, but the amount of coumarin in the pure vanilla extract is so small that this coefficient is magnified, yet is surprisingly small considering its low content. (The coefficient of variation is inversely proportional to the magnitude of the arithmetic mean and directly proportional to the standard deviation.)

Since the 0.36 values on Sample II have been determined to be far beyond the limits for the random error and are not considered, all remaining results fall well within the permitted range (Table 7, column 9). The actual and permitted range as determined by the distribution curves compare favorably also with those found for coumarin in imitation vanilla flavors.

CONCLUSIONS

The colorimetric procedures for vanillin and coumarin sent out to the collaborators have worked equally well in analyzing pure and enriched vanilla extracts and imitation vanilla flavors. The distribution curves for the analysis of both constituents in two extracts are each normal with a high concentration of values about the arithmetic mean and with the coefficients of variation being relatively small.

Very little difficulty was experienced by the collaborators in carrying out the procedures, most reporting no difficulty at all.

COMMENTS OF COLLABORATORS

F. C. Minsher: "Wedge photometer in very poor condition. . . . finally broke down completely during coumarin work. Coumarin readings had to be made on Coleman, Jr., using standards of ½ prescribed strength, and double dilution on samples."

(NOTE: He obtained 101.4% recovery on coumarin.)

A. F. Ratay: "The use of S&S hand-folded filter paper gave a cloudy filtrate. . . . This was corrected by the use of a better grade of filter paper."

All other collaborators reported no important difficulties with either the vanillin or coumarin procedures.

RECOMMENDED CHANGES IN VANILLIN AND COUMARIN METHODS

A. *Changes in the Vanillin Method:*

(1) Page 73, 4th line under "Preparation of Graph," delete "18.5 cm."

(2) Page 73, under "Determination of Sample," following the present paragraph, add a second paragraph as follows:

NOTE: Use No. 12 Whatman 18.5 cm folded filter paper or equivalent on all filtrations. When the concentration of vanillin exceeds 1.0 g per 100 ml of sample, dilute 50 ml of sample to 100 ml with water and use diluted sample. When determination is made with a photoelectric spectrophotometer, dilute samples to double the volume, and read at a wave length of 610 mμ.

B. *Changes in the Coumarin Method:*

(1) Page 74, 5th line from the top of the page under "Preparation of Graph," delete "11 cm S&S 589."

(2) Page 74, 10th line from the top of the page, delete "S&S 589."

(3) Page 74, under "Determination of Sample," following the present paragraph, add a second paragraph as follows:

NOTE: Use No. 12 Whatman 18.5 cm folded filter paper or equivalent for all filtrations. When determination is made with a photoelectric spectrophotometer, dilute samples to double the volume, use only ½ strength standards, and read at a wave length of 490 mμ.

All the above changes have been found advisable to promote more accurate analysis of vanillin and coumarin, and have been tested thoroughly.

ACKNOWLEDGMENT

The Associate Referee appreciates the assistance of the following collaborators, all of the U. S. Food and Drug Administration: John B. Wilson, Food Division; John F. Weeks, Jr., New Orleans; Garland L. Reed, Baltimore; J. E. Roe, Denver; L. W. Ferris, Buffalo; Juanita E. Breit, Minneapolis; Robert E. O'Neill, Atlanta; Harold E. Theper, St. Louis; R. H. Johnson, Portland; Janice C. Bloomingdale, Cincinnati; C. G. Cunningham, Boston; John F. Mallon, Boston; Theodore E. Byers, Cincinnati; F. C. Minsher, Philadelphia; and A. F. Ratay, Cincinnati.

RECOMMENDATIONS*

It is recommended—

(1) That the colorimetric methods for vanillin and coumarin in imitation vanilla flavors, *This Journal*, **34**, 72–4 (1951), be revised as suggested.

(2) That the revised colorimetric methods for vanillin and coumarin be adopted, first action, for pure and enriched vanilla extracts.

(3) That methods **19.6** and **19.7**, *Methods of Analysis*, 7th ed., be deleted.

(4) That methods **19.4** and **19.5** be studied collaboratively on imitation vanilla flavors and vanilla extracts.

* For report of Subcommittee D and action of the Association, see *This Journal*, 35, 58 (1952).

REPORT ON ORGANIC SOLVENTS IN FLAVORS

By ROBERT D. STANLEY (Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Associate Referee*

In accord with recommendations made by the Association that collaborative studies be made on the determination of isopropyl alcohol in extracts containing acetone, the work was continued this year.

Barthauer, Jones, and Melter (1) determined acetone in a hydrocarbon solvent in the presence of isopropyl alcohol, diisopropyl ether, and other compounds by an ultraviolet spectrophotometric method. The spectrophotometric determination of acetone in the presence of isopropyl alcohol seemed to offer an attractive solution to the problem at hand. By oxidizing a portion of the sample and measuring the acetone content, and measuring the free acetone in another portion, the difference would be proportional to the isopropyl alcohol present in the sample.

The Associate Referee's work on the absorption of ultraviolet light by acetone and isopropyl alcohol is in agreement with the work cited above and also that previously reported by others (2, 3)

REAGENTS AND EQUIPMENT

Acetone.—Reagent grade acetone was dried over calcium chloride and distilled. The purity was then checked by a determination of physical constants.

Isopropyl alcohol.—Reagent grade isopropyl alcohol was refluxed with lime and distilled, and its purity likewise checked by a determination of physical constants.

All spectral measurements were made with a Beckman DU spectrophotometer equipped with a hydrogen discharge source and matched quartz 1 cm cells.

EXPERIMENTAL

A sealed glass bulb containing 1.4033 g of acetone was placed in a 500 ml volumetric flask. The bulb was broken under water and the solution diluted to volume. The optical densities of this solution, relative to water, were measured between 210 and 350 mμ. From these data the absorption spectrum (Fig. 1) for acetone was plotted. This curve shows that the point of maximum absorption for acetone in water solution to be 265 mμ.

Three solutions of different acetone concentrations were prepared and their optical densities relative to water measured. The results are summarized in Table 1. The straight line produced when the optical densities are plotted against concentration (Fig. 2) shows that the Beer-Lambert law is satisfied.

Since this work requires an accurate estimation of the amount of acetone in the presence of various quantities of alcohols (including isopropyl

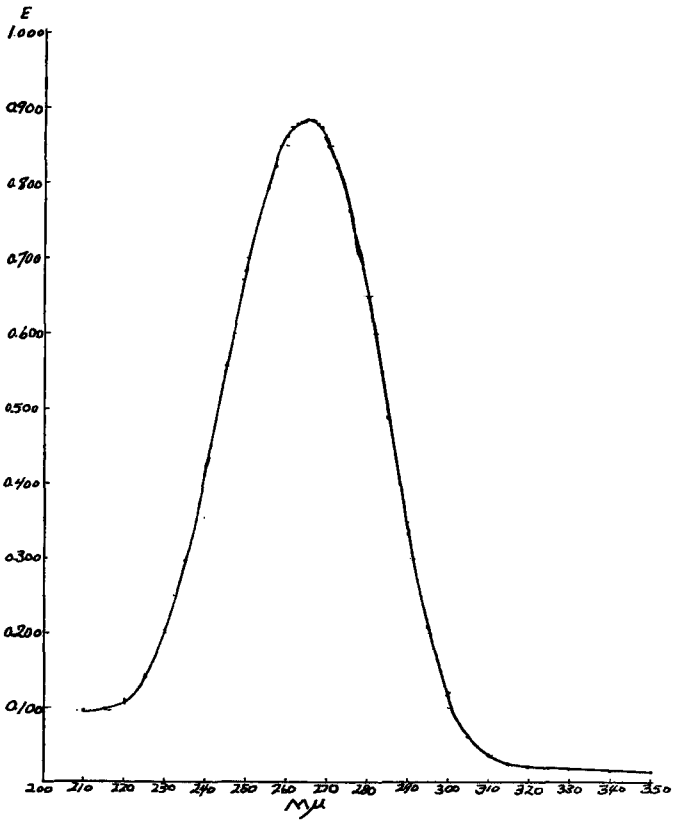

FIG. 1.—Absorption spectrum of acetone in water. 1.4033 g in 500 ml.

TABLE 1.—*Variation of optical density with concentration of solutions of acetone at 265 mμ*

ACETONE MG PER 100 ML SOLUTION	$E_{1\,cm}$ 265 mμ
77.7	0.257
155.5	0.496
310.9	0.985

and ethyl alcohol), and their oxidation products (chiefly acetic acid), it was desirable to know to what extent each of these compounds could contribute to the total light absorption at 265 mμ. Accordingly, the specific absorbancies ($E^{1\%}_{1\,cm}$) of these substances were determined and are summarized in Table 2. Likewise, the effect of changes in *p*H on the specific absorbancy of acetone was investigated and is shown in Table 3.

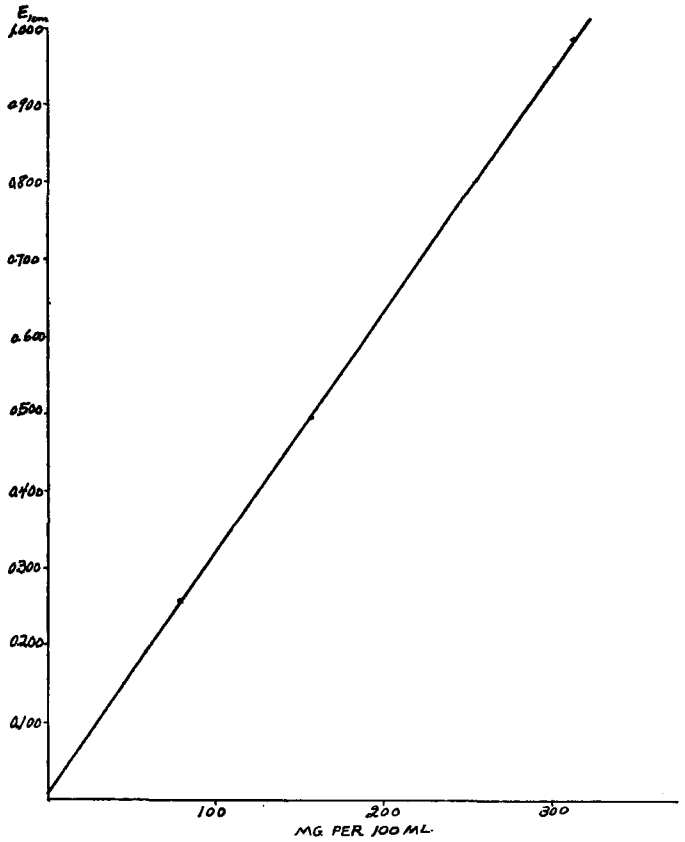

FIG. 2.—Optical density concentration curve for acetone in water at 265 mμ.

TABLE 2.—*Specific absorbancies at 265 mμ*

SUBSTANCE	SOLVENT	CONCENTRATION G PER 100 ML	$E_{1\,cm}$ 265 mμ	$E_{1\,cm}^{1\%}$
Acetone	Water	0.2807	0.862	3.07
Ethyl Alcohol	Water	10.0436	0.009	0.0009
Isopropyl Alcohol	Water	10.3415	0.007	0.0007
Acetic Acid	Water	10.23	0.037	0.0036

TABLE 3.—*Effect of pH on absorption of acetone solutions*

ACETONE G PER 100 ML	SOLVENT	$E_{1\,cm}$ 265 mμ	$E_{1\,cm}^{1\%}$
0.2348	Distilled water	0.721	3.07
0.2348	N/10 HCl	0.721	3.07
0.2348	N/10 NaOH	0.724	3.08

This preliminary work was encouraging and indicated that those substances likely to be present in the distillate from a flavor sample would not interfere with the isopropyl alcohol determination. Work was begun on analyzing isopropyl alcohol-acetone mixtures. Recoveries of 97–98 per cent were obtained from aqueous mixtures containing ethyl and isopropyl alcohols and acetone, and isopropyl alcohol and acetone.

Following this, essential oils were added. Three such mixtures were analyzed by the method submitted to the collaborators. This work is reported in Table 4.

TABLE 4.—*Analysis of isopropyl alcohol mixtures*

PRESENT		FOUND		RECOVERY (*per cent*)
ESSENTIAL OIL	ACETONE	ISOPROPYL ALCOHOL	ISOPROPYL ALCOHOL	
0.874 g lemon oil	3.676 g	3.669 g	3.612 g	98.4
3.379 g/100 ml lemon oil	25.5500 g/100 ml	39.4069 g/100 ml	37.71 g/100 ml	95.6
3.513 g/100 ml orange oil	5.883 g/100 ml	62.252 g/100 ml	59.29 g/100 ml	95.2

During the early part of this work a standard solution of acetone was prepared and its E-value determined each time an isopropyl alcohol or acetone determination was made. These values are recorded in Table 5. The average specific absorbancy value from Table 5 was used in the collaborative investigation.

TABLE 5.—*Specific absorbancies of acetone at 265 mμ*

ACETONE G/100 ML	E	$E_{1\,cm}^{1\%}$ 265 mμ
0.2807	0.862	3.07
0.3034	0.924	3.05
0.3119	0.960	3.08
0.1567	0.485	3.10
0.2360	0.729	3.09
0.2515	0.777	3.09
0.3101	0.953	3.07
0.2348	0.721	3.07
		Average 3.08

A preliminary sample (No. 1) was prepared and analyzed by two chemists at the Chicago District. Sample No. 2 was then prepared. This sample

was put into glass ampules holding slightly over 10 ml and sent out to the collaborators along with the method.[1]

The collaborators were instructed to use a 10 ml sample from one ampul for each determination. They were asked to make duplicate determinations and to report the per cent (W/V) of isopropyl alcohol. The collaborative results follow:

TABLE 7.—*Collaborative results*

COLLABORATOR[1]	SAMPLE[2]	PRESENT		FOUND	RECOVERY
		ACETONE	ISOPROPYL ALCOHOL	ISOPROPYL ALCOHOL	PER CENT
		G/100 ML	G/100 ML	G/100 ML	
1	1	6.508	61.725	59.73	96.8
2	1	6.508	61.725	58.77 58.52	95.2 94.8
2	2	12.47	51.75	48.93 49.34	94.6 95.3
3	2	12.47	51.75	51.12 50.32	98.8 97.2
4	2	12.47	51.75	48.71 48.56	94.1 93.8
5	2	12.47	51.75	49.46 49.70	95.6 96.0
6	2	12.47	51.75	46.56* 48.00†	90.0 92.8
7	2	12.47	51.75	45.58 46.10	88.1 89.1

[1] The cooperation of the following collaborators, all of the Food and Drug Administration, is gratefully acknowledged: Helen C. Barry, New Orleans District; Charles F. Bruening and Harold F. O'Keefe, Chicago District; Theodore E. Byers, Cincinnati District; R. F. Heuermann, St. Louis District; Sam H. Perlmutter, Minneapolis District.

[2] Sample 1 contained 3.002 g of oil of orange per 100 ml. Sample 2 contained 3.94 g of oil of orange per ml.

* Average of 4 values (47.29, 46.28, 46.28, 46.36) different aliquots, one distillation.

† Average of 2 values (47.88, 48.12) different aliquots, one distillation.

DISCUSSION

Most of the collaborators checked themselves quite well and the majority showed recoveries in the neighborhood of 95 per cent. Considering the volatile nature of the product, the collaborative results are considered satisfactory.

[1] Details of the method were published in *This Journal*, **35**, 78 (1952).

RECOMMENDATIONS*

It is recommended that the proposed method for the determination of isopropyl alcohol in mixtures containing acetone be adopted, first action, and that the subject be closed.

REFERENCES

(1) *Ind. Eng. Chem. Anal. Ed.*, **18**, 354 (1946).
(2) WALLS, H. J., and LUDLUM, E. B., *Trans. Faraday Soc.*, **33**, 776–81 (1937).
(3) WASHBURN, E. W., "International Critical Tables," Vol. V, pp. 365, 369, 371, 374, 376, 377, New York, McGraw-Hill Book Co., 1929.

No report was given for beta-ionone; maple flavor concentrates and imitations; or propylene glycol.

REPORT ON COSMETICS

By G. ROBERT CLARK (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

RECOMMENDATIONS†

The Referee recommends the following:

Deodorants and Anti-Perspirants.—Adoption, first action, of the methods for the determination of methenamine and phenolsulfonates, as recommended by the Associate Referee.

Cold Permanent Wave Preparations.—Adoption, first action, of the qualitative and quantitative procedures for examination of thioglycolate solutions and neutralizers, as recommended by the Associate Referee.

Continuance of the following topics:

Deodorants and Anti-Perspirants
Cold Permanent Wave Preparations
Cosmetic Creams
Mascara, Eyebrow Pencil, and Eyeshadow
Sun Tan Preparations
Hair Dyes and Rinses

The Referee further recommends that the topic *Cosmetic Skin Lotions* be discontinued.

* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 58 (1952).
† For report of Subcommittee B and action of the Association, see *This Journal*, **35**, 48 (1952).

REPORT ON MASCARAS, EYEBROW PENCILS, AND EYE SHADOWS

By PAUL W. JEWEL (Max Factor and Co., Hollywood, California), *Associate Referee*

The method described in 1949 has been improved somewhat by altering the solvents used for removing the base from the pigments. The new method is as follows:

Weigh a sample, about three grams, into a tared 50 ml centrifuge tube. Heat on the steam bath until completely melted. Add 5 ml of chloroform, and digest at the boiling point of the solvent until dispersion is complete. Then add 25 ml of benzene, heat just to incipient boiling, and centrifuge. Decant the clear liquid into a tared dish. Repeat this procedure three times, making sure that the pigments are dispersed each time. Dry and weigh the pigments. Evaporate the solvent from the solution of base, dry to constant weight at 100°C., and weigh.

Apparently, the chloroform is sufficiently better as a solvent to insure complete removal of the base, while the benzene reduces the specific gravity of the solution sufficiently to allow centrifuging.

This method gives theoretical values in the hands of the Associate Referee and will be submitted for collaborative study in the near future.

Failure to obtain theoretical values for triethanolamine and stearic acid in the extracted base, as has been noted previously,[1] appears to be due to polymer formation between these two substances during the heating and milling of the preparation. This polymerization appears to be irreversible under these conditions and will therefore constitute a consistent but not a constant source of error.

Work has begun on the cream mascaras and the so-called waterproof variety.

Most of the cream types are simply water dispersions of the old cake types but there are some interesting variations. Only one brand has been checked which uses triethanolamine, the remainder using mineral soaps. One uses what appears to be a simple solution of waxes in turpentine instead of the usual dispersion.

It is too early yet to even suggest an analytical method, but a few of the beginning efforts will be described.

Water may be determined by distilling a sample with toluene and measuring the water carried over. Examination of various brands obtained on the market showed a range from 10% to 73%.

Propylene glycol or glycerin may be determined by dispersing the sample in water in a separatory funnel, making acid with hydrochloric acid, and extracting the waxes with chloroform. Filter the aqueous phase and proceed with the malaprade method as modified by Shupe[2]. Ex-

[1] JEWEL, PAUL W., *This Journal*, **32**, 599 (1949).
[2] SHUPE, IRWIN S., *This Journal*, **26**, 249 (1943).

amination of various brands obtained on the market showed a range from 1.00 to 2.50 per cent.

Total waxes may be determined by evaporating the chloroform solution in a tared dish and weighing.

These total waxes may then be subjected to such analytical procedures as may seem indicated.

It is recommended* that work on this project be continued and that the method for cream mascara be submitted to collaborative study as soon as ready.

REPORT ON DEODORANTS AND ANTI-PERSPIRANTS

By HENRY KRAMER (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.),
Associate Referee

METHENAMINE

Several methods for the determination of methenamine were tried in the Cosmetic Laboratory. The U.S.P. XIV method was found inapplicable, and the A.O.A.C. (1) method was rejected because of the manipulative technique involved. Jones (2), using a method outlined by Shupe (3), determined methenamine by determining the formaldehyde liberated upon acid hydrolysis. This procedure proved satisfactory in the hands of the Associate Referee and was therefore submitted for collaborative study.

METHOD

REAGENTS

Sodium bisulfite.—10% w/v and 0.5% w/v.

Iodine, 0.05 *N*.

Starch indicator soln.—**5.3 (e).**

Borax-carbonate soln.—Dissolve 5.0 g Na_2CO_3 and 4.0 g $Na_2B_4O_7 \cdot 10\ H_2O$ in 100 ml of water.

PROCEDURE

Pipet an aliquot containing 150–200 mg of methenamine into a 500-ml round-bottom flask and dilute to 30 ml with water. Neutralize to litmus with either NaOH or H_2SO_4, then acidify with 1 ml of conc. H_2SO_4. Connect the flask to a water-cooled condenser and heat under reflux for 30 min. to hydrolyze the methenamine. Carefully add 160 ml of water thru the top of the condenser, and disconnect the condenser from the round-bottom flask. Connect the flask thru a Kjeldahl trap to an efficient straight-wall condenser, and distill the contents into a 200-ml volumetric flask containing 10 ml of a freshly prepared 10% $NaHSO_3$ soln. Continue the distillation until the residual volume is about 5 ml, taking care to avoid charring. Wash down the condenser with a little water and cool the distillate to room temp. Dilute the distillate to the mark with water, mix well, and allow to stand for 30 min. Pipet a 20 ml aliquot into a wide-mouth 250-ml Erlenmeyer flask, add 3–4 ml of starch indicator soln and destroy the excess bisulfite with ca 1 *N* iodine. Carefully adjust to the starch-iodine endpoint with 0.5% $NaHSO_3$ and 0.05 *N* iodine. Dilute

* For report of Subcommittee B and action of the Association, see *This Journal*, **35**, 48 (1952).

to 50 ml with water, add 10 ml of borax-carbonate soln and titrate with 0.05 *N* iodine until a permanent blue color is produced.

1 ml 0.05 *N* Iodine = 0.584 mg methenamine

An aqueous solution of methenamine, USP quality, was analyzed by both the USP XIV and the proposed method. The results are given in Table 1 and Table 2.

TABLE 1.—*Analysis by USP method*

WEIGHT METHENAMINE TAKEN	RECOVERIES	
mg	*mg*	*per cent*
801	789	98.5
1238	1223	98.8
1441	1424	98.8
	Average	98.7

TABLE 2.—*Analysis by proposed method*

WEIGHT METHENAMINE TAKEN	RECOVERIES	
mg	*mg*	*per cent*
10	9.85	98.5
10	9.88	98.8
	Average	98.7

The following solution containing a number of ingredients likely to be found in deodorants was prepared:

Glycerol, USP	10.0 grams	Sodium borate, CP	10.0 grams
Urea, CP	10.0 grams	Magnesium chloride, CP	10.0 grams
Aluminum potassium sulfate, CP	10.0 grams	Hydrochloric acid, conc.	50 ml
Calcium nitrate, CP	10.0 grams	Water q.s.	1 liter

The Associate Referee analyzed samples prepared by adding methenamine to 10 ml of this solution. The results are given in Table 3.

The collaborators were directed to prepare their samples by mixing 10 ml of the above solution with 20 ml of a solution that contained 20 mg of methenamine. Their results are given in Table 4.

The collaborators were asked to make determinations in duplicate.

Recoveries obtained by the collaborators varied from 95.9% to 99.0% with an average of 97.9%. The standard deviation of the results was 1.0%.

Collaborators who submitted results were:

C. Graichen, K. S. Heine, S. H. Newburger, of the Food and Drug Administration, Division of Cosmetics, Washington, D. C.

J. P. Traynor, Food and Drug Administration, Baltimore, Maryland; and J. H. Bornmann, Food and Drug Administration, Chicago, Illinois.

TABLE 3.—*Analysis of control samples by Associate Referee*

WEIGHT METHENAMINE TAKEN*	RECOVERIES	
mg	*mg*	*per cent*
9.87*	9.72	98.5
	9.74	98.7
19.7*	19.5	99.0
	19.5	99.0
	Average	98.8

* 10 and 20 mg respectively of the material found by the Associate Referee to contain 98.7% methenamine.

TABLE 4.—*Analysis by collaborators*

COLLABORATOR NO.	WEIGHT METHENAMINE TAKEN*	RECOVERIES	
	mg	*mg*	*per cent*
1	19.7	19.5	99.0
		19.5	99.0
2	19.7	19.2	97.5
		19.5	99.0
3	19.7	18.9	95.9
		19.4	98.5
4	19.7	19.2	97.5
		19.2	97.5
5	19.7	19.1	97.0
		19.3	98.0
		Average	97.9

* 20 mg of the material found by the Associate Referee to contain 98.7% of methenamine.

PHENOLSULFONATES

Jones (2) applied Grant's (4) modification of the U.S.P. IX Method for phenolsulfonates to anti-perspirants. A careful evaluation of this procedure indicated that it is less tedious than the A.O.A.C. method for phenolsulfonates (5) in drugs. Accordingly, the method was submitted for collaborative study.

The method is not applicable in the presence of other materials that brominate rapidly.

METHOD

REAGENTS

Potassium bromate, 0.1 *N*.—Dissolve 2.8 g potassium bromate in 1000 ml of water. Standardize against 0.1 *N* sodium thiosulfate.

Sodium thiosulfate, 0.1 *N*.

Starch indicator soln.—5.3(e).

PROCEDURE

Pipet an aliquot containing 60–125 mg of phenolsulfonic acid into a 250 ml iodine flask and dilute to ca 75 ml with water. Add 2–3 ml of conc. HCl, 2–3 g of KBr, and titrate slowly with 0.1 *N* $KBrO_3$ until an excess of 1–3 ml is present. (In the early stages of the titration the bromine formed disappears rapidly but near the end point some time is required for the bromine to react.) Stopper the flask and let stand for 10 min. If the color disappears, add more 0.1 *N* $KBrO_3$ and let stand for another 10 min. Add 2–3 g KI, shake thoroly, and titrate the liberated iodine with 0.1 *N* $Na_2S_2O_3$ using starch as indicator. The iodine liberated is equivalent to the excess of $KBrO_3$ soln added. From the net volume of $KBrO_3$ solution required in the bromination reaction, calculate the amount of phenolsulfonic acid in the sample.

1 ml 0.1 *N* $KBrO_3$ = 0.00435 g phenolsulfonic acid

EXPERIMENTAL

An aqueous solution of zinc phenolsulfonate, NF quality, was analyzed by both the A.O.A.C. procedure and the proposed method. The results are given in Tables 5 and 6.

TABLE 5.—*Analysis by A.O.A.C. method (5)*

WEIGHT PHENOLSULFONIC ACID TAKEN	RECOVERIES	
mg	*mg*	*per cent*
642	631	98.3
445	441	99.1
451	442	98.0
	Average	98.5

The following solution containing a number of ingredients likely to be found in anti-perspirants was prepared.

Glycerol, USP	10.0 grams	Magnesium chloride, C.P.	10.0 grams
Urea, C.P.	10.0 grams	Sodium borate, C.P.	10.0 grams
Calcium nitrate, C.P.	10.0 grams	Hydrochloric acid, conc.	50 ml
Aluminum chloride, C.P.	10.0 grams	Distilled water q.s.	1 liter
Zinc chloride, C.P.	10.0 grams		

The Associate Referee analyzed a number of samples consisting of 10 ml portions of this solution to which known amounts of phenolsulfonic acid had been added. The results are given in Table 7.

The collaborators were directed to prepare their samples by mixing 10 ml of the above solution with 20 ml of a solution that contained 100

TABLE 6.—*Analysis by proposed method*

WEIGHT PHENOLSULFONIC ACID TAKEN	RECOVERIES	
mg	*mg*	*per cent*
31.33	31.5	100.5
31.33	31.2	99.6
31.33	31.0	98.9
	Average	99.7
62.66	62.2	99.3
62.66	62.1	99.1
62.66	62.2	99.3
	Average	99.2
125.32	124.8	99.6
125.32	124.5	99.3
125.32	124.4	99.3
	Average	99.4

TABLE 7.—*Analysis of control samples by Associate Referee*

WEIGHT PHENOLSULFONIC ACID TAKEN	RECOVERIES	
mg	*mg*	*per cent*
31.33	31.1	99.3
31.33	31.1	99.3
31.33	31.2	99.6
	Average	99.4
62.66	62.0	98.9
62.66	62.1	99.1
62.66	62.1	99.1
	Average	99.0
125.32	124.4	99.3
125.32	124.4	99.3
125.32	124.3	99.2
	Average	99.3

mg of zinc phenolsulfonate (62.66 mg phenolsulfonic acid). Their results are presented in Table 8.

The results obtained by the five collaborators vary from 98.9% to

TABLE 8.—*Analysis by collaborators*

COLLABORATOR NO.	WEIGHT PHENOLSULFONIC ACID TAKEN	RECOVERIES	
	mg	*mg*	*per cent*
1	62.66	62.2	99.3
	62.66	62.0	98.9
2	62.66	63.1	100.7
	62.66	62.8	100.2
	62.66	62.9	100.4
	62.66	62.6	99.9
3	62.66	63.6	101.5
	62.66	64.0	102.1
4	62.66	63.2	100.9
	62.66	63.2	100.9
5	62.66	62.9	100.4
	62.66	62.8	100.2
		Average	100.4

102.1% with an average recovery of 100.4%. The standard deviation is 0.6%.

The collaborators who submitted data were:

H. E. Gakenheimer, Food and Drug Administration, Baltimore, Maryland.

C. Graichen, Food and Drug Administration, Division of Cosmetics, Washington, D. C.

L. S. Harrow, Food and Drug Administration, Division of Cosmetics, Washington, D. C.

D. D. Price, Food and Drug Administration, Baltimore, Maryland.

R. B. Smyth, Cosmetic and Colour Section, Department of National Health and Welfare, Ottawa, Ontario, Canada.

RECOMMENDATIONS*

It is recommended—

(1) That the proposed method for methenamine be adopted, first action.

(2) That the proposed method for phenolsulfonic acid in anti-perspirants be adopted, first action.

(3) That the study of anti-perspirants and deodorants be continued.

REFERENCES

(1) *Methods of Analysis*, 7th Ed., 1950, page 612.
(2) J. H. JONES (private communication).
(3) I. S. SHUPE, *This Journal*, **26**, 249 (1943).
(4) E. H. GRANT, *This Journal*, **14**, 351 (1931).
(5) *Methods of Analysis*, 7th Ed., 1950, page 617.

* For report of Subcommittee B and action of the Association, see *This Journal*, **35**, 48 (1952).

REPORT ON COLD PERMANENT WAVES

By HENRY KRAMER (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

THIOGLYCOLIC ACID

Newburger[1] has shown that the determination of thioglycolic acid by reduction with iodine compares favorably with either the titration of the carboxyl group or an analysis for total sulfur. He also demonstrated that an ammoniacal aqueous 7% solution of thioglycolic acid, sealed in a glass bottle, deteriorated only to 6.93% in four years.

QUALITATIVE TEST

Dilute 2 ml of sample to 10 ml with water, acidify with 10% acetic acid adding 5 ml in excess and shake well. Add 2 ml of 10% cadmium acetate and shake. A white gelatinous precipitate forms if thioglycolic acid is present. Add an excess of 10% ammonium hydroxide and shake. The precipitate of cadmium thioglycolate will dissolve.

QUANTITATIVE DETERMINATION

REAGENTS

Methyl red indicator soln.—**2.46 (c).**
Starch indicator soln.—**5.3(e).**
Iodine, 0.1 *N*.

PROCEDURE

Pipet an aliquot of the sample containing 250–300 mg thioglycolic acid into a wide-mouth 250-ml Erlenmeyer flask. Dilute to 50 ml with water, add 2–3 drops of methyl red indicator soln, and make slightly acid with concentrated HCl. Add 3–4 ml of starch indicator soln and titrate with 0.1 *N* iodine to a purple end point.

1 ml of 0.1 *N* iodine = 0.009209 g thioglycolic acid

(Reducing substances, other than thioglycolic acid, will interfere with the proposed method.)

The following solution, *p*H 9.2, was submitted for collaborative study.

Thioglycolic acid	65.1 grams
Ammonia	23.9 grams
Water q.s.	1 liter

The results are presented in Table 1.

The results obtained by the five collaborators vary from 97.5% to 100.8% with an average recovery of 99.7%. The standard deviation is 1.2%.

NEUTRALIZERS

Qualitative Tests for $KBrO_3$ and $NaBO_3$

(1) Note the physical characteristics of the product.
(a) $KBrO_3$ and $NaBO_3$ are white crystalline salts soluble in water.

[1] S. H. Newburger, private communication.

TABLE 1.—*Identification and recoveries of thioglycolic acid*

COLLABORATOR NO.	IDENTIFICATION OF THIOGLYCOLIC ACID	WEIGHT THIOGLYCOLIC ACID IN SAMPLE	RECOVERIES	
		mg	*mg*	*per cent*
1	Positive	651	642	98.6
		651	649	99.7
		651	653	100.3
		651	646	99.2
2	Positive	651	654	100.5
		651	653	100.3
3	Positive	651	656	100.8
		651	656	100.8
4	Positive	651	654	100.5
		651	655	100.6
5	Positive	651	635	97.5
		651	636	97.7
			Average	99.7

(2) Check the *p*H of an aqueous soln with *p*H test paper.

(a) $KBrO_3$ in aqueous soln is slightly acid.

(b) $NaBO_3$ in aqueous soln is strongly alkaline.

(3) Make a flame test in a slightly darkened room using a platinum wire.

(a) When viewed thru a cobalt glass $KBrO_3$ gives a reddish-violet flame.

(b) $NaBO_3$ gives the typical yellow sodium flame.

(4) Test for an oxidizing agent. Dissolve 0.1 g of neutralizer in 10 ml of water, acidify with conc. HCl and add 0.5 g KI.

(a) $KBrO_3$ and $NaBO_3$ liberate iodine.

(5) If tests **1, 2a, 3a,** and **4** are positive for $KBrO_3$ make the following test for bromine: To 1 ml of a 5% aqueous soln of the neutralizer in a test tube slowly add 2 ml of conc. H_2SO_4 with vigorous shaking. Note with *caution* the odor and color of the liberated gas. Cool the test tube. *Carefully* add 2 ml of CS_2 and shake. The carbon disulfide layer becomes yellow or red if bromine is present.

(6) If tests **1, 2b, 3b,** and **4** are positive for $NaBO_3$ make the following test for boron. Moisten 0.2 g of neutralizer with 1–2 drops of conc. H_2SO_4, add 2 ml of methyl alcohol, stir well, and ignite. A green flame indicates boron.

All five collaborators correctly identified a sample labeled "A" as $KBrO_3$ and a sample labeled "B" as $NaBO_3$. None of the tests were reported as obscure.

COLLABORATORS

Collaborators who submitted results for both thioglycolic acid and the "neutralizers" were:

C. Graichen, Food and Drug Administration, Division of Cosmetics, Washington, D. C.

H. I. Macomber, Food and Drug Administration, Baltimore, Maryland.
S. H. Newburger, Food and Drug Administration, Division of Cosmetics, Baltimore, Maryland.
D. D. Price, Food and Drug Administration, Baltimore, Maryland.
R. B. Smyth, Cosmetic and Colour Section, Department of National Health and Welfare, Ottawa, Ontario, Canada.

RECOMMENDATIONS*

It is recommended—

(1) That the qualitative and quantitative procedures for thioglycolic acid be adopted, first action.

(2) That the qualitative tests for sodium perborate be adopted, first action.

(3) That the qualitative tests for potassium bromate be adopted, first action.

(4) That the subject of cold permanent waves be continued.

ACKNOWLEDGMENT

Acknowledgment is due E. G. McDonough of Evans Chemetics, Inc., who kindly supplied a solution of double distilled thioglycolic acid for use in these tests.

No report was given on hair dyes and rinses; cosmetic creams; or skin lotions.

The contributed paper entitled "Determination of Resorcinol in Hair Dyes," by S. H. Newburger and J. H. Jones, was published in *This Journal*, November, 1951, p. 787.

REPORT ON STANDARDIZATION OF MICROCHEMICAL METHODS

By C. O. Willits (Eastern Regional Research Laboratory,† Philadelphia 18, Pennsylvania), *Referee*

This report gives the recommendations of the Associate Referees and the Referee, based on a collaborative study of micromethods for analysis for sulfur, nitrogen, bromine, and chlorine in organic compounds. The studies were designed to determine which of the methods described in the literature and in common use would be suitable, either in its present form or in some modified form, as the official method. The collaborators submitted not only the analytical results but also detailed descriptions of the methods used. From a statistical analysis of these data for accuracy

* For report of Subcommittee B and action of the Association, see *This Journal*, 35, 48 (1952).
† One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

and precision and a correlation of the analytical details, it is recommended†—

(1) That since the Carius and catalytic combustion procedures are equaly precise and accurate they should be studied further so that an official method can be developed for sulfur.

(2) That the catalytic combustion method for sulfur include oxygen flow rates greater than 11 ml per minute and that the long furnace be maintained at temperatures above 750°C.

(3) That the combustion tubes used in the Carius method for sulfur analysis have a volume less than 12.5 ml.

(4) That either the titrimetric or the gravimetric procedure be used for the above mentioned sulfur methods, since they are equally accurate.

(5) That further modification of the Kjeldahl method and of the pretreatment of samples be made before a method be recommended for the analysis of nitrogen in compounds containing the N-N linkage.

(6) That the method of pretreatment of samples prior to Kjeldahl nitrogen analysis used this year be further tested for compounds containing NO and NO_2 groups.

(7) Since the Pregl and Carius methods for analysis of bromine and chlorine in organic compounds are equally precise and accurate, that these two methods be studied further to develop an official method for analysis of these halogens.

REPORT ON MICROANALYTICAL DETERMINATION OF NITROGEN

(FOR N-N, NO AND NO_2 LINKAGES)

By C. L. Ogg[1] and C. O. Willits,[2] (Eastern Regional Research Laboratory,* Philadelphia 18, Pennsylvania)

The hydriodic reduction method for reducing compounds containing N–N and N–O groups before determining the nitrogen by the Kjeldahl method, proposed in the 1950 report on microchemical methods, was studied again this year. Two samples which contained these nitrogen linkages, acetone-2,-4-dinitrophenyl hydrazone and benzotriazole, were sent to those collaborators who had expressed a willingness to study the method further. The following procedure, a modification of the Friedrich method, was used in this study:

Weigh in a charging tube about 10 mg of sample and transfer to a 30 ml Kjeldahl digestion flask. Add 1 ml of hydriodic acid, sp. g. 1.70 (57%), and add boiling chips

† For report of Subcommittee C and action of the Association, see *This Journal*, 35, 53 (1952).

[1] Associate Referee.

[2] Referee.

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

to prevent bumping; gently reflux the mixture for 45 min. Cool, add 5 ml of water, and 2 ml of sulfuric acid; heat the mixture at *full heat* until the water is removed and the sulfuric acid *refluxes* up in the neck of the flask. Cool, add 2 ml, H_2O, and repeat the distillation. If iodine vapors are not removed completely, repeat the distillation step until no more iodine remains.

Cool, add 0.65 g of K_2SO_4, 40 mg of HgO, and 1 ml of H_2O; digest for 1 hour with the *sulfuric acid* refluxing 4–8 cm into the neck of the flask. (For the sample containing the ring nitrogen, add 1.3 g of K_2SO_4 and digest for 4 hours.)

Continue the determination beginning with "Cool, add minimum of distilled water . . . " in the first action procedure (*Methods of Analysis*, 7th Ed., **37.4**).

In 1949 it was found that a hydriodic acid pretreatment produces low and erratic results. Because of this, the Friedrich (hydriodic acid) method was deleted from the *Book of Methods*. The modification used this year differs from that studied in 1949 in three respects. In this year's procedure, the potassium sulfate for the Kjeldahl digestion was not added until *all* HI was removed, only one-half as much potassium sulfate (0.65 g) was added, and more emphasis was placed on removing completely all HI before beginning the digestion with potassium sulfate and mercuric oxide catalyst.

Twelve analysts reported results for sample No. 1, acetone-2, 4-dinitrophenyl hydrazone, and ten reported for sample No. 2, benzotriazole. Theoretical nitrogen percentages for the two pure compounds are 23.62 and 35.28%, respectively. Table 1 shows the results. In this table, n is the

TABLE 1.—*Results of the collaborative study of the determination of nitrogen in compounds having N-N and N-O linkages using the Kjeldahl method with HI pretreatment*

COLLABORATOR NO.	ACETONE-2, 4-DINITROPHENYL HYDRAZONE			BENZOTRIAZOLE		
	n	$\bar{X}$	s	n	$\bar{X}$	s
0	8	23.27	0.075	8	34.27	0.099
8	3	23.50	0.076	4	34.19	0.168
15	5	22.63	0.082	5	32.16	0.320
23	3	23.22	0.066	4	34.06	0.119
24	5	23.50	0.111	5	32.46	0.752
29	3	23.50	0.329	3	33.74	0.872
35	6	21.91	0.062			
37	4	23.45	0.363	6	33.34	0.642
59	2	23.73	1.017	2	28.95	0.092
1	5	23.18	0.191			
22	2	22.75	0.065	2	33.87	0.190
19	10	23.23	0.338			
36				8	31.89	0.435
$\bar{\bar{X}}$		23.16	(0.225)		32.90	(0.369)
$s_{\bar{x}}$		0.334			1.63	
Theoretical Value		23.62%			35.28%	

number of determinations reported, $\overline{X}$ is the analyst's mean value, and s is the standard deviation of his values. $\overline{\overline{X}}$ is the mean of the analyst's mean, $\overline{X}$'s. For sample No. 1 this was 23.16%, as opposed to a theoretical value of 23.62%. Six of the twelve analyst's means were within 0.2% of the true value; all the other six values were lower than the theoretical value by more than 0.2%. The standard deviation of the mean, $s_{\bar{x}}$, was 0.334, whereas the mean of the individual standard deviations was 0.225, showing that on the average, the results of each analyst were more precise than were the averages of all analysts.

The corresponding values for sample No. 2, which contains three nitrogens linked together in a ring compound were also determined. The value for the grand mean, $\overline{\overline{X}}$, was 32.90%, as compared with a theoretical value of 35.28%. The standard deviation of the means, $s_{\bar{x}}$, was 1.63, and the mean of the individual s values was 0.369. Collaborator 19, not listed in Table 1, reported no values for Sample No. 2 but said that his results ranged from 16.03 to 24.42%. These data show that the nitrogen values obtained for this sample by the proposed method were much poorer than those for sample No. 1.

In the previous study of this method of pretreatment (1), methyl orange was the test sample, and a mean value of 11.02% nitrogen was obtained as compared with a value of 11.50% by the Dumas method. The standard deviation of all the values reported was 0.496. Although the nitrogen values for methyl orange deviated more from the theoretical value than did those obtained this year for sample No. 1, they were closer to the theoretical value than those for sample No. 2.

Although the results have neither a $\overline{\overline{X}}$ value sufficiently near to the theoretical value for nitrogen nor a sufficiently low standard deviation of the mean values to indicate good precision among analysts, they do indicate that the differences in accuracy and precision were more closely related to the nature of the compounds analyzed than to the methods used. The results obtained for acetone-2, 4-dinitrophenyl hydrazone, which has one N–N linkage and two NO_2 groups, were better than those for benzotriazole, which has the N–N–N linkage, and for methyl orange, which has one N–N linkage and one amine group. This indicates that the method may be more satisfactory for compounds containing N–O linkages than those containing N–N linkages.

RECOMMENDATIONS*

The referees recommend that the method used in this year's study be tested on compounds containing only NO and NO_2 groups and that a search be made for another modification of the Kjeldahl method or pretreatment suitable for determining nitrogen in compounds containing the N–N linkage.

* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 53 (1952).

Collaborators on nitrogen analysis were:

Alicino, J. F., Squibb Institute for Medical Research
Brown, L. E., Southern Regional Research Laboratory
Brown, Wm. L., Eli Lilly and Company
Browning, B. L., The Institute of Paper Chemistry
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Means, J. A., Charles Pfizer and Company, Inc.
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Van Etten, C. H., Northern Regional Research Laboratory

LITERATURE CITED

(1) WILLITS, C. O., and OGG, C. L., *This Journal*, **32**, 561 (1949).

REPORT ON MICROANALYTICAL DETERMINATIONS OF BROMINE AND CHLORINE

By AL STEYERMARK (*Associate Referee*) and MARIAN B. FAULKNER
(Hoffmann-La Roche Inc. Nutley, New Jersey)

Since no previous work had been done toward establishing an official micromethod for bromine or chlorine in organic material, this year's study followed the pattern used for the previously reported carbon and hydrogen investigations (2, 5). Two samples, bromoacetanilide[1] and chloroacetanilide,[1] were sent to each analyst who expressed a willingness to participate, and each was asked to analyze the samples by the method currently in use in his laboratory. Detailed questionnaires, one for each of the three combustion methods, catalytic, Carius, and Parr bomb, were sent with the samples, with instructions that the analyst return along with his results the questionnaire that pertained to his method. The collaborator was requested to send all of the values obtained, so that a statistical analysis of the data could be made, to compare the different combustion methods and also to show the effect of different materials and operations on the results obtained by any one of the three combustion methods.

CATALYTIC COMBUSTION METHOD

Bromine and chlorine results obtained by the catalytic combustion procedure were reported by ten and eleven collaborators, respectively. Ten analysts reported 51 values for bromoacetanilide, and eleven analysts reported 51 values for chloroacetanilide. Although the analysts used a

[1] The samples were Eastman Kodak Company material which was not further purified, since in the referee's laboratory they were found on analysis to give respective halogen values which differed from the theory by amounts well within the limits of the allowable error (±0.3%) (1, 4).

TABLE 1.—*Bromine—catalytic combustion*

COLLABORATOR NUMBER:		1	9	17	24	28	30	40	60	61	64	$\bar{\bar{X}}$	$S_{\bar{x}}$
Sample— Bromoacetanilide (37.33% Bromine)	n $\bar{X}$ S $\bar{X}$—Theory	4 37.41 0.145 +0.08	8 37.31 0.227 −0.02	8 37.32 0.205 −0.01	3 37.33 0.068 0.00	2 37.30 0.143 −0.03	7 37.40 0.113 +0.07	6 37.24 0.190 −0.09	9 37.45 0.181 +0.12	2 37.38 0.083 +0.05	2 37.65 0.463 +0.32	37.38 (0.181) (+0.05)	0.114
Combustion tube	Quartz Other	*	*	*	*	*	*	*	*	*	*		
Absorber	Spiral Grote	*	*	*	*	*	*	*	* *	*	*		
Absorber connection	Joint No Joint	*	*	*	*	*	*	*	*	*	*		
Contact catalyst	Platinum Star Platinum Gauze	*	* *	*	*	*	*	*	*	*	*		
Platinum catalyst behind sample	Yes No	*	*	*	*	*	*	*	*	*	*		
Long furnace temperature	750°C. and over Less than 750°C.	*	*	*	*	*	*	*	*	*			
Sample burner	Electric Gas	*	*	*	*	*	*	*	*	*	*		
Sample burner temp.	750°C. and over Less than 750°C.	*	*	*	*		*	*	*	*	*		
Burner operation	Mechanical Manual	*	*	*	*	*	*	*	*	*	*		
Absorbing solution	H_2O_2-KOH-NaOH $NaHSO_3$+Na_2CO_3	*	*	*	*	*	*	*	*	*	*		
Sample weight range	2–10 mg Over 10 mg	*	*	*	*	*	*	*	*	*	*		
Burner moved over boat	Once Twice	*	*	*	*	*	*	*	*	*	*		
Distance burner moved	Over 11 cm Less than 11 min.	*	*	*	*	*	*	*	*	*	*		
Sweeping time	Over 11 min. Less than 11 min.	*	*	*	*	*	*	*	*	*	*		
Total analysis time	Over 31 min. Less than 31 min.	*	*	*	*	*	*	*	*	*	*		
Bromine determined	Gravimetric Titrimetric	*	*	*	*	*	*	*	*	*	*		
Oxygen flow rate	Over 11 ml/min. Less than 11	*	*	*	*	*	*	*	*				

TABLE 2.—*Chlorine—catalytic combustion*

COLLABORATOR NUMBER:		1	9	17	24	28	30	31	40	60	61	64	$\bar{\bar{X}}$	$S_{\bar{x}}$
Sample— Chloroacetanilide (20.91% Chlorine)	n $\bar{X}$ S $\bar{X}$—Theory	4 20.59 0.124 −0.32	8 20.73 0.301 −0.18	8 20.91 0.178 0.00	3 21.03 0.264 +0.12	2 20.71 0.389 −0.20	8 21.03 0.179 +0.12	4 20.81 0.026 −0.10	4 20.99 0.109 +0.08	6 21.03 0.142 +0.12	2 20.93 0.103 +0.02	2 21.05 0.221 +0.14	20.89 (0.185) (−0.02)	0.158
Combustion tube	Quartz Other	*	*	*	*	*	*	*	*	*	*	*		
Absorber	Spiral Grote	*	*	*	*	*	*	*	*	*	*	*		
Absorber connection	Joint No Joint	*	*	*	*	*	*	*	*	*	*	*		
Contact catalyst	Platinum Star Platinum Gauze	*	*	*	*	*	*	*	*	*	*	*		
Platinum catalyst behind sample	Yes No	*	*	*	*	*	*		*	*	*	*		
Long furnace temperature	750°C. and over Less than 750°C.	*	*	*	*	*	*	*	*	*	*	*		
Sample burner	Electric Gas	*	*	*	*	*	*	*	*	*	*	*		
Sample burner temp.	750°C. and over Less than 750°C.	*	*	*	*		*	*	*	*	*	*		
Burner operation	Mechanical Manual	*	*	*	*	*	*	*	*	*	*	*		
Absorbing solution	H_2O_2-KOH-NaOH $NaHSO_3$+Na_2CO_3	*	*	*	*	*	*	*	*	*	*	*		
Sample weight range	2–10 mg Over 10 mg	*	*	*	*	*	*	*	*	*	*	*		
Burner moved over boat	Once Twice	*	*	*	*	*	*	*	*	*	*	*		
Distance burner is moved	Over 11 cm Less than 11 cm	*	*	*	*	*	*	*	*	*	*	*		
Sweeping time	Over 11 min. Less than 11 min.	*	*	*	*	*	*	*	*	*	*	*		
Total analysis time	Over 31 min. Less than 31 min.	*	*	*	*	*	*	*	*	*	*	*		
Chlorine determined	Gravimetric Titrimetric	*	*	*	*	*	*	*	*	*	*	*		
Oxygen flow rate	Over 11 ml/min. Less than 11	*	*	*		*	*		*	*	*			

catalytic combustion method that was basically the same, there was considerable variation in material and operation in the methods used. Tables 1 and 2 show summaries of the analytical data reported, as well as a condensation of the information obtained from the completed questionnaires for bromine and chlorine, respectively. In these tables, n is the number of halogen (bromine and chlorine) values reported by each analyst, $\overline{X}$ is the mean of his data, and S is the standard deviation of his data,

$$S = \text{standard deviation} = \sqrt{\frac{\Sigma(X-\overline{X})^2}{n-1}}.$$

(X = individual values.)

The symbols $\overline{\overline{X}}$ and $S_{\bar{x}}$ are used for the mean of all $\overline{X}$'s and the standard deviation of the $\overline{X}$'s respectively. Under the $\overline{\overline{X}}$ column, the values in parentheses are the means of the standard deviations, S, and the means of $\overline{X}$-theory for the halogens (bromine and chlorine).

The $\overline{\overline{X}}$'s differ from the theoretical values by only $+0.05\%$ for bromoacetanilide and -0.02% for chloroacetanilide. There was also but little difference between the standard deviation of the means, $S_{\bar{x}}$, for the two samples, which were 0.114 and 0.158, respectively.

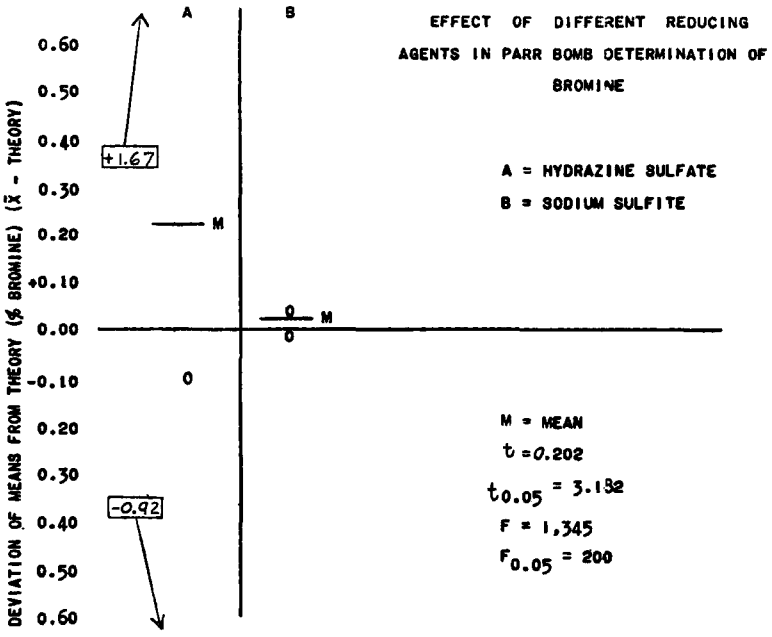

FIG. 1.—Effect of different reducing agents in Parr bomb determination of bromine.

The effect of the variations within a method was studied to see if the data indicated that one procedure would produce more accurate results than the alternate. The variables whose alternate procedures were compared were those listed in Tables 1 and 2. The data for the two alternate procedures for each variable studied were plotted in the same way as Figure 1.[2] For each set of data the student's t value was calculated using the formula (3)

$$t=\bar{x}\sqrt{\frac{n_a n_b(n_a+n_b-2)}{(n_a+n_b)\left[\Sigma(X_a-\overline{X}_a)^2+\Sigma(X_b-\overline{X}_b)^2\right]}}$$

Where $\bar{x}=\overline{X}_a-\overline{X}_b$; n_a and n_b are the number of values in groups a and b,

TABLE 3.—*Effect of variations within the catalytic method for bromine*

VARIATION		NUMBER OF COLLABORATORS	t	$t_{0.05}$
Temperature at center of long burner	Below 750°C.	4	0.6555	2.306
	Above 750°C.	6		
Temperature of sample burner	Below 750°C.	5	0.7422	2.365
	Above 750°C.	4		
Total time of combustion	Less than 30 min.	6	0.8070	2.306
	More than 30 min.	4		

TABLE 4.—*Effect of variations within the catalytic method for chlorine*

VARIATION		NUMBER OF COLLABORATORS	t	$t_{0.05}$
Temperature at center of long burner	Below 750°C.	5	0.3992	2.262
	Above 750°C.	6		
Temperature of sample burner	Below 750°C.	4	0.4465	2.306
	Above 750°C.	6		
Total time of combustion	Less than 30 min.	6	0.9333	2.262
	More than 30 min.	5		

[2] Only figures for critical values are given. Non-critical data are given in tables.

respectively, X_a and X_b are the individual values in the two groups; $\overline{X}_a$ and $\overline{X}_b$ the means of the values for the two groups. If the calculated t value was greater than the critical value ($t_{0.05}$) obtained from a table of student's t, the difference between the two groups of data was critical at the 95% level and the procedure whose mean was nearer the theoretical value was considered to be the better. No such comparisons had t values that were critical. Tables 3 and 4 give the t values for the comparisons made.

CARIUS COMBUSTION METHOD

The data furnished by the thirteen collaborators who used the Carius method for bromoacetanilide and the twelve collaborators who used the Carius method for chloroacetanilide were treated in the same manner as those for the catalytic combustion method. Tables 5 and 6 show summaries of the data and also the variables with alternate procedures which were studied statistically. In these tables, the symbols are the same as used in Tables 1 and 2.

The $\overline{\overline{X}}$'s differ from the theoretical values by only +0.02% for bromoacetanilide and −0.07% for chloroacetanilide. There was also but little difference between the standard deviation of the means, $S_{\bar{x}}$, for the two samples, which were 0.187 and 0.139, respectively.

The effect of the variations within a method was studied to see if the data indicated that one procedure would produce more accurate results than the alternate. The variables whose alternate procedures were compared were those listed in Tables 5 and 6. The data for the two alternate procedures for each variable studied were plotted in the same way as Figure 1. For each set of data, the student's t value was calculated (above formula). No such comparisons had t values that were critical. Tables 7 and 8 give the t values for the comparisons made.

PARR BOMB COMBUSTION

Bromine and chlorine results obtained by the Parr bomb combustion method were reported by five collaborators. Five analysts reported 35 values for bromoacetanilide and 36 values for chloroacetanilide. Tables 9 and 10 show summaries of the data and also the variables with alternate procedures which were studied statistically. In these tables, the symbols are the same as used in Tables 1 and 2.

The $\overline{\overline{X}}$'s differ from the theoretical values by only +0.14% for bromoacetanilide and −0.12% for chloroacetanilide. There was some difference between the standard deviation of the means, $S_{\bar{x}}$, for the two samples, which were 0.942 and 0.135, respectively. The effect of the variations within a method was studied to see if the data indicated that one procedure would produce more accurate results than the alternate. The variables whose alternate procedures were compared were those listed in Tables 9 and 10. The data for the two alternate procedures for each vari-

TABLE 5.—*Bromine—Carius combustion*

COLLABORATOR NUMBER:		0	15	22	29	31	35	37	46	49	50	59	62	63	$\bar{\bar{X}}$	$S_{\bar{x}}$
Sample— Bromoacetanilide (37.33%) Bromine	n $\bar{X}$ S $\bar{X}$—Theory	4 37.41 0.098 +0.08	8 36.94 0.436 −0.39	5 37.24 0.172 −0.09	3 37.71 0.487 +0.38	4 37.27 0.149 −0.06	7 37.46 0.220 +0.13	3 37.43 0.178 +0.10	4 37.29 0.149 −0.04	4 37.52 0.257 +0.19	8 37.34 0.103 +0.01	2 37.15 0.264 −0.18	16 37.39 0.228 +0.06	4 37.34 0.187 +0.01	37.35 0.225 (+0.02)	0.187
Combustion temperature (max.)	300°C. and over Less than 300°C.	*	*	*	*	*	*	*	*	*	*	*	*	*		
Combustion tube volume (calc.)	9 ml and over Under 9 ml	*	*		*	*	*	*	*	*	*	*	*	*		
Sample weight range	2–10 mg Over 10 mg	*	*	*	*	*	*	*	*	*			*	*		
Nitric acid	Fuming Concentrated	*	*		*	*	*	*	*	*	*	*	*	*		
Volume of nitric acid	Less than 0.3 ml 0.3 ml and over	*	*		*	*	*	*	*	*	*	*	*	*		
Amount of silver nitrate	25 mg and less Over 25 mg	*	*		*	*	*	*	*	*		*	*	*		
Combustion time	Less than 5 hrs. Over 5 hrs.	*	*	*	*	*	*	*	*	*	*	*	*	*		
Bromine determination	Gravimetric Titrimetric	*	*	*	*	*	*	*		*	*	*	*	*		

TABLE 6.—*Chlorine—Carius combustion*

COLLABORATOR NUMBER:		0	15	22	29	35	37	46	49	50	59	62	63	$\bar{\bar{X}}$	$S\bar{x}$
Sample— Chloroacetanilide (20.91% Chlorine)	n $\bar{X}$ S $\bar{X}$—Theory	7 20.75 0.261 −0.16	8 20.58 0.371 −0.33	5 20.88 0.172 −0.03	2 20.90 0.312 −0.01	7 20.56 0.400 −0.35	3 20.89 0.040 −0.02	2 20.87 0.112 −0.04	4 20.83 0.152 −0.08	8 20.92 0.101 +0.01	2 20.95 0.050 +0.04	18 20.98 0.243 +0.07	4 20.94 0.160 +0.03	20.84 (0.198) (−0.07)	0.139
Combustion temperature (max.)	300°C. and over Less than 300°C.	*	*	*	*	*	*	*	*	*	*	*	*		
Combustion tube volume (calc.)	9 ml and over Under 9 ml	*	*		*	*	*	*	*	*	*	*	*		
Sample weight range	2–10 mg Over 10 mg	*	*	*	*	*	*	*	*			*	*		
Nitric acid	Fuming Concentrated	*	*		*	*	*	*	*	*	*	*	*		
Volume of nitric acid	Less than 0.3 ml 0.3 ml and over	*	*		*	*	*	*	*	*	*	*	*		
Amount of silver nitrate	25 mg and less Over 25 mg	*	*		*	*	*	*	*		*	*	*		
Combustion time	Less than 5 hrs. Over 5 hrs.	*	*	*	*	*	*	*	*	*	*	*	*		
Chlorine determination	Gravimetric Titrimetric	*	*	*	*	*	*		*	*	*	*	*		

TABLE 7.—*Effect of variations within the Carius method for bromine*

VARIATION		NUMBER OF COLLABORATORS	t	$t_{0.05}$	F	$F_{0.05}$
Maximum temperature of furnace	Below 300°C.	4	1.499	2.201	1.15	4.07
	Above 300°C.	9				
Amount of silver nitrate	Less than 25 mg	6	1.4773	2.262		
	More than 25 mg	5				
Calculated volume of combustion tube	Under 9 ml	6	0.1167	2.228		
	Over 9 ml	6				

TABLE 8.—*Effect of variations within the Carius method for chlorine*

VARIATION		NUMBER OF COLLABORATORS	t	$t_{0.05}$
Maximum temperature of furnace	Below 300°C.	4	1.3057	2.228
	Above 300°C.	8		
Amount of silver nitrate	Less than 25 mg	5	0.3717	2.306
	More than 25 mg	5		
Calculated volume of combustion tube	Under 9 ml	5	0.4812	2.262
	Over 9 ml	6		

able studied were plotted in the same way as Figure 1. For each set of data the student's t value was calculated (above formula).

The only apparent variables that the analyst used were hydrazine sulfate or sodium sulfite as the reducing agent. The plot of the data for the two variables is shown in Figure 1. Calculation of the t value due to poor precision shows that the difference between the means obtained by the two procedures is not critical ($t=0.202$, $t_{0.05}=3.182$). However, calculation of F value (3), where $F=(S_{\bar{x}})^2_a/(S_{\bar{x}})^2_b$, did show that the difference in precision is critical in favor of sodium sulfite ($F=1345$, $F_{0.05}=200$). On the other hand, the same comparison for chlorine did not show that there was any difference in precision: ($t=1.2194$, $t_{0.05}=3.182$, $F=9$, $F_{0.05}=200$).

TABLE 9.—*Bromine—Parr bomb combustion*

COLLABORATOR NUMBER:		14	19	24	45	65	$\bar{\bar{X}}$	$S_{\bar{x}}$
Sample— Bromoacetanilide (37.33% bromine)	n $\bar{X}$ S $\bar{X}$—Theory	8 37.24 0.318 −0.09	8 39.00 2.410 +1.67	6 37.32 0.130 −0.01	8 36.41 0.993 −0.92	5 37.37 0.008 +0.04	37.47 (0.963) (+0.14)	0.942
Capacity of bomb	2.5 ml 22–50 ml	*	*	*		*		
Amount of KNO_3	20 mg Over 200 mg	*	*	*	*			
Amount of Na_2O_2	1.5 g Over 3 g	*	*	*	*	*		
Amount of sugar	Under 75 mg 75 mg and over	*	*	*	*	*		
Reducing agent	Hydrazine sulfate Sodium sulfite	*	*	*	*	*		
Amount of silver nitrate	Under 10 ml 10 ml and over	*	*	*		*		
Bromine determined	Titrimetric Gravimetric	*	*	*	*	*		

TABLE 10.—*Chlorine—Parr bomb combustion*

COLLABORATOR NUMBER:		14	19	24	45	65	$\overline{\overline{X}}$	S^2
Sample— Chloroacetanilide (20.91% chlorine)	n $\overline{X}$ S $\overline{X}$—Theory	8 20.88 0.349 −0.03	8 20.58 0.728 −0.33	7 20.91 0.210 0.00	8 20.73 0.205 −0.18	5 20.84 0.105 −0.07	20.79 (0.319) (−0.12)	0.135
Capacity of bomb	2.5 ml 22–50 ml	*	*	*		*		
Amount of KNO_3	20 mg Over 200 mg	*	*	*	*			
Amount of Na_2O_2	1.5 g Over 3 g	*	*	*	*	*		
Amount of sugar	Under 75 mg 75 mg and over	*	*	*	*	*		
Reducing agent	Hydrazine sulfate Sodium sulfite	*	*	*	*			
Amount of $AgNO_3$	Under 10 ml 10 ml and over	*	*	*		*		
Chlorine determined	Titrimetric Gravimetric	*	*	*	*	*		

TABLE 11.—*Comparison of methods*

DETERMINATION OF	COMPARISON OF	NUMBER OF COLLABORATORS	t	$t_{0.05}$
Chlorine	Carius vs. Parr	12 / 5	0.3947	2.131
Chlorine	Catalytic vs. Parr	12 / 5	0.1220	2.131
Chlorine	Carius vs. Catalytic	12 / 11	0.8080	2.080
Bromine	Carius vs. Catalytic	13 / 10	0.4494	2.080

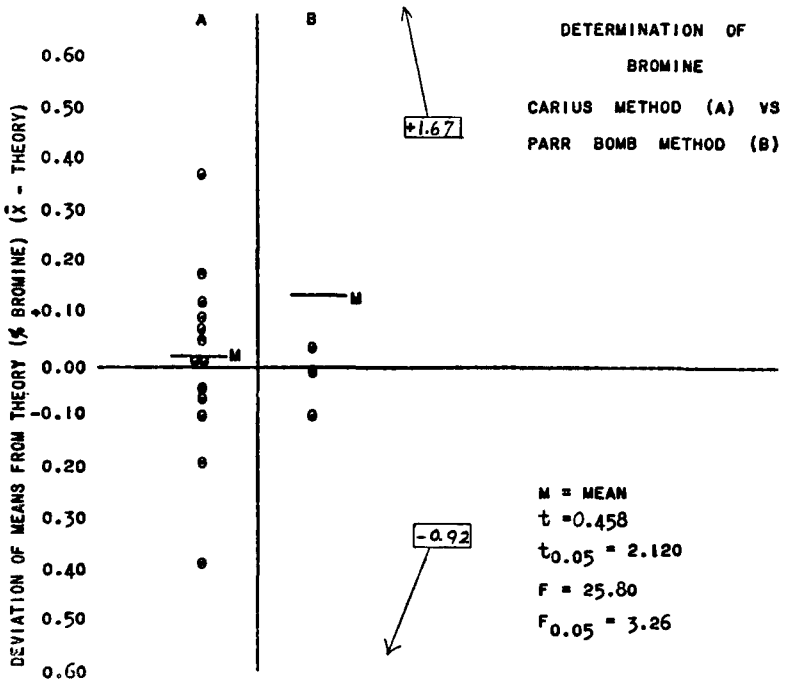

FIG. 2.—Carius method (A) *vs.* Parr bomb method (B).

COMPARISON OF METHODS

Comparisons were made between the various methods to see whether or not a method or methods gave more accurate or precise results than the other or others. Comparisons were made between the Carius and catalytic combustion methods, the Carius and Parr bomb methods, and the catalytic and Parr bomb methods for both bromine and chlorine. As shown in Table 11 for the determination of chlorine, no one method is more precise than the others. However, for the determination of bromine, such is not the case. Figure 2 shows the comparison between the Carius combus-

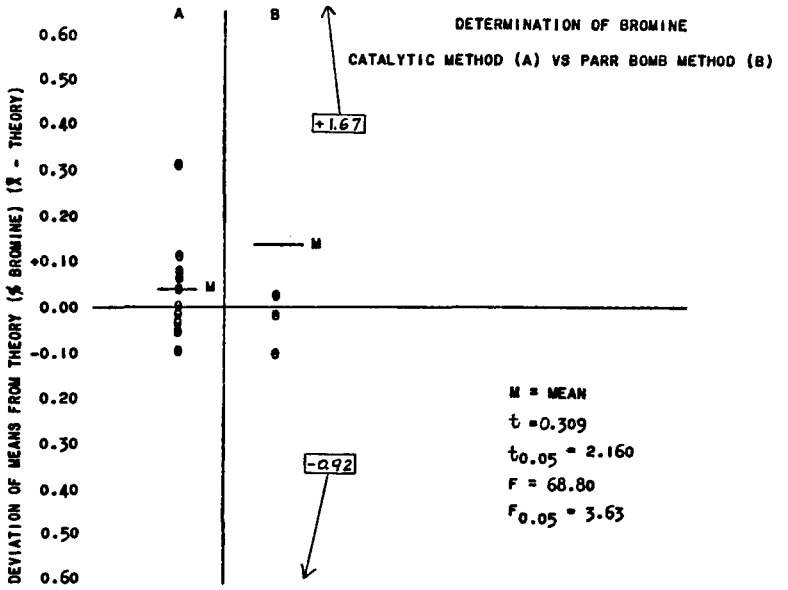

FIG. 3.—Catalytic method (A) *vs.* Parr bomb method (B).

tion and Parr bomb method ($t=0.458$, $t_{0.05}=2.120$, $F=25.80$, $F_{0.05}=3.26$). Figure 3 shows the comparison between the catalytic combustion and the Parr bomb method ($t=0.309$, $t_{0.05}=2.160$, $F=68.80$, $F_{0.05}=3.63$). In neither of these is the t value critical due to poor precision of the Parr method. However, the F value in each case shows that the difference in precision is critical and in favor of the Carius and catalytic methods respectively, over the Parr bomb method.

CONCLUSIONS

1. Because of poor precision in the determination of bromine by the Parr bomb method, a comparison of the reducing agents hydrazine sulfate and sodium sulfite did not yield a critical t value. However, calculation

of the F value showed that the difference in precision is critical. Sodium sulfite gave a much greater precision.

2. Parr bomb versus Carius method for bromine—calculation of the t value did not give a critical value due to poor precision (Fig. 2). However, calculation of the F value did show that the difference in precision is critical in favor of the Carius method.

3. Catalytic combustion versus Parr bomb for bromine—calculation of the t value did not give a critical value because of poor precision indicated from Fig. 3. However, calculation of the F value did show that the difference in precision is critical in favor of the catalytic combustion.

RECOMMENDATIONS*

(1) The Parr bomb method is not precise enough to be considered further.

(2) The Referee recommends either the Carius or catalytic combustion methods since both are equally precise and accurate. Both of these methods (Carius and catalytic) should be studied further for the development of a procedure which can be adopted as first action.

COLLABORATORS

The collaborators on the bromine and chlorine analyses were:

Alicino, J. F., Squibb Institute for Medical Research; Bier, M., Fordham University; Boos, R. N., Merck and Company; Bronk, L. B., General Electric Co.; Brown, W. L., Eli Lilly & Co.; Brunner, A. H., Ansco; Dearing, A. W., Hunter College; Dorfman, L., Ciba Pharmaceutical Co.; Dutton, C. D., Picatinny Arsenal; Feldman, J. R., General Foods Corp.; Flanders, C. A., West Virginia University, Agricultural Experiment Station; Grodsky, J., Ortho Research Foundation; Jones, G. A., E. I. duPont de Nemours & Co.; Ketchum, D. E., Eastman Kodak Co.; Kinsey, D., International Resistance Co.; Koch, C. W., University of California; Kuck, J. A., American Cyanamid Co.; Lohr, L. J., General Aniline & Film Corp.; McCard, R. Savacool, Smith, Kline & French Lab.; Means, J. A., Chas. Pfizer & Company, Inc.; Ogg, C. L., Eastern Regional Research Lab.; Paulson, R. A., National Bureau of Standards; Rush, C. A., Edgewood Arsenal; Steyermark, Al, Hoffmann-La Roche Inc.; Streeter, K. B., Sharp & Dohme, Inc.; Sundberg, O. E., American Cyanamid Company, Calco Div.; Throckmorton, W. H., Tennessee Eastman Corp.; Van Etten, C. H., Northern Regional Research Lab.

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* For report of Subcommittee C and action of the Association, see *This Journal*, **35**, 53 (1952).

REPORT ON MICROANALYTICAL DETERMINATION OF SULFUR

By C. L. Ogg (Eastern Regional Research Laboratory,* Philadelphia 18, Pennsylvania), *Associate Referee*

Since no previous work had been done toward establishing an official micromethod for sulfur in organic material, this year's study followed the pattern used for the previously reported (1, 2) carbon and hydrogen investigations. Two samples, benzyl-iso-thiourea hydrochloride and sulfanilamide, were sent to each analyst who expressed a willingness to participate and each was asked to analyze the samples by the method currently in use in his laboratory. Detailed questionnaires, one for each of the three combustion methods—catalytic, Carius, and Parr bomb—were sent with the samples, with instructions that the analyst return along with his results the questionnaire that pertained to his method. The collaborator was requested to send all the values obtained, so that a statistical analysis of the data could be made both to compare the different combustion methods and to show the effect of different materials and operations on the results by any one of the three combustion methods.

CATALYTIC COMBUSTION METHOD

Results obtained for sulfur by the catalytic combustion procedure were reported by eleven collaborators. Nine analysts reported thirty-nine values for benzyl-iso-thiourea hydrochloride, and fifty-two values were reported for sulfanilamide by all eleven collaborators. Although the eleven used a catalytic combustion method that was basically the same, there was considerable variation in material and operation in the methods used. Table 1 shows a summary of the analytical data reported as well as a condensation of the information obtained from the completed questionnaires. In this table, n is the number of sulfur values reported by each analyst, $\overline{X}$ is the mean of his data, and s is the standard deviation of his data. The symbols $\overline{\overline{X}}$ and $s_{\bar{x}}$ are used for the mean of all analysts' means, $\overline{X}$'s, and the standard deviation of the $\overline{X}$'s, respectively. Under the $\overline{X}$ column, the values in parentheses are the means of the standard deviations, s, and the means of the values of $\overline{X}$-theory for sulfur.

The two samples used as standards were purified by repeated recrystallization until they were shown to be pure by carbon, hydrogen, and nitrogen analyses in which the official A.O.A.C. micromethods were used. The $\overline{\overline{X}}$'s differ from the theoretical values by only $+0.05\%$ for benzyl-iso-thiourea hydrochloride and -0.02% for sulfanilamide. There was also but little difference between the standard deviation of the means, $s_{\bar{x}}$, for the two samples, which were 0.135 and 0.126, respectively.

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

TABLE 1.—*Sulfur—catalytic combustion*

COLLABORATOR NO.		9	24	28	30	37	50	60	61	45	1	51	$\bar{\bar{X}}$	$s\bar{x}$
Sample No. 1 Benzyl-isothiourea hydrochloride (15.82% sulfur)	n	8	2	2	6	4		8	3		4	2		
	$\bar{X}$	15.83	15.84	15.70	15.89	15.93		15.81	16.10		15.70	16.03	15.87	0.135
	s	0.179	0.071	0.022	0.168	0.052		0.153	0.205		0.180	0.036	(0.118)	
Sample No. 2 Sulfanilamide (18.62% sulfur)	n	8	2	2	8	3	8	8	2	4	4	3		
	$\bar{X}$	18.49	18.63	18.49	18.44	18.77	18.51	18.59	18.85	18.58	18.69	18.57	18.60	0.126
	s	0.285	0.247	0.036	0.117	0.149	0.082	0.119	0.031	0.059	0.250	0.087	(0.113)	
Sample No. 1	$\bar{X}$-theor. value	+0.01	+0.02	−0.12	+0.07	+0.11		−0.01	+0.28		−0.12	+0.21	(+0.05)	
Sample No. 2	$\bar{X}$-theor. value	−0.13	+0.01	−0.13	−0.18	+0.15	−0.11	−0.03	+0.23	−0.04	+0.07	−0.05	(−0.02)	
Combustion tube	Quartz	*	*		*		*	*	*	*		*		
	Pyrex			*		*					*			
Absorber	Spiral	*	*	*	*	*	—		*	*	*			
	Grote							*				*		
Absorber connection	₹ Joint	*		*	*	*	*		*	*	*			
	No joint		*					*				*		
Contact catalyst	Pt. star	*					*			*				
	Pt. gauze		*	*	*	*		*	*		*	*		
Pt. catalyst behind sample	Yes			*							*	*		
	No	*	*		*	*	*	*	*	*				
Long furnace temperature	750°C. and over	*	*	*				*		*		*		
	Less than 750°					*	*		*		*			
Sample burner	Electric	*	*	—	*	—	*			*		*		
	Gas			*		*		*	*		*			
Sample burner	750°C. and over	*	*		*				*	*		*		
	Less than 750°						*	*			*			

COLLABORATOR NO.		9	24	28	30	37	50	60	61	45	1	51	$\bar{\bar{X}}$	$s_{\bar{x}}$
Sample No. 1 Benzyl-isothiourea hydrochloride (15.82% sulfur)	n	8	2	2	6	4		8	3		4	2		
	$\bar{X}$	15.83	15.84	15.70	15.89	15.93		15.81	16.10		15.70	16.03	15.87	0.135
	s	0.179	0.071	0.022	0.168	0.052		0.153	0.205		0.180	0.036	(0.118)	
Sample No. 2 Sulfanilamide (18.62% sulfur)	n	8	2	2	8	3	8	8	2	4	4	3		
	$\bar{X}$	18.49	18.63	18.49	18.44	18.77	18.51	18.59	18.85	18.58	18.69	18.57	18.60	0.126
	s	0.285	0.247	0.036	0.117	0.149	0.082	0.119	0.031	0.059	0.250	0.087	(0.113)	
Sample No. 1	$\bar{X}$-theor. value	+0.01	+0.02	−0.12	+0.07	+0.11		−0.01	+0.28		−0.12	+0.21	(+0.05)	
Sample No. 2	$\bar{X}$-theor. value	−0.13	+0.01	−0.13	−0.18	+0.15	−0.11	−0.03	+0.23	−0.04	+0.07	−0.05	(−0.02)	
Burner operation	Mechanical	*	*		*		*					*		
	Manual			*		*		*	*	*	*			
Absorbing solution	H_2O_2		*	*		*	—	*	*		*			
	Other	*			*					*		*		
Sample weight range	2 to 10 mg.			*		*	*		*		*			
	10 mg. and over	*	*		*			*		*		*		
Burner moved over boat	Once	*	*		*		*		*	*	*	*		
	Twice			*		*		*						
Distance burner moved	More than 11 cm.	*		*		*			*	*				
	Less than 11		*		*		*	*			*	*		
Sweeping time	More than 11 min.	*		*			*							
	Less than 11		*		*	*		*	*	*	*	*		
Total analysis time	More than 31 min.	*		*		*	*			*	*			
	Less than 31		*		*			*	*			*		
Sulfate determined	Gravimetrically		*			*	*	*	*					
	Titrimetrically	*		*	*					*	*	*		
Oxygen flow rate	More than 11 ml/min	*		*	*		*	*		*				
	Less than 11		*			*			*		*	—		

* Indicates which of the alternate procedures was used by each collaborator.

The effect of the variations within a method was studied to see whether the data indicated that one procedure would produce more accurate results than the alternate. The variables whose alternate procedures were compared were those listed in Table 1. The data for the two alternate procedures for each variable studied were plotted as shown in Fig. 1.

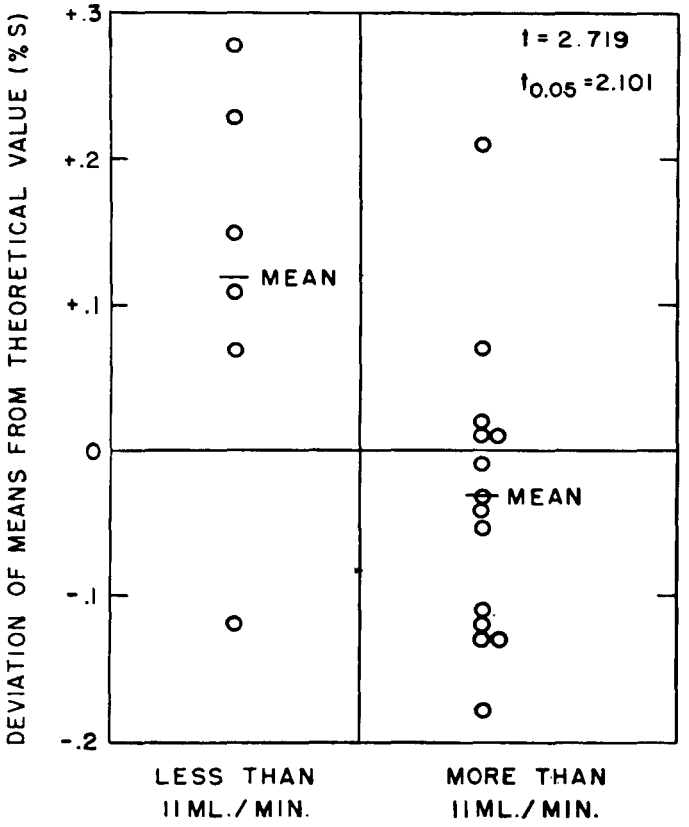

FIG. 1.—Effect of the oxygen flow rate on the determination of sulfur by the catalytic combustion method.

Visual inspection of this plot quickly showed whether there was any marked difference in the data obtained by either of the alternate procedures. When it appeared that a marked visual difference did exist, Student's t value was calculated by the formula

$$t=\bar{x}\sqrt{\frac{n_a n_b(n_a+n_b-2)}{(n_a+n_b)\left[\Sigma(X_a-\overline{X}_a)^2+\Sigma(X_b-\overline{X}_b)^2\right]}}$$

where $\bar{x} = \overline{X}_a - \overline{X}_b$; n_a and n_b are the number of values in groups a and b, respectively; X_a and X_b are the individual values in the two groups; $\overline{X}_a$ and $\overline{X}_b$ the means of the values for the two groups. If the calculated t value was greater than the critical value, $t_{0.05}$, obtained from a table of Student's t, the difference between the two groups of data was critical at the 95% level, and the procedure whose mean was nearer the theoretical value was considered to be the better. Only two such comparisons

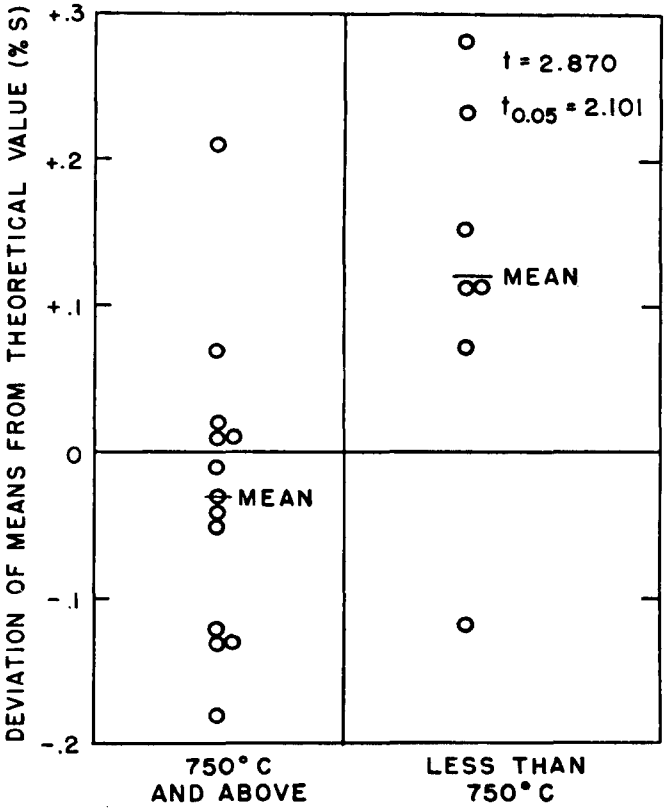

FIG. 2.—Effect of the long furnace temperature on the determination of sulfur by the catalytic combustion method.

had critical t values. These were the oxygen flow rate and the temperature of the long furnace. The data for these comparisons are presented graphically in Figures 1 and 2. The means for the values obtained by using oxygen flow rates below and above 11 ml. per minute were +0.12% and −0.03% from theory, respectively. The calculated t value of 2.719 when compared with a $t_{0.05}$ of 2.101 showed a significant difference between the means. The mean values obtained by using long furnace tem-

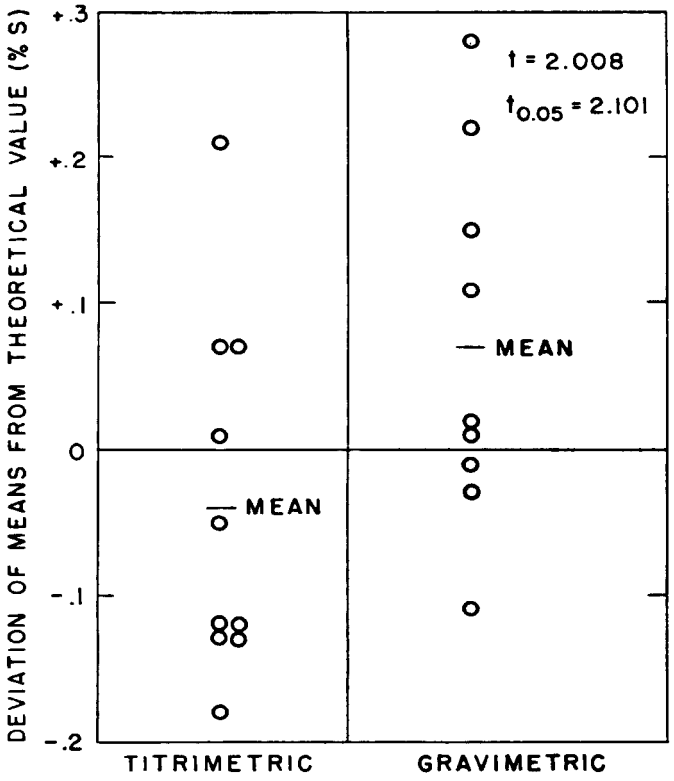

FIG. 3.—Effect of the method of sulfate analysis on the determination of sulfur by the catalytic combustion method.

peratures of 750°C. and over, as opposed to less than 745°C., were −0.03 and +0.12% from theory, respectively. Comparison of the calculated t value, 2.870, with a $t_{0.05}$ of 2.101 showed the difference between the two means to be significant. It was also noted that the two procedures, slow oxygen flow and low furnace temperature, which might lead to incomplete combustion, were associated with too high sulfur values. One other interesting comparison of results was that obtained by the gravimetric and titrimetric procedures. Although the difference between these two methods was not critical at the 95% level, the t value was so high that the comparison could not be ignored. The data for this comparison, shown in Figure 3, will be referred to later under the discussion of the results obtained by the Carius method.

CARIUS COMBUSTION METHOD

The seventy-nine values furnished by the seven collaborators who used the Carius method were treated in the same manner as those for the cata-

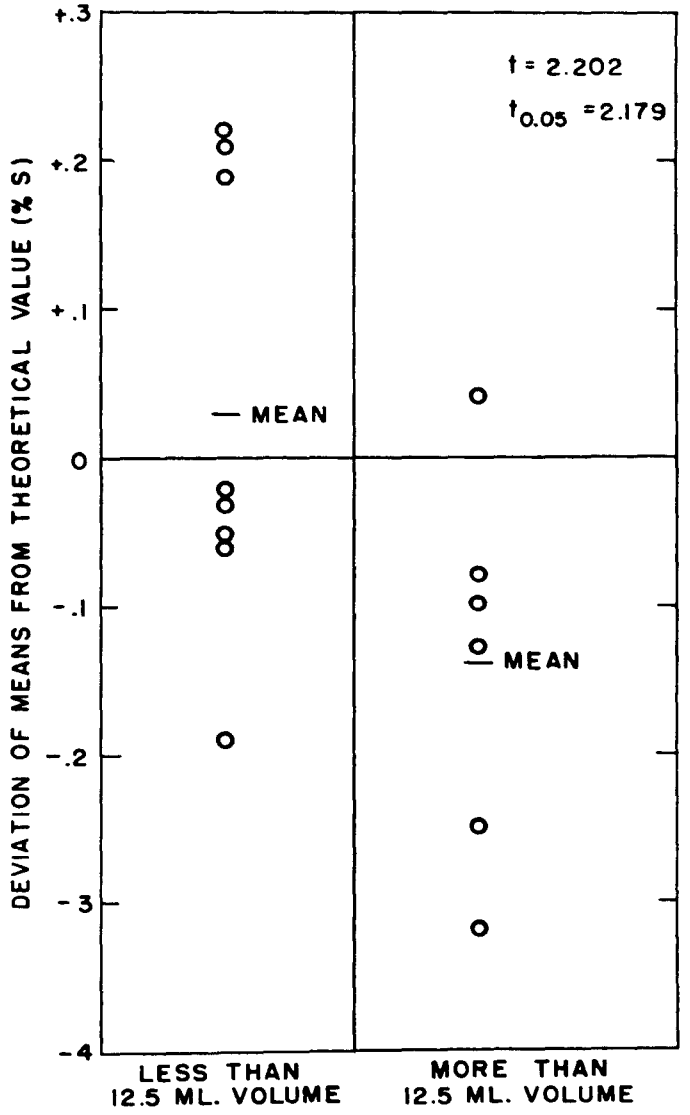

FIG. 4.—Effect of the volume of combustion tube on the determination of sulfur by the Carius combustion method.

lytic combustion method. Table 2 shows a summary of the data and also the variables with alternate procedures, which were studied statistically. The grand mean, $\bar{\bar{X}}$, for benzyl-iso-thiourea hydrochloride was 15.79%, which is 0.03% less than the theoretical value of 15.82%; that of sulfanil-

TABLE 2.—*Sulfur—Carius combustion*

COLLABORATOR NO.			0	17	46	49	50	59	63	$\bar{\bar{X}}$	$s_{\bar{x}}$
Sample No. 1 Benzyl-iso-thiourea hydrochloride (15.82% sulfur)		n $\bar{X}$ s	8 15.74 0.064	8 15.75 0.228	6 15.86 0.200	3 15.63 0.127	8 15.76 0.061	3 15.79 0.085	4 16.01 0.097	15.79 (0.123)	0.119
Sample No. 2 Sulfanilamide (18.62% sulfur)		n $\bar{X}$ s	8 18.37 0.084	8 18.30 0.498	5 18.49 0.141	3 18.83 0.139	8 18.57 0.174	3 18.60 0.165	4 18.87 0.127	18.57 (0.190)	0.209
Sample No. 1, Sample No. 2,	$\bar{X}$—theor. value X—theor. value		−0.08 −0.25	−0.07 −0.32	+0.04 −0.13	−0.19 +0.21	−0.06 −0.05	−0.03 −0.02	+0.19 +0.22	(−0.03) (−0.05)	
Combustion temperature	300°C. and over Less than 300°		*	*	*	*	*	*	*		
Combustion tube volume (cal. from length and I.D.)	More than 12.5 ml. Less than 12.5 ml.		*	*	*	*	*	*	*		
Sample weight range	2 to 10 mg. More than 10 mg.		*	*	*	*	*	*	*		
Nitric acid	Fuming Concentrated		*	*	*	*	*	*	*		
Volume of nitric acid	0.15 to 3 ml. More than 3 ml.		*	*	*	*	*	*	*		
Reagent added	NaCl or KCl Others		*	*	*	*	*	*	*		
Combustion time	2 to 5.5 hrs. More than 5.5 hrs.		*	*	*	*	*	*	*		
Sulfate determination	Gravimetric Titrimetric		*	*	*	*	*	*	*		

* Indicates which of the alternate procedures was used by each collaborator.

amide was 18.57%, which is 0.05% less than the theoretical value of 18.62%. The standard deviations, $s_{\bar{x}}$, for the sulfur values of the two samples showed a greater difference than those obtained by the catalytic combustion study. The difference, however, was not critical at the 95% level when the F test was applied, $F = (s_{\bar{x}})^2_a/(s_{\bar{x}})^2_b$. Since the data were those of only seven collaborators with a total of fourteen averages, and these data were obtained by using methods where a large number of variables existed, the results of this statistical treatment must be viewed as only indicating a possible trend; they cannot be regarded as conclusive. Nevertheless, it was believed that the statistical treatment of the data was worthwhile and was the best way to obtain any indication of the effect of the variables on the sulfur values. The data obtained under the condition (procedure) of each variable were plotted as shown in Figures 1, 2 and 3, and the t values were calculated for each of these comparisons when the data after visual inspection appeared to be sufficiently different. Only one of the calculated t values was found to exceed the value for $t_{0.05}$, showing that only in one instance a significant difference in the results was obtained by two procedures. This difference was the volume of combustion tubes greater than 12.5 ml versus volumes less than 12.5 ml. The volumes were calculated from the length and inside diameter of the tubes used. Figure 4 shows the data for this comparison. It can be seen that tubes with volumes greater than 12.5 ml lead to low results, since the mean value for the data obtained with the larger tubes was 0.14% less than the theoretical value, whereas with the smaller tube the mean was only 0.03% higher than the theoretical value.

The comparison of gravimetric versus titrimetric methods for the sulfate determination gave a t value only slightly less than the critical value. Figure 5 shows the data for this comparison. For a better statistical comparison of the data by gravimetric and titrimetric procedures for sulfate analysis, all the data obtained by the catalytic combustion and the Carius combustion methods were combined. Figure 6 shows the combined data. The new t value, 2.978, compared with $t_{0.05}$ of 2.037 showed that the difference between the data for the two methods is highly significant. Although the difference is significant, there is but little choice between the gravimetric and the titrimetric methods based on the deviation of the mean sulfur value from the theoretical sulfur value, since the deviation of the gravimetric values was +0.08% and that of the titrimetric values was −0.06%. The gravimetric method gave high results, probably because of adsorption and co-precipitation with the barium sulfate precipitate. The results of the titrimetric method may be lower because the true stoichiometric point may not be reached, owing to the slowness of the indicator in reaching equilibrium when the titration nears the end point.

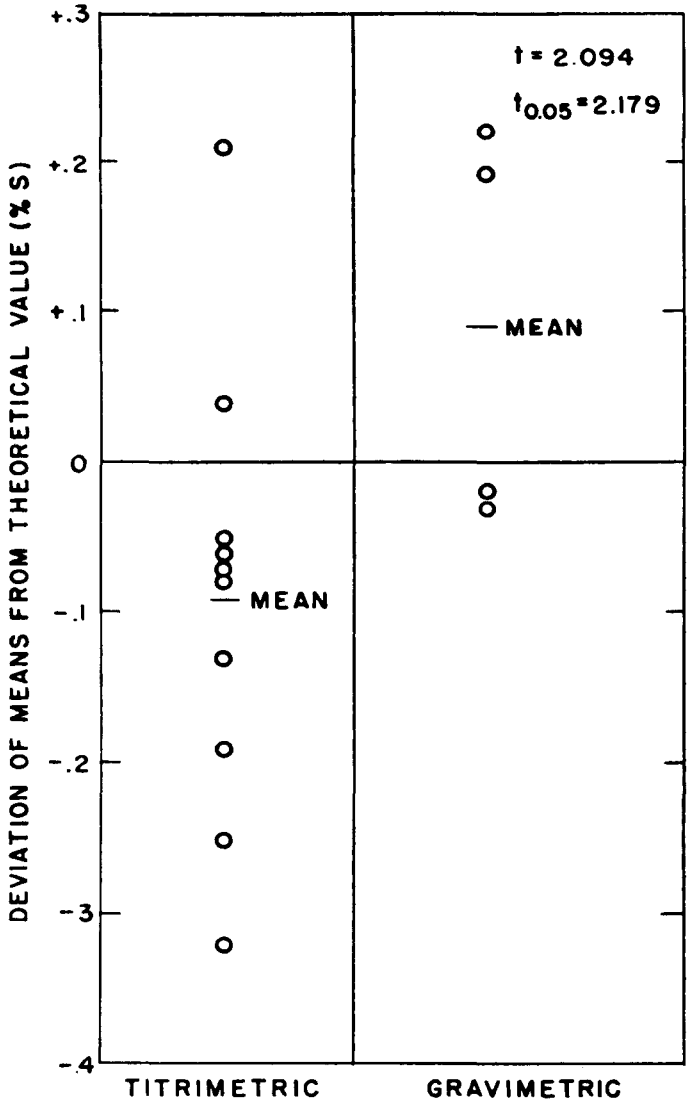

FIG. 5.—Effect of the method of sulfate determination on the determination of sulfur by the Carius combustion method.

PARR BOMB COMBUSTION

Seven collaborators reported fifty-four sulfur values, but no comparison of procedures using the *t* test was feasible because of the similarity of most of the procedures. Table 3 presents a summary of the data reported. The

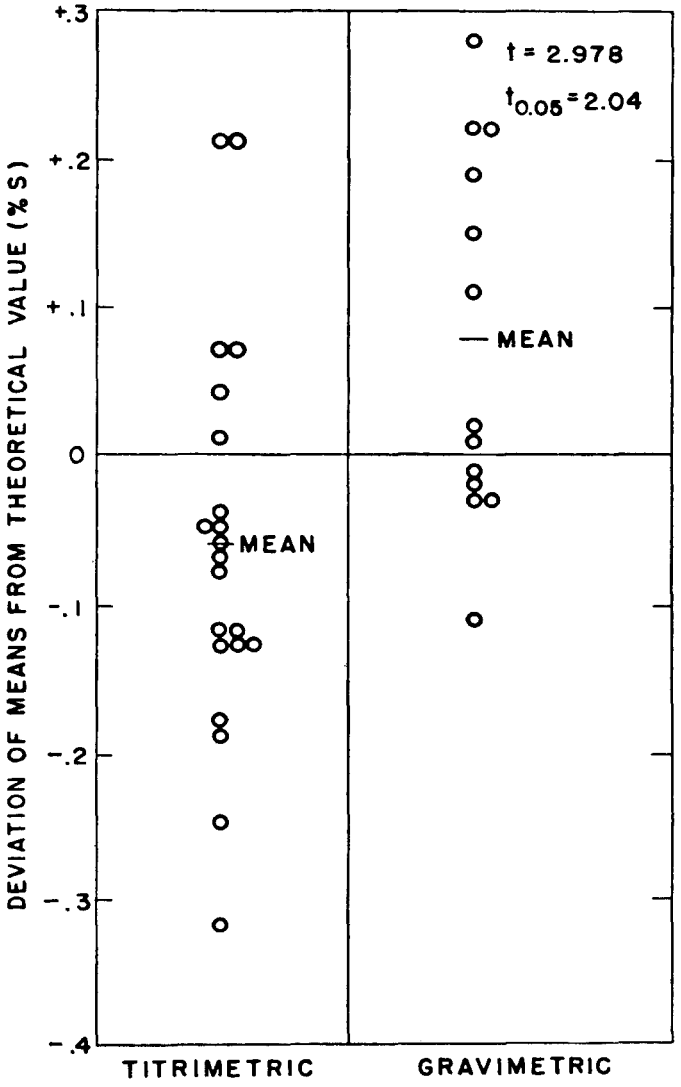

FIG. 6.—Effect of the method of sulfate analysis on the determination of sulfur. (Combined catalytic and Carius combustion data).

grand mean, $\overline{\overline{X}}$, of the individual means, $\overline{X}$'s, for the sulfur values for the two compounds were only +0.06 and −0.03% from the theoretical values, but the standard deviations of the means $s_{\bar{x}}$ were 0.391 and 0.176. This difference was found to be critical at the 95% level by applying the F test, $F = (s_{\bar{x}})^2_a/(s_{\bar{x}})^2_b$. F calculated was 4.93, and $F_{0.05}$ was 4.28.

TABLE 3.—*Sulfur—Parr bomb combustion*

COLLABORATOR NO.			8	15	24	29	44	65	23	$\bar{\bar{X}}$	$s_{\bar{x}}$
Sample No. 1 Benzyl-iso-thiourea hydrochloride (15.82% sulfur)		n		6	1	4	4	4	4		
		$\bar{X}$		16.50	15.73	15.83	15.44	15.98	15.79	15.88	0.391
		s		0.488	—	0.101	0.211	0.097	0.174	(0.224)	
Sample No. 2 Sulfanilamide (18.62% sulfur)		n	2	8	3	4	4	5	4		
		$\bar{X}$	18.53	18.60	18.73	18.70	18.24	18.64	18.72	18.59	0.176
		s	0.318	1.470	0.026	0.116	0.059	0.060	0.109	(0.342)	
Sample No. 1	X—theor. value			+0.70	−0.09	+0.01	−0.38	+0.16	−0.03	(+0.06)	
Sample No. 2	$\bar{X}$—theor. value		−0.09	−0.02	+0.11	+0.08	−0.38	+0.02	+0.10	(−0.03)	

Thus far, only comparisons have been made of data obtained by alternate procedures for a variable in one of the three methods. To determine whether any one of the methods gave more accurate or precise results than the others, the t and F tests were applied to the three possible pairs of data, that is, Carius versus Parr, Carius versus catalytic, and Parr versus catalytic combustion. The data for sample No. 1 and sample No. 2 were compared separately, and the combined data for the two samples were compared. No critical t values were obtained, so there were no significant differences in the means, $\overline{X}$'s, obtained by the three methods. Neither were there any critical F values when the one high sulfur value of 16.5 for benzyl-iso-thiourea hydrochloride by the Parr bomb method was discarded. When this value was included, critical F values were obtained when the data for sample No. 1 by the Parr method were compared with those for the other two. Thus, when this single value was included in the calculations, the results by the Parr method for benzyl-iso-thiourea hydrochloride were less precise than those by the other two methods.

Collaborators on sulfur analysis were:

Alicino, J. F., Squibb Institute for Medical Research; Bier, Milan, Fordham University; Bronk, L. R., General Electric Company; Brown, L. E., Southern Regional Research Laboratory; Brown, Wm. L., Eli Lilly and Company; Browning, B. L., The Institute of Paper Chemistry; Brunner, A. H., Ansco; Dorfman, Louis, Ciba Pharmaceutical Company; Dutton, C. D., Picatinny Aarsenal; Feldman, J. R., General Foods Corporation; Jones, G. A., E. I. duPont de Nemours and Company; Ketchum, D. F., Eastman Kodak Company; Koch, C. W., University of California; Lohr, L. J., General Aniline and Film Corporation; Ogg, C. L., Eastern Regional Research Laboratory; Rush, C. A., Edgewood Arsenal; Savacool, Ruth, Smith, Kline and French; Scafe, E. T., Sacony Vacuum Oil Company; Steyermark, Al, Hoffmann-La Roche, Inc.; Streeter, K. B., Sharpe and Dohme; Sundberg, A. E., Calco-American Cyanamide Company; Throckmorton, W. H., Tennessee Eastman Corporation; Van Etten, C. H., Northern Regional Research Laboratory.

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MONDAY-AFTERNOON SESSION

REPORT ON PRESERVATIVES AND ARTIFICIAL SWEETENERS

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

Mr. Wilson has reported his studies on quaternary ammonium compounds and sucaryl ® in four papers* and has summarized his work on the quaternary ammonium compounds in his report on that subject.

The Associate Referees on monochloracetic acid and thiourea believe that further work could be done even though the methods are at the present time official. It is recommended that these studies be continued.

The Associate Referee on artificial sweeteners proposes a petroleum ether extraction of P-4000 from an alkaline solution with organoleptic identification of the residue; he also proposes a barium sulfate test for sucaryl after treatment with barium chloride and sodium nitrite. The Referee considers that the collaborative results with these tests justify their adoption as first action.

The substitution of hydrogen chloride gas for concentrated hydrochloric acid in the La Parola-Mariani test for dulcin could be made more specific by including directions for generating the gas, or a statement that the test will work with concentrated hydrochloric acid with reduced sensitivity. The Referee considers the substitution of hydrogen chloride gas for concentrated hydrochloric acid a departure from the test adopted as first action and the method should be retested before adoption as official.

The subject of qualitative tests for fluorides was proposed last year, but since there were no volunteers, the Referee tried a comparison of the official etching method and the Gettler method (which has as its reaction the formation of hexagonal pink crystals of sodium fluosilicate). This alternate method shows good sensitivity, and it is recommended that the two methods be submitted to collaborative study.

The use of hydrogen peroxide in milk infected with *Brucella Melitensis* (1) brings this preservative again to the fore. With the possibility that it may be used for milk or chocolate milk drinks, it is recommended that an Associate Referee be appointed to study a method for its detection and/or determination in milk products.

Nothing has been reported on the hydroxybenzoic acids, since it was the opinion of Committee C that the detection of these compounds is adequately covered by the present methods for benzoic acid.

Much activity has been recorded in the past year in the development of preservatives for soy sauce; and the following were indicated as effective in the prevention of mold growth: .005% (2) Bu-5 hydroxyorsellinates; .005% thymol; .003% *p*-chlorothymol; .001% *o*-chlorothymotinaldehyde

* (See pages 455–469, incl.)

(3). In Japan, antiseptics (4) for foodstuffs have also been investigated with the proposed use of hendecylenic acid, or esters of higher alcohols containing 7–11 carbon atoms with capric acid or *p*-tolylthiocarbamate acid esters, or variations of salicylic acid (5). In Germany, *p*-methoxy-*o*-benzoylbenzoic acid (6) is recognized as a sweetening agent. Switzerland uses bromine (7) containing preservatives in fruit juices with 1 p.p.m. permissible limit. The Swiss are also trying a plant-derived antibiotic on milk.

The writer wishes to acknowledge the possibility of the use of these preservatives and artificial sweeteners, and hopes to keep up to date with methods, if and when they become a problem instead of a possibility. The investigation (8) of vanillic acid esters was started when two food journals offered enthusiastic claims for them, but the Associate Referee found in his study that there was no commercial supply of the chemicals. We must conserve the efforts of our Associate Referees.

Recent literature contains many new uses for products now on the market. For instance:

Chlorine dioxide for the preservation of non-cereal foods, (fruits, vegetables, meats, and fish) (9).

Peracetic (CH_3COOOH) germicidal washes and sprays to reduce mold count in tomato and grape juices (10).

Dehydroacetic acid (3-acetyl, 6-methyl,1,2,pyran,2,4-dione) as a dip or impregnating material for wrappers on butter and cheese, in fruit juices, bread, or to sprinkle on surface of meats (11).

Propionates for dipping fruits, berries, and vegetables, and wrapping butter as well as dipping it (12).

Sulfites on peeled potatoes (13).

The emulsifiers Tween and Span are recommended in 1% concentrations as a chocolate preservative to avoid bloom deterioration. (The problem whether emulsifiers should be considered preservatives is referred to Committee D) (14).

Cationic detergent (15) for the prevention of mold growth in shell eggs.

Waterproofing marshmallows with vapor of monomethyldiethoxysilane (16).

In the report of the British Joint Committee on Preservative Regulations it was recommended that the term "Preservative" (16) be amended, to bring under control the addition of substances to foods for other purposes, such as antioxidants, prevention of staling, anti-mold agents, stabilizers, etc. A list was submitted of substances which appeared to be added to food in various countries for the purposes mentioned.

The decision as to what should be included in the chapter on preservatives and artificial sweeteners as preservatives has been under discussion for several years. It appears that others are having similar considerations. Any expression of opinion from members of the A.O.A.C. on this subject will be welcomed by the present Referee and Committee D.

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(15) C. A., **44**, 9581a.
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REPORT ON QUATERNARY AMMONIUM COMPOUNDS

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

Since there were no available methods for the identification of the various quaternary ammonium compounds and no convenient method for the determination of these substances in the sanitizing agents widely used by physicians, surgeons, and dentists, which are sold in dilutions of from 1–500 up to 1–15,000, the Associate Referee used the available time in the study of these aspects of the subject.

The results of this study are incorporated in the two papers entitled "Determination of Quaternary Ammonium Compounds as Reineckates," by John B. Wilson, and "Identification of Certain Quaternary Ammonium Compounds as Reineckates," by A. H. Tillson, Wm. V. Eisenberg, and John B. Wilson.*

The Associate Referee believes that these methods should be subjected to collaborative study.

RECOMMENDATIONS†

It is recommended—

(1) That collaborative study be made of the method for "Determination of Quarternary Ammonium Compounds as Reineckates" by John B. Wilson, as given at this meeting.

(2) That collaborative study be made of the method for "Identification of Certain Quaternary Ammonium Compounds as Reineckates" by A. H. Tillson, Wm. V. Eisenberg, and John B. Wilson, as given at this meeting.

(3) That collaborative study be continued on the following quantitative methods for the determination of quaternary ammonium compounds:

* (See pages 455–465, incl.)
† For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 59 (1952).

(a) Method for Fruit Juices. *This Journal*, **29**, 318 (1946).
(b) Shorter Method for Fruit Juices. *Ibid.*, **29**, 319 (1946).
(c) Method for Bottled Sodas. *Ibid.*, **29**, 323 (1946), subject to increasing the volume of bromophenol blue reagent to 5–10 ml.
(d) Method for Milk. *Ibid.*, **29**, 324 (1946) on samples containing preservative quantities of quaternary ammonium compounds.
(e) Method for Mayonnaise, Salad Dressings, and Sandwich Spreads. *Ibid.*, **29**, 323 (1946).
(f) Method for Pickles and Relishes. *Ibid.*, **29**, 326 (1946).
(g) Method for Shrimp. *Ibid.*, **33**, 670 (1950).

REPORT ON ARTIFICIAL SWEETENERS

By WILLIAM S. COX (Food and Drug Administration, Federal Security Agency, Atlanta, Ga.), *Associate Referee*

The most important development in the field of artificial sweeteners in the past few years is the commercial development of sodium cyclohexylsulfamate (1), or sucaryl sodium ®. This compound (in dilute solutions) is only about 75 times as sweet as sugar, as compared with the factors of about 250 for dulcin, 400 for saccharin and 4,000 for P-4000 (Propoxy 2-amino, 4-nitrobenzene). However, its toxicity (2) is extremely low and it has the advantage that its taste is a true sweet taste with no aftertaste, similar to that sometimes noted when saccharin is used.

In compliance with the recommendations, the work for the current year consisted of collaborative studies of methods for the detection of the artificial sweeteners: dulcin, P-4000 and cyclohexylsulfamate sodium.

A series of samples was prepared and submitted to collaborators, as follows:

A base solution, which corresponded to a decarbonated ginger-ale, was prepared by dissolving sugar, citric acid, and benzoic acid in water and adding a commercial ginger-ale flavor extract. The finished solution contained about 10% sugar, 2% citric acid, 0.1% benzoic acid, and a trace of flavoring.

To this base solution, known amounts of the artificial sweeteners were added, two or three being present in the various samples. In order to determine the sensitivity of the tests, two different amounts of the dulcin and P-4000 were used, one concentration being 10 p.p.m. and the other 25 p.p.m. The sucaryl sodium ®, where used, was present in the amount of 50 p.p.m.

The samples were prepared so that there were four sets of identical solutions, as follows:

Solution A contained 10 p.p.m. dulcin and 50 p.p.m. sucaryl sodium ®.
Solution B contained 25 p.p.m. dulcin and 10 p.p.m. P-4000.
Solution C contained 25 p.p.m. dulcin and 25 p.p.m. P-4000.
Solution D contained 10 p.p.m. dulcin, 10 p.p.m. P-4000 and 50 p.p.m. sucaryl sodium ®.

TABLE 1.—*Collaborators' results*

SAMPLE	SWEETENERS PRESENT	NUMBERS												
		1	2	3	4	5	6	7	8	9	10	11	12	13
A.	P-4000 No													
(a) by sweet taste		#	#	#				#	?	#				
(b) by anaesthetic action									+					
(c) by melting point									**					
	Dulcin (1 mg)													
(a) A.O.A.C. 27.9[1]		+	−	−				+	+	−				
(b) A.O.A.C. 27.10 (benzaldehyde)		?	?	?				+	+	−				
(c) A.O.A.C. 27.10 (anisaldehyde)		+	+	*				+	+	?				
	Cyclohexylsulfamate Sodium (5 mg)	+	+	+				+	+	+				
B.	P-4000 (1 mg)													
(a) by sweet taste			−		+	+			+		#	+		−
(b) by anaesthetic action			−		−	?			+			?		−
(c) by melting point			**		?	**			**			**		**
	Dulcin (2.5 mg)													
(a) A.O.A.C. 27.9			?		−	+			+		−	+		−
(b) A.O.A.C. 27.10 (benzaldehyde)			+		?	−			+		−	?		?
(c) A.O.A.C. 27.10 (anisaldehyde)			+		+	+			+		?	+		+
	Cyclohexylsulfamate Sodium: No		−		−	−			−		−	−		−
C.	P-4000 (2.5 mg)													
(a) sweet taste				+	+		+			+	+		+	+
(b) anaesthetic action				+	?		+			+	+		+	+
(c) melting point				+	**		**			+	**		**	+
	Dulcin (2.5 mg)													
(a) A.O.A.C. 27.9				−	−		−			−	−		−	−
(b) A.O.A.C. 27.10 (benzaldehyde)				?	?		−			+	−		−	?
(c) A.O.A.C. 27.10 (anisaldehyde)				*	+		?			+	−		+	+
	Cyclohexylsulfamate Sodium: No			−	−		−			−	−		−	−
D.	P-4000 (1 mg)													
(a) sweet taste		+				+	+	+				+	#	
(b) anaesthetic action		+				+	−	−				+		
(c) melting point		**				**	**	**				**		
	Dulcin (1 mg)													
(a) A.O.A.C. 27.9		+				+	−	+				?	−	
(b) A.O.A.C. 27.10 (benzaldehyde)		+				−	−	+				−	−	
(c) A.O.A.C. 27.10 (anisaldehyde)		+				+	+	+				+	?	
	Sucaryl Sodium (5 mg)	+				+	+	+				+	+	

+—Present.
−—Not present.
?—Doubtful.
#—No yellow residue, reported negative.
*—No anisaldehyde available for test.
**—Residue insufficient or unsuitable for melting point determination.

[1] *Methods of Analysis*, 7th Ed., 1950.

METHODS USED IN COLLABORATIVE WORK

I. P-4000 (Propoxy-2-amino, 4-nitro benzene) (3)

Make 100 ml of sample slightly alkaline (*p*H: 7.5–8.0) and extract with three 25 ml portions of low-boiling petroleum ether (b.p. 40–55°), shaking vigorously each time. (Retain aqueous solution for II.) Evaporate the petroleum ether at 45° in a small beaker or evaporating dish.

P-4000 is a yellow-orange crystalline compound, melts at 49°, and has an intensively sweet taste, as well as a very strong anaesthetic effect.

Test any yellow residue for:

(1) Sweet taste.
(2) Anaesthetic action (on tongue).
(3) Melting point.

II. Dulcin

Using the still alkaline solution from I, treat as directed in *Methods of Analysis* (7th Ed.) **27.8, 27.9,** and **27.10.** (Retain the extracted solution for III.)

III. Sucaryl Sodium ® (*Sodium Cyclohexylsulfamate*) (1)

Make the solution (from II) acid with 10 ml HCl, add 0.2 g $NaNO_2$. (Filter solution, if not clear.) Add 1 g $BaCl_2$. A turbidity or faint white precipitate, due to barium sulfate, indicates sucaryl sodium ®.

INSTRUCTIONS TO COLLABORATORS

1. In the test for the detection of P-4000, report the test as negative if no yellow residue is recovered. If such a residue is recovered, report negative, positive, or doubtful results for taste, anaesthetic action, and melting point determination. For II and III, report the tests as positive, negative, or doubtful.

2. In I, if low-boiling petroleum ether is not available, petroleum ether with a slightly higher range may be used. If it is, please report its boiling point range.

3. In II, **27.10,** the Associate Referee desires that *both* benzaldehyde and anisaldehyde be used (on separate portions) and both tests reported.

4. Collaborators are urged to comment on clarity of methods, difficulties encountered, and results obtained.

COMMENTS OF COLLABORATORS

Most collaborators commented on the gummy residue obtained in part I, which made it impracticable to obtain a melting point. Two of the collaborators were able to recover enough crystals to check the melting point with good results.

Almost all collaborators commented on the colors obtained in part II. The colors *will* vary considerably, depending on the concentration of the dulcin present.

One collaborator reported that the residue from part I gave a test for dulcin, with anisaldehyde, which indicated that some dulcin was extracted by the petroleum ether. He also suggested the use of HCl gas in method **27.10,** rather than using a drop HCl. (Associate Referee's comment: This suggestion is a very good one, as the dulcin-anisaldehyde (or benzaldehyde) would react in fairly high concentrations, not being diluted by the HCl solution. It will definitely give more intense colors.)

Several collaborators recommended the use of authentic materials as controls.

INTERPRETATION OF RESULTS

In order to fairly evaluate these results, it must be kept in mind that extremely small amounts of the sweeteners were present in all instances.

This was deliberately so for P-4000, which is about 4000 times as sweet as sugar. Each milligram would be equivalent to 4 grams of sugar, so it would usually be present in the range of 10–100 p.p.m.

As to P-4000, the results would indicate that by this method the vast majority of analysts would detect the presence of the sweetener by its sweet taste when only 1 milligram is recovered, and by its anaesthetic effect when only 2.5 milligrams are recovered. The gummy nature of the residue and the low melting point of P-4000 make its detection by melting point very difficult.

As to dulcin, the results by the Deniges-Tourrou test and by the benzaldehyde test (modified La Parola-Mariani test) are rather inconclusive in the range of 1–2.5 milligrams. However, dulcin is only 250 times as sweet as sugar, and dulcin would usually be present in the amounts of 200–2000 p.p.m., or 20 to 200 milligrams per 100 ml.

The anisaldehyde test (the true La Parola-Mariani test) gives satisfactory tests even in the low range present in these samples, since only 1 of 12 collaborators who used this test was unable to obtain positive results either with 1 or 2.5 milligrams present.

As for cyclohexylsulfamate sodium, the results are excellent even though the amounts of the sweetener present are extremely small as compared to that which would normally be present if the sweetener were used.

For all these tests, it is recommended that small amounts of authentic material should be run concurrently as controls.

RECOMMENDATIONS*

1. That the following method be adopted for the detection of propoxy-2-amino 4 nitrobenzene (P-4000):

Organoleptic test.—Make alkaline (*p*H 7.5–8.0) with 10% NaOH 200 ml of liquid food or aqueous extract of 200 g of solid food or semi-solid product, **27.41 (c)**, and extract 3 times with 25 ml portions of petroleum ether. Wash combined petroleum ether extracts once with 5 ml of H_2O, transfer to small beaker or empty dish, allow ether to evaporate spontaneously, and taste residue. (Presence of as little as 5 mg of P-4000 per liter or kg of original material may be detected by its intensely sweet taste, or as little as 12.5 mg/l or kg of original material may be detected by its strong anaesthetic effect).

2. That the following method be adopted for the detection of cyclohexylsulfamate (sucaryl sodium ®):

Sodium nitrite test.—Add to 100 ml of sample or of aqueous extract, prepared as directed in **27.45 (c)**, 2 g of $BaCl_2$. Allow to stand 5 min. and filter. Acidify with 10 ml HCL and add 0.2 g $NaNO_2$. A white precipitate ($BaSO_4$) indicates presence of cyclohexylsulfamate.

3. That the following changes be made in Method **27.10:**

La Parola-Mariani test (5).—Expose dry residue, **27.8,** to HCl gas for 5 min.

* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 59 (1952).

and add 1 drop anisaldehyde. Presence of dulcin is indicated by development of orange-red to blood-red color. (Presence of as little as 25 mg/l or kg of original sample can usually be detected by this test.)

4. That the La Parola-Mariani test for the detection of dulcin be made official.

5. That methods for estimation of dulcin, cyclohexylsulfamate sodium and P-4000 be studied collaboratively.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation to the following collaborators, all members of the Food and Drug Administration: A. J. Shingler, Atlanta; H. P. Bennett, New Orleans; H. E. Gakenheimer, Baltimore; Rosa de Francesco, Philadelphia; David Firestone, New York; Mary C. Harrigan, Boston; J. H. Bornmann, Chicago; Juanita E. Breit, Minneapolis; Janice C. Bloomingdale, Cincinnati; D. W. McLaren, Buffalo; L. W. Ferris, Buffalo; M. L. Dow, St. Louis; and F. E. Yarnell, Kansas City.

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(3) HOEKE, *Analyst*, **72**, 548 (1947).

REPORT ON MONOCHLOROACETIC ACID

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

RECOMMENDATIONS*

It is recommended—

(1) That the collaborative study of the method for monochloroacetic acid in beverage bases containing halogenated weighting oils, *This Journal*, **34**, 345 (1951), be continued.

(2) That further work be done on the determination of monochloroacetic acid in fruit juices other than orange juice.

REPORT ON THIOUREA IN FOODS

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

For some time past the use of thiourea as a preservative was thought to be a dead issue, but it seems that periodically new uses for it in food and dietary preparations arise.

* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 59 (1952).

TABLE 1.—*Reactions of various constituents of food concentrates with hydrated sodium cyanoferrate in a weak acid solution*

SUBSTANCE†	COLOR REACTION			
	5 MIN	30 MIN	2 HRS	16–24 HRS
Thiourea	blue developing rapidly	deep blue	deep blue	blue
Thiamin HCl	yellow	greenish yellow	light green	
Thiamin + thiourea		blue	blue	
Methionine	yellow	yellowish green	light green	
Methionine + thiourea	yellow	greenish blue	blue	
Cystine	yellow	yellowish green	light green	
Cystine + thiourea	blue	blue	blue	
Cysteine	amber	yellow	light green	cloudy light blue
Cysteine + thiourea	greenish blue	blue	blue	blue
Ascorbic acid		green		
Ascorbic acid + thiourea	yellowish green	green	greenish	greenish
Niacinamide	yellow	yellowish green	greenish	
Niacinamide + thiourea	green	greenish blue	blue	
Water blank	greenish yellow	pale greenish yellow	light chartreuse	

† When the reaction was tested in the presence of thiourea the other substances usually had a concentration 5 to 10 times that of the thiourea.

A short time ago samples of a vitamin-mineral concentrate which contained thiourea as a preservative were found on the market.

The presence of the various vitamins, amino acids, and salts, made it difficult to determine if and how much thiourea was present. Several constituents inhibited the reaction with sodium cyanoferrate hydrate. It was also questionable whether some other constituents might produce a similar reaction with the reagent. The particular substances of special interest in this respect were those having sulfur linkages such as thiamin and the sulfur containing amino acids, cystine and cysteine. The results of the tests made on a number of these materials alone and in the presence of thiourea are given in Table 1.

The results show that none of the materials tested gave a ready positive blue reaction with hydrated sodium cyanoferrate[1] (modified Grote reagent) such as that given by thiourea. Cysteine did develop a somewhat similar color on long standing. A number of substances inhibited the thiourea reaction with the reagent to a greater or less degree. In particu-

[1] *This Journal*, **32**, 100 (1949).

lar, these were methionine, niacinamide, and ascorbic acid. Depending on the relative amounts of the materials, the thiourea may be entirely obscured in the presence of these materials. In the presence of such substances, where the color develops very slowly, there may be a slight possibility of confusing the cysteine and thiourea tests.

In this case the materials can be separated quite effectively by dissolving some of the sample in water and extracting the solution several times (4–6) with about 5 volumes (1 vol. soln to 5 vols. solvent) of a 1 to 1 mixture of $CHCl_3$ and butanol.

The extracts are then extracted, in turn, three times with 40–50 per cent of their volume of water. The water extract is concentrated, made to volume, and tested for thiourea. Five extractions will give about 89 per cent recovery.

It is recommended* that work on determination of thiourea be continued.

No report was given for fluorides; dulcin; or 1-propoxy, 2-amino, 4-nitrobenzene.

REPORT ON EXTRANEOUS MATERIALS IN FOODS AND DRUGS

By KENTON L. HARRIS (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Referee*

RECOMMENDATIONS†

After a lapse of several years during which little work was done on manure fragments in dairy products, the newly-appointed Associate Referee has undertaken a re-evaluation of the manure fragment procedures. Progress has been made during the past year. The Referee concurs in the recommendation made in the report on extraneous materials in dairy products that a collaborative study be conducted on the new method.

Two new methods are given in the report on extraneous materials in nut products, which are recommended to replace methods **35.23** and **35.22 (b)** and **(c)**. The Referee concurs in the first action recommendation.

The report on sediment tests in milk and cream continues the work of the Associate Referee in clarifying the standard sediment pad procedures. This work has resulted in a steady improvement in procedures over the past several years, and the Referee concurs in the recommendation that the new method be subjected to collaborative study with a view to making it official. *Methods of Analysis*, 7th Ed. should be corrected by inclusion of the correct procedure for **35.9(c)**.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 59 (1950).
† For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 57 (1952).

The Referee concurs in the report of the Associate Referee for extraneous materials in vegetable products. The method involving a 2-liter separator should be studied as an alternate procedure to the present **35.67(b).** Further comparative studies are needed on the 2-liter and 6-liter separators. Method **35.80** should be changed as recommended. The procedure for rot fragments should be included as recommended. The procedure for fly eggs and maggots in spaghetti sauce needs further study.

Changes are recommended by the Associate Referee on baked products, prepared cereals, and alimentary paste for sections **35.2(b), 35.4(a), 35.28, 35.28(b), 35.29, 35.36(d),** and **35.37.** The Referee concurs in these recommendations.

The proposed new methods for spices concern sections **35.83, 35.84, 35.85, 35.86,** and **35.87.** Experience has shown that the revisions will permit the use of larger samples, give cleaner separations, and more complete recoveries. These methods, involving the use of two new treatments, are largely the result of work by Maryvee G. Yakowitz, who has been investigating the adaptation of pancreatin digestion and synthetic detergents to various filth methods. It is recommended that collaborative data on these methods be obtained during the coming year.

The Referee concurs in the recommendations in the report for fruit products, that **35.48** and **35.55** should be made official, and two new methods for "drupelet berries" should be adopted, first action.

REPORT ON EXTRANEOUS MATERIALS IN DRUGS, SPICES, AND MISCELLANEOUS PRODUCTS

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

The following methods for estimating the amount of filth in spices are proposed revisions of methods **35.83, 35.84, 35.85, 35.86,** and **35.87.** An important feature of the proposed methods is that a larger sample is taken for examination, compared with the present A.O.A.C. methods.

No collaborative data have been assembled to compare the amount of filth recovered by the new procedures relative to results obtained on the same material using the old procedures. However, experience obtained in the use of the new methods has led to the conclusion that the new methods give better recoveries of the filth elements present in the spice than that obtained through the use of the present methods numbered as above.

35.83 *Ground cinnamon.*

(a) *Heavy filth and sand.*—Weigh 2 g of sample into 50 ml centrifuge tube and add ca 45 ml of CCl_4. Centrifuge 5 min. at 800 r.p.m. in International Size 1 Type SB Centrifuge, using No. 240 head with arm length of 5.25″ or its equivalent. Stir layer at top of liquid and repeat centrifuging. Decant ca ⅔ of liquid and floating layer and add fresh CCl_4 up to 45 ml. Mix thoroly and again centrifuge. Decant as

much of liquid and floating layer as possible without disturbing residue in centrifuge tube. Wash residue onto 11 cm ashless filter with CCl_4. Examine under low-power microscope for insect excreta, rodent excreta, and other filth. Ignite filter and residue in tared crucible and determine sand and soil.

(b) *Light filth.*—Weigh 50 g of sample into 600 ml beaker. Add 300–400 ml of H_2O. Stir until smooth. Add the filtered aqueous extract from 5 g of pancreatin and mix. Adjust to *p*H 8 with Na_3PO_4 soln. Readjust the *p*H after about 15 min. and again after about 45 min. Add 5 drops of formaldehyde and digest overnight at 37–40°C. Cool. Transfer the digested material to a 2-liter Wildman trap flask and add water to make 800 ml volume. Trap off twice with 25 and 15 ml gasoline, respectively, in the usual manner. Combine trappings in beaker. Transfer contents of beaker to trap flask and fill with H_2O. Stir and after 30 min. trap off into beaker and filter. Examine microscopically.

35.84 *Tumeric.*

Light filth.—Weigh 25 g into 400 ml beaker. Add 300 ml of $CHCl_3$-CCl_4 mixture (1+1), stir thoroly, and allow to stand 15 min. with occasional stirring. Transfer the mixture onto 15 cm filter paper in Büchner funnel and rinse with solvent. Dry overnight or in an oven at 80°C. for 1 hr.

Transfer the dry residue to a 600 ml beaker. Add 300–400 ml of H_2O. Stir until smooth. Add the filtered aqueous extract from 5 g of pancreatin and mix. Adjust to *p*H 8 with Na_3PO_4 soln. Readjust the *p*H after about 15 min. and again after about 45 min. Add 5 drops of formaldehyde and digest overnight @ 37–40°C. Transfer the digested material to a 2-liter Wildman trap flask and add water to make 800 ml volume. Trap off twice with 25 and 15 ml gasoline, respectively, in the usual manner. Combine trappings in beaker. Transfer contents of beaker to trap flask and fill with H_2O. Stir and after 30 min. trap off into beaker and filter. Examine microscopically.

35.85 *Onion and Garlic Powder.*

Light and heavy filth.—Weigh 50 g into 250 ml, hooked-lip beaker. Add 200 ml of CCl_4, stir thoroly, and allow to stand 30 min. with occasional stirring. Decant plant tissue onto 15 cm filter paper in Büchner funnel. Add 100 ml of CCl_4 and repeat decanting until practically no plant tissue remains with sand and soil on bottom of beaker. Transfer residue in beaker to ashless filter paper with stream of CCl_4 from wash bottle and examine for filth. If there is appreciable quantity of residue, place filter paper in tared crucible, ignite, and determine sand and soil.

Dry the residue of plant tissue from the Büchner funnel overnight or in an oven at 80°C. for 1 hr. Transfer to a 2-liter Wildman trap flask. Add 250 ml Tween 80[1]—60% alcohol soln. (40 ml Tween 80+210 ml 60% alcohol). Mix well and let stand 15–30 min. Add 60% alcohol to make 800 ml volume. Trap off twice in 60% alcohol with 75 and 35 ml gasoline, respectively, in the usual manner. Allow to stand 1 to 1½ hrs. for each of the two extractions, however, and avoid stirring except for a few circular upward strokes immediately after having filled the flask with 60% alcohol prior to the standing period. Filter and examine.

35.86 *Ground Black and White Pepper.*

Light and heavy filth.—Weigh 50 g into 600 ml beaker. Add 400 ml of CCl_4 and allow beaker to stand at least 1 hour, with occasional stirring. Decant pepper and solvent onto 15 cm filter paper in Büchner funnel, leaving any heavy residue of

[1] Polyoxyethylene sorbitan monooleate; Atlas Powder Company, Wilmington, Delaware.

sand and soil in beaker. Repeat decanting with CCl_4 if necessary to secure practically complete separation of spice materials from any heavy residue. Transfer residue from beaker to ashless filter and examine for filth. If there is appreciable quantity of residue, place filter paper in tared crucible, ignite and determine sand and soil. Wash spice material in the Büchner funnel with $CHCl_3$ and dry overnight or in an oven at 80°C.

Transfer the dry residue to a 600 ml beaker. Add 300–400 ml of H_2O, stirring until smooth. Add the filtered aqueous extract from 5 g of pancreatin and mix. Adjust to *p*H 8 with Na_3PO_4 after about 15 min. and again after about 45 min. Add 5 drops of formaldehyde and digest overnight at 37–40°C. Cool. Transfer the digested material to a 2-liter Wildman trap flask and add water to make 800 ml volume. Trap off twice with 25 and 15 ml gasoline, respectively, in the usual manner. Combine trappings in beaker. Transfer contents of beaker to trap flask and fill with H_2O. Stir and after 30 min. trap off into beaker and filter. Examine microscopically.

35.87 *Ground Capsicums (red and cayenne pepper, chili powder, paprika, etc.).*

Light and heavy filth.—Isolate large elements of filth, such as large larvae, adult insects, clumps of webbing, and insect and rodent excreta pellets by sifting pepper thru No. 10 sieve. Filth is retained on sieve.

Weigh 50 g of pepper into 600 ml beaker and add 400 ml of petroleum benzine. Boil gently 30 min. on electric hot plate, adding petroleum benzine occasionally to keep volume constant. Decant petroleum benzine onto smooth 15 cm filter papers in Büchner funnel. Add 400 ml of CCl_4 and allow to stand 30 min., with occasional stirring. Decant pepper and solvent onto same 15 cm filter paper in Büchner funnel, leaving any heavy residue of sand and soil in beaker.[2] Proceed as directed in **35.86** above, beginning "Transfer residue from beaker to ashless filter . . . etc."

It is recommended* that the above modifications be adopted. Additional work should be undertaken to see if the proposed techniques might apply to other spices and crude drugs.

REPORT ON EXTRANEOUS MATERIALS IN DAIRY PRODUCTS

By DOROTHY B. SCOTT (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Associate Referee*

During the past year the recommendations of Committee D were carried out in part.

Two samples of cheese, one containing only feed plant fragments and the other containing barnyard soil, were sent to the sixteen districts and one substation of the Food and Drug Administration.

Fifty analysts participated in the examinations. They were told the composition of each sample and were asked to count the plant fragments in both and the percentage of dung fragments in the sample containing barnyard soil. The table of characteristics of plant and dung fragments

[2] Repeat decanting with CCl_4 if necessary to secure practically complete separation of spice materials from any heavy residue.

* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 57 (1952).

given in the report of the Associate Referee at the 1950 meeting of the Association of Official Agricultural Chemists was used for reference.

The majority of the results were satisfactory.

A new method was developed during the year, using a neutral water solution of three dyes—eosin, wool violet, and a blue dye, Color index 518. The Color Certification Branch of the Cosmetic Division of the Food and Drug Administration worked out this stain.

The new method may be used to determine dung fragments in all dairy products, since the two predominant characteristics, that is, the lack of cell contents and the surface deposit of mucilage on the dung fragments, will be present.

The characteristic brought out by R. E. Duggan, "Method for the Identification of Cow Manure in Dairy Products," *This Journal*, **27**, 331 (1944), showing that plant fragments which have not undergone digestion will stain deeply, is also noted with the Graichen-Harrow stain. However, in this new method, the stain is used primarily to show the cell contents and the mucilage.

The most important characteristic for identification of a dung fragment is the lack of digestible nutrients.

In ruminants with four compartments in their compound stomachs, digestion of the plant tissue cell contents is drastic.

Digestion begins in the mouth where the food is crushed, ground, and mixed with slimy saliva just enough to make it easily swallowed. After the animal's appetite is satisfied, it seeks a quiet place and proceeds to "chew its cud." The enzyme ptyalin in the saliva digests the starch.

The enzymes of the digestive system of ruminants are unable to break down the cellulose and lignin cell walls of the plant. These are partially digested by the bacteria in the first three compartments of the compound stomach. The nutrients are then set free and are acted upon by the enzymes secreted in the fourth compartment and the intestines. Certain protozoa found in the food mass in the first compartment or paunch also aid in the digestion.

Simple sugars are absorbed directly from the stomach but most carbohydrates are carried into the intestines where they are acted on by the pancreatic enzymes.

Proteins are first attacked by the enzymes pepsin and rennin, in the fourth stomach. The food mass here is acid, due to the hydrochloric acid present. When it passes into the intestines it becomes alkaline before it can be acted upon by the pancreatic enzymes.

The proteins are broken down further in the intestines by the enzymes trypsin of the pancreatic juice and erepsin of the intestines.

Fats are digested in the small intestine, being acted upon by the enzyme lipase of the pancreatic juice after being emulsified through the aid of the bile salts of the liver.

The mineral nutrients are dissolved first by the acid of the gastric juice, and those freed by enzymic action are absorbed in the small intestine.

The more or less solid residue accumulates in the rectum and is voided as feces. These then contain the portions of food which have not been digested, largely lignified cellulose or crude fiber that has escaped bacterial action. Roughages contain large quantities of lignified cellulose with relatively little protein, starch, or fat.

The bacteria and enzymes of the digestive system are unable to break down the lignified walls. Also resistant are some conductive vessels of leaves and seed coats. Small seeds may pass through the digestive system intact. Cereal grains, if not broken up in mastication, may also pass through undigested.

The feces also contain large numbers of bacteria. It is estimated that an average of approximately 30 per cent of the feces on a dry basis are bacteria. These will be in the mucilage on the outside of the dung fragments and will be readily visible at a magnification of 400×.

Residue from the bile and other digestive fluids, worn out cells from the intestinal lining, dirt, and mucus are in the mucilage of the feces.

Therefore, mucilage on the surface of dung fragments is another important diagnostic characteristic.

Microscopic examinations of some decayed plant material at low power in an alcohol wetting mixture will show a slimy coating somewhat resembling the mucilage on dung fragments. Examination of a large number of various kinds of decomposed, decayed, and weathered plant material showed fragments from this type of material to contain much cell contents. The breakdown of tissue was not nearly as drastic as that of digested dung fragments. These fragments were usually brown in color and generally showed mold filaments and mold spores on the surface. Bacteria are also present but not in the quantities found in the mucilage of dung. With experience, after staining and examining at high power, the difference in the composition of the coating can be readily seen. The mucilage of dung consists mainly of bacteria, whereas in the slimy material of decayed plant tissue mold and mold spores predominate.

Plant fragments which appear to be empty of cell contents at low magnification, when stained and viewed at a high power, will show stained cell contents and lack of mucilage.

It is to make these digestible nutrients more easily seen at high power and to make the microorganisms in the mucilage on the dung fragments recognizable that a staining technique has been developed.

METHOD

1. Examine the residue or filth contaminants from cheese on filter paper after wetting with a 1–1 alcohol-glycerine soln. Use 30× magnification. Use 60×–70× to confirm the mucilage on dung fragments.

Most fragments will be identified by direct examination, given in 1 above.

* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 57 (1952).

Plant fragments which have not gone thru the digestive system of the ruminant will have the following characteristics:

A. Much cell contents—opaque.
B. Clean on the surface—no mucilage.
C. Ends of fragments clean cut and containing cell contents.
D. No loose spirals and other conductive vessels.

Dung fragments will show these characteristics:

A. Lack of cell contents—translucent.
B. Mucilage on surface in varying quantities.
C. Ends of fragments jagged and fibrous.
D. Easily disintegrated.
E. Consist mostly of conductive vessels.
F. Loose spiral vessels.

The following fragments require additional study as given below:

A. Fragments which appear to be translucent, lacking cell contents when viewed at low power, and have no external coating of mucilage or dirt.

B. Fragments which contain many cell contents, may be dark brown in color, may be pigmented but have a coating in alcohol which resembles mucilage.

C. Any other fragments which appear to have characteristics of dung fragments but are doubtful.

2. *Staining technique:*

A. Remove the plant fragments from the filter paper to a drop of water on a slide.

B. Transfer to a drop of Lugol's iodine soln and immerse for one min.

C. Wash in another drop of water.

D. Stain for 15 min. in a 10% soln of Graichen-Harrow stain.

E. Wash in a drop of water, then remove to a drop of 95% alcohol. Remove to a slide from alcohol and allow to dry.

F. Remove to a drop of clove oil on a slide. As the alcohol evaporates, the tissue will become impregnated with the oil.

G. Place a cover glass on the plant fragments and examine first at 100×, then 400×.

The use of fine jeweler's tweezers (Dumont No. 5) is a necessity in handling small plant fragments. Hanging drop slides are useful though not necessary.

Dung fragments will be almost *devoid* of cell contents. The fragments themselves will be translucent, but the dirty appearance is due to the external coating of mucilage. They may have fibrous and jagged ends. In general, they do not stain as deeply as the plant fragments containing digestible nutrients. The lignified cellulose, which constitutes a large part of the plant tissue of dung fragments, may be colored the light brown color of the iodine solution. Adhering cheese fragments stain red. Digestible cellulose stains blue.

The mucilage coating on the surface of the dung fragments should be brought into focus at 400× and studied. Numerous small bacteria can be seen, as well as other microorganisms and the finely divided dirt particles found in dung.

Plant fragments which contain much cell contents will stain deeply. Those undigested plant fragments such as stem tissue which may appear to contain no nutritive elements when viewed at low power magnification, will on staining show cell contents when viewed at high power.

In conclusion, a dung fragment must be almost entirely free of cell contents. It will have surface contamination in varying quantities. When stained as in the method, the lignified cellulose may be abundant and colored a yellow or light brown. When the mucilage is examined at high power, it will be found to contain abundant bacteria and other microorganisms. Some dung fragments which have escaped complete digestion will contain cell contents. A study of dung from cows, sheep, and goats show these to be a small percentage of the total dung.

In feed plant fragments, there may be a very few consisting entirely of vessels, tracheids, and fibers with no parenchymatous tissue attached which normally contain no cell contents. If they have not gone through the digestive system of the ruminant they will have no surface contamination. These should not be confused with dung fragments.

The stain used was developed by Charles Graichen and Lee S. Harrow of the Color Certification Branch. Thanks are extended to them and to L. C. Mitchell of the Food Division for help in obtaining the stain.

The composition of the stains follows:

(1) *Lugol's iodine soln*:

Iodine	1 gram
Potassium iodide	2 grams
Water	300 ml

(2) *Graichen-Harrow stain*:

Eosin Y S	0.2%
Wool Violet 5 BN	0.05%
Blue Color Index No. 518	0.15%
Solvent—Water	

RECOMMENDATIONS*

It is recommended—

(1) That the staining technique be studied further.

(2) That collaborative study be made of the method for detection of manure fragments, using the Graichen-Harrow stain.

* For report of Subcommittee D and action of the Association, see *This Journal*, 35, 57 (1952).

REPORT ON EXTRANEOUS MATERIALS IN NUT PRODUCTS

By MARYVEE G. YAKOWITZ (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Associate Referee*

This report covers two subjects: Filth in shredded coconut and light filth in Spanish peanuts.

It is recommended* that method **35.23** for extraneous matter in coconut be deleted, and that a new method be adopted as first action. The proposed method has been found to yield better recovery than the present **35.23** procedure and has been subjected to prolonged general use. Details of the method have been published in *This Journal*, **35**, 94 (1952).

Present methods **(35.22(b)** and **(c))** for the determination of light filth in nut products have been unsatisfactory for Spanish peanuts, on account of the abundant amounts of peanut skins which rise with the gasoline layer and hinder filth recovery. A new method has been developed by the Associate Referee and subjected to collaborative testing. Details of the method have been published in *This Journal*, **35**, 94 (1952).

Duplicate 100 g samples of Spanish peanuts each containing 20 rodent hair fragments (1–2 mm) were examined as unknowns by five collaborators using the new procedure. The results obtained are shown in Table 1.

TABLE 1.—*Recovery of rodent hairs from Spanish peanuts*

COLLABORATOR	SAMPLE A			SAMPLE B		
	1ST EXTRACT	2ND EXTRACT	TOTAL	1ST EXTRACT	2ND EXTRACT	TOTAL
1	16	2	18	14	3	17
2	12	1	12	10	1	11
3	19	0	19	17	1	18
4	13	4	17	16	0	16
5	8	3	11	6	4	10
Average			15.4			14.4
Per cent recovery			77.0			72.0
Per cent recovery Samples A and B			74.5			

Collaborator 2 indicated that four or five peanut skins floated to the top. No other comments concerning interferences or difficulties with the method were reported.

* For report of Subcommittee D and action of the Association, see *This Journal*, 35, 57 (1952).

In general the results are better than average for rodent hair recovery procedures. It is recommended* that the above method for light filth in Spanish peanuts be adopted, first action.

The Associate Referee wishes to thank the following collaborators of the Food and Drug Administration who kindly cooperated: Manion M. Jackson, Philadelphia; Flora Y. Mendelsohn, Los Angeles; Robert E. O'Neill, Atlanta; Garland L. Reed, Baltimore; Hymen D. Silverberg, St. Louis.

REPORT ON EXTRANEOUS MATERIALS IN BAKED PRODUCTS, PREPARED CEREALS, AND ALIMENTARY PASTES

By J. FRANK NICHOLSON (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Associate Referee*

A revision of the method for the preparation of pancreatin solution, **35.2(d),** has been suggested. The proposed method is much simpler in procedure and gives better results than the present method. The revision has been published in *This Journal,* **35,** 94 (1952).

It has also been found that a new procedure suggested by Mrs. Yakowitz of the Microanalytical Branch gives better recovery in extractions using the Wildman trap flask, **35.4(a).** The method is given in detail in *This Journal,* **35,** 94 (1952).

In section **35.28(a)** (p. 714) the following corrections and revisions have been suggested:

In the third paragraph, to the sentence beginning "Add to trap flask," insert "60%" before the first "alcohol."

In **35.28(b)** make the second sentence read "Cool somewhat, neutralize to ca *p*H 6 with dilute NaOH soln, bring to *p*H 7–8 with dilute Na_3PO_4 soln, cool to 35–40° and digest with pancreatin as directed in **(a).**"

The first action method, **35.29,** should be revised to read as given in *This Journal,* **35,** 95 (1952).

35.36(d). This method should read "proceed as directed under **35.33.**"

35.37. In next to last sentence change "80% alcohol" to "60% alcohol."

It is recommended† that the above changes be made.

* For report of Subcommittee D and action of the Association, see *This Journal,* **35,** 57 (1952).
† For report of Subcommittee D and action of the Association, see *This Journal,* **35,** 57 (1952).

REPORT ON EXTRANEOUS MATERIALS IN FRUIT PRODUCTS

By W. G. HELSEL (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Associate Referee*

RECOMMENDATIONS*

It is recommended that section **35.48,** Rot in Apple Butter, be made official (final action), since it applies an official method **(35.64)** to this product.

It is recommended that section **35.55,** Mold in Frozen Strawberries, be made official. This method has been used regularly by analysts of the Food and Drug Administration and in industry and has been found to be satisfactory.

It is also recommended that the following methods for rot be adopted, first action:

In blackberries, raspberries, and other drupelet berries (frozen):

Pulp thawed berries thru cyclone with screen openings 0.027″ in diam. and mix thoroly. Mix 25 g of pulp with 50 g of 3% pectin soln **(35.2 (b)).** Make mold count as directed in **35.64.**

In blackberries, raspberries, and other drupelet berries (canned):

Drain the berries 2 min. on a No. 20 sieve. Pulp, dilute, and make mold count as directed above for frozen berries.

REPORT ON EXTRANEOUS MATERIALS IN VEGETABLE PRODUCTS

By FRANK R. SMITH (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Associate Referee*

In the section on Extraneous Materials in Vegetable Products there are a few changes to be made this year. One of these is relatively minor, and one is the inclusion of a method for the identification of rot fragments in tomato products. The inclusion of some plates of photographs will be of material aid in the use of this method. Another method has been found useful in the examination, for fly eggs and maggots, of a series of samples of tomato sauce from canned spaghetti. The changes and new methods are as follows:

35.67(b). In fourth line insert "l" between "6" and "separator." This is a typographical error and can be corrected in an errata sheet.

35.80(a). Morton I. Riefberg of Minneapolis District U. S. Food and Drug Administration has found that a considerable amount of filtering time is saved and a cleaner paper is secured if the trapped off portion

* For report of Subcommittee D and action of the Association, see *This Journal.* 35. 57 (1952).

is washed on a No. 200 sieve prior to transferring to filter paper. The starch and castor oil which ordinarily clog the filter paper are washed through the sieve with hot water and the oily mass is dispersed with hot alcohol. The material passing through the No. 200 sieve was examined and found to contain no filth.

It is recommended that the method be changed as follows: After the sentence in line seven ending "heat trapped off portion nearly to boiling," insert "Transfer contents of beaker onto a No. 200 sieve, wash beaker thoroly with hot alcohol to insure complete transfer. Wash material on sieve with hot alcohol from a wash bottle to eliminate oily mass." In line eight change "pour oil and H_2O mixt." to "transfer residue" and in line 10, delete "to dissolve oil and speed filtration."

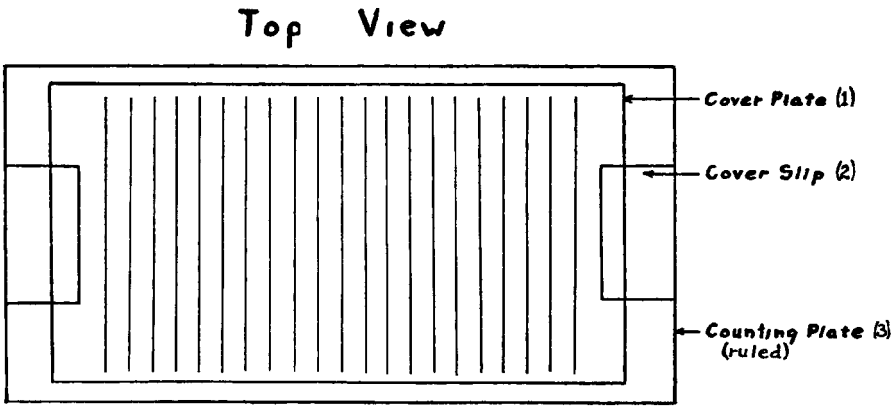
The following method has been found applicable over a period of eight years by a number of analysts. The inclusion of photographs of rot fragments will do much to clarify the type of material to be counted.

IDENTIFICATION OF TOMATO ROT FRAGMENTS

Weigh 10 g of juice (5 g of puree or catsup or 2 g of paste) and transfer with 100 ml of H_2O to 400 ml beaker. Add ca 2 ml of saturated aqueous crystal violet soln (10% crystal violet in alcohol may replace the aqueous soln when latter fails), stir, and allow to stain 3 min. Add ca 200 ml of H_2O, stir, and pour thru No. 60 sieve ca 7.5 cm in diam., held in horizontal position. Pour material over entire surface of sieve, using glass rod held against lip of pouring beaker, with lower end of rod ca 2 cm from screen. If sample weight specified does not drain rapidly, reduce size of sample. Rinse beaker with 200 ml of H_2O and pour rinse H_2O over tomato debris on sieve, using glass rod as before. Tilt sieve to ca 30° angle and wash debris to lower part with ca 100 ml of H_2O. Allow debris to drain and transfer to bottom of graduated tube, ca 12×3 cm, by means of spatula. Transfer remaining debris by washing down with water from a dropper and immediately taking up debris in wash water before it has run thru the screen. When completely transferred, make volume of H_2O and debris to 10 ml with H_2O. Add sufficient pectin soln **35.2 (b)** to bring volume to 20 ml. Mix stained suspension well, measure out two separate 0.5 ml portions, spread over 2 counting slides, and cover with special cover slip. Examine each slide with Greenough-type microscope, **35.1 (k)**, using magnification of 40–45 diam. with transmitted light. (Rot fragments are tomato tissue to which mold filaments are attached. Some may appear as almost solid masses of mold (see Plates).) Count number of rot fragments on each of the 2 slides, add, and multiply by 2 (10 g sample), 4 (5 g sample), or 10 (2 g sample) to obtain number of rot fragments/g of product.

It is recommended that this method be included as a first action method.

Counting plate and cover for rot fragments.—Plates are of glass 1.5–4.0 mm thick and covers are ca 1.5 mm thick. Dimensions of plates 55×100 mm; rulings: crosswise, parallel lines 4.5 mm apart, with one 15 mm space at each end. One-half of square cover-slip ca 22 mm on a side and ca 0.25 mm thick is fastened at each end of counting plate by balsam to separate it from the cover-plate. Covers are 50 mm ×85 mm.



Rot Fragment Counting Slide.

Matthew L. Dow of St. Louis District has developed the following method and found it useful in the recovery of fly eggs and maggots in the tomato sauce from canned spaghetti:

FLY EGGS AND MAGGOTS IN SPAGHETTI SAUCE

Empty the contents of the entire can on an 8 inch No. 8 sieve nested on an 8 inch 100 mesh sieve, wash the interior of the can thoroly with water and pour over the spaghetti on the sieve. Wash the spaghetti under a cold water tap until all of the sauce and any material adhering to the spaghetti has been washed thru the No. 8 sieve. Transfer the material on the 100 mesh sieve to a 600 ml beaker, dilute with water at a temp. of 37°C., add pancreatin extract **35.2 (d)** and digest for 1–2 hrs. after adjusting the *p*H of the soln to 8. Filter thru 10xx bolting cloth and examine at ca 10 diam.

The above method should also be tried in the examination of the sauce from pork and beans and of tomato soup. It is recommended that further work be done on this method as opportunity is presented.

RECOMMENDATIONS*

It is recommended—

(1) That the procedure for rot fragments in tomato products described in the Associate Referee's report be made first action.

(2) That method **35.80(a)** for insect fragments in canned corn be modified as described in the Associate Referee's report and made first action.

* For report of Subcommittee D and action of the Association, see *This Journal*, 35, 57 (1930).

REPORT ON SEDIMENT TESTS IN MILK AND CREAM

By CURTIS R. JOINER (Food and Drug Administration, Federal Security Agency, St. Louis, Mo.), *Associate Referee*

The method for the preparation of regular standard disks that was adopted as first action in 1949 (2) is given below. Through an error this method was not published in the Seventh Edition of *Methods of Analysis* (4). Instead, the original tentative method (5) was printed. The following method should replace par. **35.9(c):**

METHOD

35.9 (c) *Preparation of standard sediment disks.*—Make a uniform mixture of oven dried (100°) materials which meet following screening specifications, using U. S. Standard Sieves. Grind all materials by hand with mortar and pestle.

	per cent
Cow manure, thru No. 40	53
Cow manure, thru No. 20, retained on No. 40	2
Garden soil, thru No. 40	27
Charcoal, thru No. 40	14
Charcoal, thru No. 20, retained on No. 40	4

Place 2.00 g of above mixture in 100 ml volumetric flask and moisten with 5 ml 1% aerosol soln or other suitable wetting agent, add 46 ml 0.75% gum soln such as carob bean **(35.2 (c))**, bring level of liquid just into neck of flask by adding 50% by weight sucrose soln, let stand at least 30 min., add few drops alcohol and dilute to volume with the sucrose soln. Mix thoroly, pour into 250 ml beaker or 6–8 fl. oz. screw-capped jar, and stir with small motor stirrer at speed (ca 200–300 r.p.m.) such that mixture is thoroly agitated, but so that very little air is whipped into the suspension. Observe with light. Place blade of stirrer so that fine particles do not accumulate in small eddies at bottom of beaker.

Transfer (while stirring) 10 ml portion (200 mg standard sediment) with large tipped, graduated pipet to a volumetric flask and make up to one liter with 50% by weight sucrose soln. When thoroly mixed, each ml contains 0.2 mg sediment. Mix contents of flask, pour into 1500 ml beaker and stir with suitable motor stirrer as directed above. If particles accumulate on side of beaker, wash down with portions of sediment suspension or push under with tip of pipet. While stirring, pipet definite volumes of sediment mixture and add to ¾ pint filtered sweet skimmed milk. Mix thoroly and pass mixture thru standard sediment disk in filtering device having filtering area measuring $1\frac{1}{8}''$ in diameter. Pour milk gently down side of filtering apparatus and filter with very little or no suction. Wash container promptly with ¼ pint filtered skimmed milk. Let last portion of milk flow thru pad with no suction applied. If sediment does not appear to be evenly distributed over pad, add 15 or 20 ml milk and let it filter thru without any suction. Repeat this until sediment appears to be evenly distributed. Suck air thru disk for ca 1 min. to remove excess milk. For permanent record mount and spray disks with 40% HCHO soln or with alcoholic soln containing 2.5 g each of menthol and thymol in 100 ml. Alternatively, if most of milk is removed by thoro aspiration, no preservative is needed. Dried pads may be coated with colorless plastic cement diluted with 1–3 volumes of acetone so that mixture is thin enough to pour easily. If acetone dissolves pigment from paper and stains pads, place them on flat glass plate for treating with diluted cement. Move pads while drying, to prevent sticking to glass. When pads are almost

dry place a light weight (petri dish, etc.) directly on them to prevent curling. Pads may be mounted with the plastic cement. (Standard disks made from manure containing large amount of chlorophyll cannot be coated with plastic cement, as solvent will extract chlorophyll and stain pad green. Use this method of preserving pads only if there is no leaching of pigment from the sediment on addition of diluted plastic cement.) Following above method, prepare series of disks containing sediment remaining from 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0 and 14.0 mg standard mixture. Mark disks to show quantity of sediment (mg) used to prepare each pad. Do not use as a standard any pad on which sediment is not evenly distributed.

For purpose of comparison, entire series of disks may be used, but it will usually be found more convenient to select a few disks denoting variations in grade that are applicable to particular investigation being made. If grading charts are prepared and reports made, chart and report must indicate whether mixed or off-bottom sample was used. If standards are to be handled or used for any appreciable length of time, place them under glass, transparent plastic sheets, or other suitable materials. In using standards, grade sediment disk of sample to nearest standard disk, regardless of whether actual quantity of sediment is above or below standard. Disregard gross pieces of material (whole flies, large chunks of manure, etc.) in grading pads, but list their presence separately.

"FINE" SEDIMENT PADS

Several regulatory officials have indicated that there is a need for a "fine" set of standard sediment pads to be used for the sediment tests in milk in conjunction with, and in addition to, the regular standard pads. The latter were adopted as first action (1, 2) by this Association. The "fine" standards are needed for grading sediment from retail market milk and sediment from milk for manufacturing purposes when the sediment is very finely divided. Such pads are difficult to grade with the regular standards. For these reasons the Associate Referee undertook to develop a method for preparing an acceptable set of fine sediment standards.

Fifteen different sediment mixtures varying in composition and in particle size were made up. A number of different methods of preparing the standards were tried out, using the basic techniques of the method for preparing the regular standard pads (1, 3). As a result of this work the following conclusions were reached: 1. The fine standards should be made from materials that would at least pass through a U. S. No. 140 sieve. 2. When using sediment of that degree of fineness, the porosity and surface texture of the disks are of major importance. 3. The use of milk in the preparation of standard pads is not necessary. 4. Fine standard sediment pads should not be washed after filtration of the sediment suspension, because the washing carries more of the sediment through the pad. 5. When standard pads are prepared without washing them, the sucrose remaining on the particles of sediment cause the pad to appear darker than a comparable disk prepared without using sucrose in the dilute suspension. These factors were made use of in arriving at the composition of the sediment mixture and the method for preparing the pads.

The method for the preparation of fine standard sediment pads is given

below. A method for testing disks and tentative specifications for them are included: (It could logically appear as par. **35.9(e)** in *Methods of Analysis*).

Preparation of Fine Standard Sediment Disks.—Prepare the following mixture, using oven-dried (100°) materials and U. S. Standard sieves.

	Per cent
Cow manure	66.0
Garden soil	28.0
Charcoal	6.0

(All materials thru No. 140 and retained on No. 200 sieve.)

Proceed as directed in **(c)**, beginning, "Place 2.00 g of above mixture . . . ," except to use H_2O instead of 50% sucrose for diluting the 10 ml aliquot to 1 liter.

Where **(c)** states "While stirring, pipet definite volumes . . . ," proceed as follows: Determine approx. capacity of funnel on filtering apparatus by pouring water into assembled apparatus with filter flask air outlet closed. Include H_2O that filters thru as part of capacity of funnel. While stirring, pipet aliquots of sediment suspension into beakers. Add water to make total volume 20 to 50 ml less than capacity of funnel, using a max. total volume of 400 ml and a min. of 60 ml. With filter flask air outlet closed to prevent filtration, mix diluted aliquot and pour into funnel fitted with a wet standard disk that passes tests given below. Add 20 to 50 ml H_2O to beaker and rinse by swirling. Pour into funnel, keeping lip of beaker touching surface of H_2O if possible. (Rinse H_2O should nearly fill funnel if capacity is 450 ml or less.) Open flask air outlet. After H_2O has filtered thru pad, apply vacuum and aspirate disk for ca 1 min. Remove pad and let dry in covered dish. If sediment is not evenly distributed, pad must be discarded. After some practice with method, ca 75% of pads prepared should be acceptable. No preservative is required. Pads may be coated with diluted plastic cement as in **(c)**.

Test sediment disks as follows: Filter 12 mg standard sediment mixture (60 ml aliquot) thru pad as directed above, using clean flask to catch filtrate. Transfer filtrate to beaker. Rinse flask three times with H_2O and add rinsings to beaker. Filter filtrate thru 7 or 9 cm. S&S No. 589 White Ribbon Paper (or equivalent) that has been washed with ca 200 ml H_2O, dried to constant weight at 100°C. and cooled in covered dish in desiccator before weighing. Rinse beaker and paper thoroly with H_2O. Dry paper to constant weight as directed above. Average weight of sediment passing thru 3 or more disks should not exceed 2.2 mg. In addition, standard disk should not appear to have sediment buried beneath surface.

DISCUSSION OF COLLABORATIVE WORK

By taking part in a preliminary collaborative study, several chemists in this laboratory helped in the writing of the method so that it could be used with good results by inexperienced analysts. Later, collaborative work was carried out with a standard sediment mixture composed of 50 per cent of materials through a U. S. No. 200 sieve and 50 per cent through a U. S. No. 140 and retained on the No. 200 sieve. After results were received from several of the laboratories, it was apparent that the sediment disks on the market were too porous to be used with such a fine mixture. Accordingly, a new sediment mixture was prepared in which the

portion passing through the U. S. No. 200 sieve was eliminated. The average weight of the latter sediment mixture that passed through 15 sediment disks of one type was 1.95 milligrams (from 12 milligrams filtered). With the finer of the two mixtures this figure was approximately 3.0 milligrams for the same type of pad.

Several collaborators who had prepared acceptable standard disks with the finer mixture found it almost impossible to prepare good disks with the mixture containing no fraction that passed through a U. S. No. 200 sieve. Thus, it was necessary to re-write the directions completely. Five analysts in this laboratory and one in another city prepared excellent duplicate sets of standard pads using the modified instructions with the coarser of the two mixtures. Two of the analysts also prepared their own mixtures and made up satisfactory sets of pads. This method is the one that is presented above. Because of limited time no further collaborative study was possible.

A limited amount of work was done on the preservation of standard sediment pads to make them suitable for use in field work. Through the courtesy of a commercial fabricator of plastics, some work was done to determine whether or not it is feasible to mount standard disks in clear plastic by high pressure lamination. It was found that the fine sediment standards could be laminated without altering their appearance if the disks were free of milk and sucrose. However, when the regular standard disks were treated in this manner, the larger particles of sediment were mashed flat by the high pressure used in the process and this changed the appearance of the pads.

SUMMARY

1. A method for preparing fine standard sediment disks is presented.

2. A method for testing the sediment disks to be used and a tentative standard for them are presented. When less porous, but hard-surfaced, rapid filtering disks are developed and marketed commercially, this tolerance should be lowered.

3. Six different analysts (five of them in one laboratory) prepared excellent duplicate sets of pads using the method presented.

4. Some work has been done on preserving standard pads for use in the field by mounting them directly in plastic by pressure lamination.

RECOMMENDATIONS*

It is recommended—

(1) That method **(c)** as presented above be substituted for method **35.9(c)** on page 706 of the Seventh Edition of *Methods of Analysis*.

(2) That the method for the preparation of fine standard sediment disks, as given above, be studied further during the coming year.

* For report of Subcommittee D and action of the Association, see *This Journal*, **35**, 58 (1952).

ACKNOWLEDGMENTS

The writer wishes to thank the following members of the staff of the Food and Drug Administration for participating in the collaborative work: John F. Weeks, Jr., New Orleans District; George E. Keppel, Minneapolis District; John H. Bornmann, Chicago District; Frank H. Collins, Cincinnati District; and eight analysts of the St. Louis District.

Thanks are also due to Carl J. Tabor, of Tires, Inc., St. Louis, Mo., for his kindness in doing the pressure laminating referred to above.

REFERENCES

(1) *This Journal*, **33**, 552 (1950).
(2) *Ibid.*, p. 50.
(3) *Ibid.*, **32**, 324 (1949).
(4) *Methods of Analysis, A.O.A.C.*, 7th Ed. (1950), p. 706, **35.9 (c).**
(5) "Changes in Methods of Analysis," *This Journal*, **31**, 93 (1948).

No report was given for extraneous material in beverages, or in miscellaneous materials.

REPORT ON MEAT AND MEAT PRODUCTS

By R. M. MEHURIN (Meat Inspection Division, Bureau of Animal Industry, Department of Agriculture, Washington 25), *Referee*

The Associate Referees on starch in meat products, and on creatin, have reported methods which they recommend for further study. No report was received from the Associate Referee on the detection of horsemeat in ground meat.

The changes recommended below involve no change in reagents, but the use of the single reagent does result in a saving of time. The procedure has been widely adopted.

RECOMMENDATIONS*

It is recommended—

(1) That the following changes be made in the first action method for nitrites **(23.15, 23.16)**, and the revised method be adopted, first action.

Paragraph **23.15** (p. 361–362): Delete reagents **(a)** and **(b)** and substitute the following:

(*a*) *Modified Griess reagent.*—Dissolve 0.5 g sulfanilic acid in 150 ml 15% acetic acid. Boil 0.1 g alpha-napthylamine in 20 ml H_2O until dissolved and pour while hot into 150 ml 15% acetic acid. Mix the 2 solns and store in brown glass bottle.

Paragraph **23.16** (p. 362). Change reagent **(c)** to **(b).**

Paragraph **23.16.** Delete, starting with the sentence in line 8, "Filter,

* For report of Subcommittee C and action of the Association, see *This Journal*, **35**, 52 (1952).

dil. suitable aliquot to mark . . . " to the end of the paragraph, and substitute the following:

Filter, dilute aliquot to mark in 50 ml volumetric flask, add 2 ml reagent, mix, and allow color to develop for 1 hour. Transfer suitable portion of soln to photometer cuvette and determine density at a wave length of 520 millimicrons, setting the instrument to zero density by means of a blank consisting of 50 ml H_2O plus 2 ml reagent. Determine nitrite present by comparison with standard curve prepared as follows: Dilute suitable volumes of standard nitrite soln to mark in 50 ml vol. flasks, add 2 ml reagent, and proceed as above.

(2) That work be continued on the proposed method for starch in meat products as submitted by the Associate Referee.

(3) That work be continued on the method for creatin in meat products as proposed by the Associate Referee.

(4) That work be continued on the proposed chemical method and on a serological method for horsemeat in ground meat.

REPORT ON STARCH IN MEAT PRODUCTS

By F. J. STEVENS and R. A. CHAPMAN (*Associate Referee*) (Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada)

The method for the determination of starch in meat products given in the 6th Edition of the *Methods of Analysis*, (1) was based on saponification of the sample with alcoholic potassium hydroxide, and precipitation of the starch with alcohol, followed by determination of the reducing sugars after hydrolysis with sulfuric acid. However, it was found that many modified starches were not completely precipitated by the alcohol, with the result that low values were frequently obtained. Therefore, Mehurin (2) recommended that this procedure be revised and the original method be deleted from the 7th Edition.

A method based on the Schirokow and Milowidowa (3) procedure has been used in these Divisions for a number of years with satisfactory results. It involves hydrolysis of the entire meat sample with hydrochloric acid, followed by the determination of reducing sugars by a modified Fehling's method (4). However, by this procedure all reducing sugars present after hydrolysis are calculated as starch. Therefore, to employ this method for the determination of starch only, in meat products, it was necessary to develop a technique for the removal of all reducing sugars and other carbohydrates which would yield reducing sugars on hydrolysis.

A number of solvents were investigated as extractants for dextrose, sucrose, lactose, and maltose in meat products. Concentrations of alcohol varying from 65% to 85% were employed. It was found that 99.3% of wheat starch could be recovered when 75% alcohol was employed as an

extractant, but a recovery of only 22.7% was obtained on a modified wheat starch. Higher concentrations of alcohol did not entirely remove the lactose added to meat samples. Lower concentrations resulted in incomplete recoveries of wheat starch. Similar results were obtained with acetone. The use of 75% alcohol containing varying amounts of barium chloride, calcium chloride, polyoxyethylene monostearate, and polyoxyethylene sorbitan monooleate, as starch precipitants, gave satisfactory results with a number of starches, but low recoveries were obtained on other samples. It was finally concluded that the separation of soluble sugars from the starches by the use of selective solvents was not feasible.

A solution of potassium ferrocyanide and zinc acetate were first employed by Carrez (5) for the separation of lactose from interfering proteins in milk. Later, Van Voorst (6) used the same reagents for the precipitation of starch from aqueous solutions in the determination of reducing sugars in a number of food products. Preliminary experiments indicated that this technique might be applicable to the determination of starch in meat products. It was found that, in the presence of the heavy gelatinous precipitate of zinc ferrocyanide which formed on the addition of these two reagents, it was possible to remove dextrose, lactose, sucrose, maltose, and reagent dextrin (Merck) from aqueous suspensions of meat products without extracting the starch. Therefore the use of this reagent was investigated more extensively.

Van Voorst (6) has recommended the use of 1 ml of 15% aqueous potassium ferrocyanide and 1 ml of 30% aqueous zinc acetate in 50 ml of solution. In this investigation this concentration was found satisfactory and resulted in the most rapid filtration. A solution consisting of 0.5 ml of each of these reagents in 100 ml of water was employed in the washing of the residue after centrifuging. It was necessary to prepare the potassium ferrocyanide solution freshly in order to avoid the formation of significant amounts of ferricyanide ion. (Low results obtained with a ferrocyanide solution which had been standing at room temperature for 2 weeks were attributed to the oxidation of ferrocyanide to ferricyanide ion which subsequently interfered with the determination of the reducing sugars.)

Schirokow and Milowidowa (3) recommended the use of 10% hydrochloric acid under reflux for the hydrolysis of potato starch. In this investigation more satisfactory results were obtained by conducting the hydrolysis in an open centrifuge bottle. This procedure resulted in an increase in the concentration of acid during the period of hydrolysis, and this higher concentration for a short time resulted in a more complete hydrolysis of the starch without any increase in the destruction of glucose.

It has been assumed that Schirokow and Milowidowa (3) employed a solution consisting of 10% by volume of concentrated hydrochloric acid or approximately 1.2 *N*, although the original publication is not clear on

this point. To determine the effect of acid concentration on the hydrolysis of wheat flour and potato starch, 0.4 gram of the carbohydrate material was added to 9.6 grams of ground meat and the samples were carried through the entire procedure with both 1.2 *N* and 1.5 *N* hydrochloric acid. The results are shown in Figure 1. These data indicate that higher results are obtained with 1.5 *N* hydrochloric acid, and that optimum values are returned after approximately 1.75 hours. Therefore, these conditions were employed in all subsequent experiments. It should be pointed out that

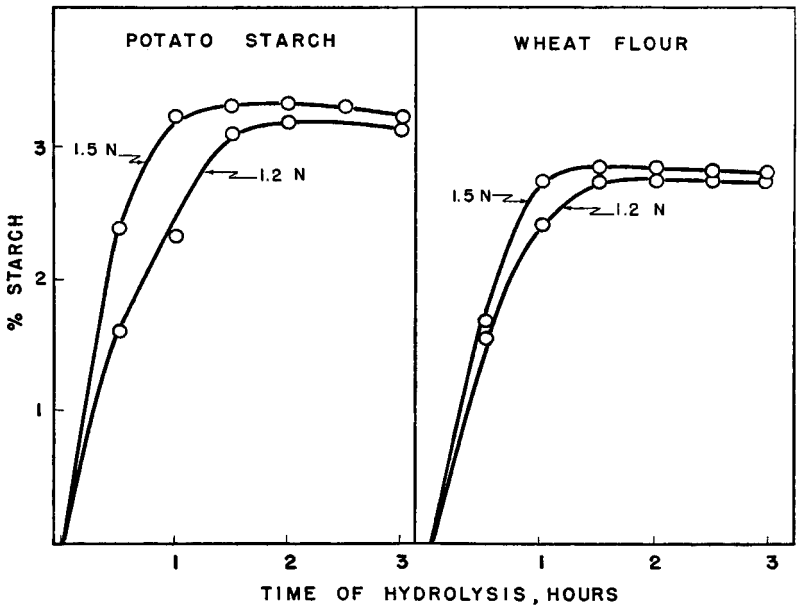


FIG. 1.—The effect of acid concentration on the hydrolysis of potato starch and wheat flour in the presence of meat.

there is some destruction of glucose during the hydrolysis and that the optimum values given for 1.5 *N* hydrochloric acid represent only about 96.5 and 95.3% of the total carbohydrate content of the potato starch and wheat flour, respectively. However, improved recoveries were obtained by some collaborators, and hence it appears that the conditions of hydrolysis require further investigation.

As previously indicated, it was necessary to remove all traces of sugars and dextrins from the sample prior to hydrolysis in order to avoid high values for starch. Three extractions, with 100 ml of solution for the first extraction, and two additional washings with 25 ml, were required. It was necessary to agitate the suspension continually in order to obtain complete removal of the sugars. This agitation could be achieved by

mechanical shaking or stirring, or by hand-shaking. Since equipment for mechanical shaking or stirring might not be available, shaking by hand was adopted, and it was found that intermittent shaking for an initial period of 15 minutes followed by two 10-minute periods was satisfactory. Since it was found on repeated determinations on meat samples that a blank titration of 0.15 ml was obtained, this correction has been incorporated in the formula for the calculation of percentage starch. On the basis of the foregoing considerations the following method was developed.

METHOD

REAGENTS

(*a*) *Zinc acetate soln.*—Dissolve 30 g of $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ in water and dilute to 100 ml.

(*b*) *Potassium ferrocyanide soln.*—Dissolve 15 g of $K_4Fe(CN)_6 \cdot 3H_2O$ in water and dilute to 100 ml.

(*c*) *Copper sulphate soln.*—Dissolve 40.0 g of $CuSO_4 \cdot 5H_2O$ in water and dilute to 1 liter.

(*d*) *Alkaline tartrate soln.*—Dissolve 200 g of Rochelle salt and 150 g of NaOH in hot water, filter, and dilute to 1 liter.

(*e*) *Standard dextrose soln.*—Dissolve 0.40 g of pure dextrose in water and dilute to 200 ml.

(*f*) *Starch indicator soln.*—Mix 1 g of powdered starch with 20 ml of cold water. Pour the mixture into 500 ml of boiling water and boil for 10 min.

(*g*) *Phosphotungstic acid soln.*—Dissolve 20 g of phosphotungstic acid in water and dilute to 100 ml. Filter.

DETERMINATION

Extraction and hydrolysis.—Weigh 10 g of finely comminuted and thoroly mixed sample into a 250 ml heat-resistant centrifuge bottle. Add 100 ml of water, 2 ml of zinc acetate soln and 2 ml of potassium ferrocyanide soln. Stopper tightly, extract the sample by shaking vigorously for 1 min., and allow to stand for 2 min. Repeat the shaking and standing periods five times. Then centrifuge for 15 min. at 1500 r.p.m. Decant thru a No. 54 Whatman or similar filter paper, retaining as much of the residue as possible in the centrifuge tube. To the residue add 25 ml of a freshly-prepared soln containing 1 ml of the zinc acetate soln and 1 ml of the potassium ferrocyanide soln in 200 ml. Shake vigorously for 1 min. and allow to stand for 2 min. Repeat 3 times. Centrifuge for 10 min. at 1500 r.p.m., and decant thru the same filter paper. Repeat this last extraction with a further 25 ml of the zinc acetate-potassium ferrocyanide washing soln. Rinse stopper with water.

After centrifuging and filtering, transfer filter paper with residue to the centrifuge bottle and add 90 ml of 1.50 *N* HCl. Suspend bottle in an open boiling water bath so that the level of water in the bath is ca the level of the soln within the centrifuge bottle. Do not reflux. Hydrolyze for exactly 1.75 hours, maintaining the water level of the water bath at its original position and stir the contents of the bottle occasionally.

Cool immediately. Make just alkaline to litmus with 20% NaOH soln (about 27 ml) and then add 10 ml of 1+2 HCl. Transfer soln to 200 ml phosphoric acid flask, rinse centrifuge bottle with 15 ml of phosphotungstic acid soln, followed by several 10 ml portions of water. Make up to volume with fat layer, if any, just above the 200 ml mark. Stopper, shake, and allow to stand for ca 30 min. Filter thru a Whatman No. 1 or similar filter paper.

Determination of reducing sugars.—Pipet 20 ml of filtrate into a 200 ml heat-resistant Erlenmeyer flask. Add 20 ml of the copper sulphate reagent and 20 ml of the alkaline Rochelle salt reagent. Hold flask over burner in an inclined position and heat to boiling. Boil for ca 1 min. Cool immediately under cold running water, transfer to a 200 ml volumetric flask, make up to volume with water, stopper, and shake.

Pipet 40 ml of the foregoing soln into a 125 ml Erlenmeyer flask. Add 20 ml of 10% potassium iodide soln and 4 ml of 1+3 H_2SO_4 soln. Titrate with ca 0.025 *N* $Na_2S_2O_3$ soln, adding 4 ml of the starch indicator and ca 0.5 g of solid ammonium thiocyanate when the yellow iodine color has almost disappeared. One drop of the $Na_2S_2O_3$ soln should change the color from blue to white or a faint lilac shade for at least 1 min. Carry out a determination on the reagents using 20 ml of water instead of the filtrate, starting at "Determination of Reducing Sugars." Similarly conduct a determination on 20 ml of the standard dextrose soln, starting at the same point.

Calculate percentage of starch by following formula:

$$\frac{(A-0.15)-B}{(A-0.15)-(C-0.15)}\times 4\times 0.9 = \text{per cent starch},$$

where

A = Reagent titration in ml.
B = Sample titration in ml.
C = Standard dextrose titration in ml.
0.15 = The average blank obtained on meat samples carried through the entire procedure.
4 = 20 ml of the standard dextrose solution represents 4% of dextrose.
0.9 = Factor to convert dextrose to starch.

RESULTS

A number of materials which might be employed as meat binders were added to meat samples at a level of 5 per cent, and the percentage of starch was determined by the above procedure. Dextrose, sucrose, dextrin, and soya flour were also included in the experiment. A standard value was obtained for each material by omitting the extraction procedure and determining reducing sugars after hydrolysis. The results are shown in Table 1. These data indicate a satisfactory recovery of the starch

TABLE 1.—*Recovery of carbohydrate materials added to meat calculated as starch*

MATERIAL	STANDARD	SAMPLE	RECOVERY
	per cent	*per cent*	*per cent*
Wheat Flour	3.56	3.52	98.8
Wheat Starch	4.23	4.28	101.2
Modified Wheat Starch			
A	4.19	4.11	98.1
B	4.34	4.37	100.7
Potato Starch			
A	4.28	4.34	101.4
B	4.28	4.37	102.1
Tapioca Starch	4.36	4.38	100.4

with an average value of 100.4 per cent. It should be pointed out, however, that while there was no loss of starch through the extraction procedure, this experiment gave no indication of the destruction which might occur during hydrolysis.

Zero values were obtained with meat samples to which 5 per cent of dextrose, sucrose, buttermilk powder, skim milk powder, dextrin, and soya flour had been added. Pork and beef liver were also carried through the procedure, and no reducing sugars were found as compared to approximately 10 per cent prior to extraction. These results indicated that the foregoing procedure gave satisfactory recoveries with starch-containing materials. Carbohydrates other than starch were completely removed.

Therefore, the method was submitted to collaborative study. Samples of potato starch, wheat flour, and skim milk powder were sent to collaborators with instructions to add these materials to ground meat at levels of 2.5 and 5%. The samples of wheat flour and potato starch were found to contain, on analysis and calculation of carbohydrate by difference, 74.5 and 86.0% of starch, respectively. The starch content of the milk powder sample was, of course, zero but contained approximately 50 per cent of lactose. The results of the collaborative study are given in Table 2. These data indicate that satisfactory values can be obtained by the proposed method. However, there appear to be some factor or factors which are not adequately controlled, for while there was reasonable agree-

TABLE 2.—*Results of collaborative study*

MATERIAL	STARCH ADDED	STARCH FOUND, PER CENT				AVERAGE STARCH	RECOVERY
		ANALYST					
		1	2	3	4		
	per cent					*per cent*	*per cent*
Wheat Flour	3.73	3.49	3.76	3.60	3.53	3.62	97.1
		3.54	3.76	3.64	3.62		
	1.86	1.68	1.88	2.01	1.55	1.77	95.2
		1.79	1.84	1.92	1.51		
Potato Starch	4.30	4.22	4.32	4.64	4.53	4.46	103.7
		4.19	4.36	4.74	4.71		
	2.15	2.14	2.12	2.35	2.05	2.14	99.5
		2.06	2.04	2.39	2.00		
Skim milk Powder	0	0.0	0.0	0.23	0.0	—	—
		0.02	0.0	0.18	0.0		
	0	−0.02	0.0	0.04	0.0	—	—
		0.0	0.0	0.04	0.0		

ment within a laboratory, significant differences appeared between laboratories. Collaborator No. 2, who obtained the most satisfactory results, stated that he had used a glass rod to break up the samples at the beginning of the hydrolysis. Subsequent work has indicated that the variations reported may be attributed to (a) the use of potassium ferrocyanide and zinc acetate solutions which had been allowed to stand for more than 3 days and (b) a prolonged period of standing prior to hydrolysis.

RECOMMENDATIONS*

It is recommended that this method be given further study. Particular attention should be paid to the possibility of shortening the lengthy extraction procedure and improving the conditions of hydrolysis.

The assistance of the following collaborators is gratefully acknowledged.

C. E. Hynds, State Food Laboratory, Albany, New York.

F. J. Stevens, Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada.

J. T. Janson, Division of Chemistry, Science Service, Department of Agriculture, Ottawa, Canada.

Ernest S. Windham, Army Medical Service Graduate School, Army Medical Center, Washington 12, D. C.

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REPORT ON CREATIN IN MEAT PRODUCTS

BY J. M. MCCOY (Meat Inspection Division, Bureau of Animal Industry, Department of Agriculture, Washington 25, D. C.), *Associate Referee*

Results of collaborative tests of a direct method for the determination of creatin in meat extracts are reported.[1]

Two samples of meat extract and one sample of an aqueous solution of creatin were sent to six collaborators for analysis by the proposed method. (See Table 1.)

Sample A consisted of 5 g of a sample of imported meat extract dis-

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* For report of Subcommittee C and action of the Association, see *This Journal*, **35,** 52 (1952).
[1] EGGLETON, ELSDEN, and GOUGH, *Biochem. Jour.*, **37,** 526 (1943).

solved in water, made up to 1000 ml, and 0.5 ml of oil of cassia added as a preservative.

Sample B, same as A, except from a different lot of meat extract.

Sample C consisted of an aqueous solution containing 40 micrograms of creatin per ml.

The method employed and a tabulation of results reported by collaborators are given below. The data indicate that while the method shows promise, further refinement will be necessary before consistent results can be expected.

TABLE 1.—*Creatin sample—Results*

ANALYST	A	B	C
A	250 p.p.m.	80 p.p.m.	41 p.p.m.
	263	84	37.8
	263	86	39
	258.7 ave.	83.3 ave.	39.3 ave.
B	260	88	36
	260	90	36
	260	89	37
	260 ave.	89 ave.	36.3 ave.
C	227	72	37
	230	70	35
	240	72	36
	232 ave.	71 ave.	36 ave.
D	226	71	38
	224	70	38
	230	74	38
	227 ave.	72 ave.	38 ave.
E	268	78	40
	268	79	40
	274	79	40
	270 ave.	78.7 ave.	40 ave.
F	270	86	41
	270	86	40
	285	86	40
	273 ave.	86 ave.	40.3 ave.

DETERMINATION OF CREATIN

REAGENTS

Diacetyl.—1% soln diluted 1 to 10 before use.

Stock alkali.—30 g NaOH plus 80 g Na_2CO_3 in 500 ml H_2O.

Alpha naphthol (purified by steam distillation).—2% in stock alkali soln (prepared immediately before use).

Standard soln.—20 micrograms creatin per ml.

METHOD

To a 25 ml volumetric flask add aqueous soln containing not more than 200 micrograms creatin. Add 5 ml alpha naphthol reagent, followed by 1 ml diacetyl reagent. Shake and allow to stand 15 min., make up to volume with water, mix, and read density immediately at 525 millimicrons on spectrophotometer, setting instrument to zero with blank consisting of 5 ml alpha naphthol reagent and 1 ml diacetyl reagent made up to 25 ml with water. Determine creatin present by comparison with standard curve.

PREPARATION OF STANDARD CURVE

To a series of 25 ml volumetric flasks add 0, 1, 2, 3, 4, and 5 ml standard creatin soln and proceed as above. Plot density *vs* concentration of creatin in micrograms. The resulting curve should be a straight line passing thru the origin.

It is recommended* that further work be done with the proposed method for the direct determination of creatin in meat products.

No report was given on defatted milk products in meat products or on horsemeat in ground meats.

REPORT ON GUMS IN FOODS

By M. J. Gnagy (Food and Drug Administration, Federal Security Agency, Los Angeles, California), *Referee.*

The Associate Referee on gums in catsup and related products has presented a progress report showing much promise of success. The method, which involves the use of a pectinase to destroy the pectin is about ready for collaborative work.

The Associate Referee on gums in starchy salad dressings has submitted a method for the detection of gum acacia, gum karaya, gum tragacanth, locust bean (carob bean) gum, and guar gum in amounts as low as 0.1 per cent. His work is supported by collaborative study. The Referee concurs in the Associate Referee's recommendation.

No reports were received from the Associate Referee on alignates in soft curd cheese and the Associate Referee on gums in cacao products. At the present time there is no Associate Referee on gums in frozen desserts.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 52 (1952).

RECOMMENDATIONS*

It is recommended—

(1) That the method for the detection of gums in catsup and related products be submitted to collaborative study.

(2) That the method for the detection of gums in starchy salad dressing be adopted, first action.

(3) That work be done by the Associate Referee on gums in cheese on the detection of algin.

(4) That work be continued on the detection of gums in cacao products.

(5) That the subject, detection of gums in frozen desserts, be assigned.

(6) That work be continued on detection of the other emulsifying agents which are permitted by the Federal standard for salad dressing.

REPORT ON GUMS IN CATSUP AND RELATED TOMATO PRODUCTS

By Theron E. Strange (U. S. Food & Drug Administration, Federal Security Agency, Portland, Oregon), *Associate Referee*

The detection of added gums in catsup and related tomato products is complicated by the presence of naturally occurring pectinous materials. Also, the high concentration of fibrous matter in catsup mechanically interferes with the removal of gums. A review of the literature on the general subject of gums in foods disclosed very little information that might be helpful in attacking this problem. It was the suggestion of the Referee on Gums in Foods that a promising approach to the problem would be to utilize a pectinase to destroy the pectinous materials then to precipitate and purify the gums in the usual manner.

Following the receipt of a communication from Glenn H. Joseph (California Fruit Growers Exchange, Research Dept., Corona, Calif.), suggesting that the pectin might be destroyed by digestion with about 10% of Pectinol 10-M at about *p*H 4.0 and 40°C., a quantity of the pectinase was obtained from the Rohm & Haas Co., and a limited investigation into the various phases of the problem was undertaken, utilizing a sample of catsup that was on hand and was assumed to be gum-free, since the solids were relatively high (30–35%). Tragacanth gum was selected for the study because it is commonly used and is somewhat more difficult than some of the other gums to recover.

Some observations of the Associate Referee as a result of the investigation to date are:

A. *Extraction of added gums from catsup.*

It appears highly desirable, if not essential, to extract the catsup first with a volatile fat solvent. Zylene or benzene followed by petroleum ether

* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 52 (1952).

appear to be satisfactory. Residual solvent is removed by heating, followed by extraction of the gum with water.

B. *Digestion with pectinase.*

An apparently satisfactory digestion was obtained in 50 ml. volume with 0.25 g of Pectinol 10-M at *p*H 4.0 and 40°C., overnight. There may be some destruction of tragacanth, but a positive test was obtained on a 0.1% solution.

C. *Separation of gum.*

Apparently adequate purification was obtained by alcohol precipitation and trichloracetic acid treatment, as in the method for gums in soft curd cheeses, *This Journal*, **34**, 71 (1951).

D. *Detection of gum.*

The following tests and reagents were found satisfactory:

Bial's Orcinol Test (probably the best).—Physical and Chemical Methods of Sugar Analysis, Browne and Zerban, page 716.

Phloroglucinol Test.—Ibid., page 715.

Benzidine Test.—Ibid., page 722.

Benedict's Test for reducing sugars.

The new organic reagent anthrone ("Microne" brand used).[1]

The Associate Referee has succeeded in clearly and unmistakably detecting by the following method the presence of tragacanth gum when 0.1 g was dispersed in 100 g of catsup. Based on very limited investigation, it is hoped that acacia, karaya, and carob bean gums, carboxymethylcellulose, and sodium CMC may be detected in like manner. Algin and gum ghatti have not been studied, but the method is probably not applicable to algin.

Method for Gums in Catsup

APPARATUS

(1) *Stirring device.*—Motor stirrer equipped with a circular disk propeller of diameter permitting insertion thru the top of a 250 ml centrifuge bottle, with the pitch of the propeller vanes and speed of the motor adjusted to give vigorous agitation to the interface between sample and solvent without surface turbulence.

(2) *A 2-hole rubber stopper,* (to fit the 250 ml centrifuge bottles, equipped with two glass tubes, one bent and inserted just thru the stopper, and the other inserted well down into the centrifuge bottle and constricted so as to create a partial vacuum and at the same time vigorously ruffle the surface of the gum soln when the bent tube is connected to a suction line.

(3) *Boiling water bath.*—A large beaker or other container with a basket or other means of holding centrifuge bottles clear of the bottom.

(4) *Electric incubator,* or other means of maintaining a temp. of 40°C. overnight.

REAGENTS

Volatile solvents.—Benzene, Zylene, petroleum ether.

Diatomaceous filter aid.—Supercel, Celite, etc.

[1] Scientific Apparatus and Methods (Vol. 4, Winter 1950–1951, page 31), E. H. Sargent & Co.

Alcohol.—95%.

Alcohol.—70%.

Acetic acid.—Reagent, glacial.

Pectinase.—Pectinol 10-M, Rohm & Haas Co., Special Products Dept., Washington Square, Philadelphia 5, Pa.

Trichloracetic acid soln.—50%.

Potassium aluminum sulfate soln.—5%.

Hydrochloric acid.—Reagent, concentrated.

Sodium hydroxide soln.—10%.

Benedict's soln.—*This Journal*, **34**, 71 (1951).

Bial's orcinol soln.—Physical & Chemical Methods of Sugar Analysis, Browne and Zerban, page 716.

Phloroglucinol soln.—*Ibid.*, page 715.

Benzidine soln.—*Ibid.*, page 722, but diluted to 1 g in 100 ml soln.

Anthrone reagent.—"Microne" brand, 0.2% soln in conc. sulphuric acid.

TREATMENT OF SAMPLE

Weigh 100 g of catsup into 250 ml centrifuge bottle, add about 30 ml water and 50 ml zylene or benzene. Insert propeller of stirrer to a point below the interface of liquids and stir about 15 min. or until sample and solvent form a uniform heterogeneous mixture but not an emulsion. Counterbalance and centrifuge. Siphon off solvent layer. Add 50 ml zylene or benzene, stir, centrifuge, and siphon off solvent layer. Repeat extraction, using 50 ml petroleum ether. Heat carefully with stirring in hot water bath (finally at boiling) to drive off residual solvent. (If this is carefully done, the pulp will settle to the bottom, leaving an almost clear upper liquid layer when centrifuged.) Centrifuge, and siphon off liquid layer. Add two 20 ml portions of water, stirring well, centrifuging, and siphoning off liquid layer after each addition. If light solid matter swirls, it will prove helpful to add a small quantity (1–2 ml) of Supercel to the centrifuge bottle, swirling it gently a few times and re-centrifuging. Combine the aqueous extractions (probably about 130–160 ml) in a beaker of suitable size and precipitate with 4 volumes of alcohol. Let stand overnight or until clear.

ENZYME DIGESTION

Decant the supernatant liquid and transfer residue with aid of stirring rod and jet of 70% alcohol to the 250 ml centrifuge bottle in which catsup was extracted, after thoroly rinsing out tomato material with hot water but without wiping or drying interior of bottle. Counterbalance, centrifuge, and decant liquid. Add 40–50 ml hot water to the precipitating beaker to remove any remaining residue and (reheat if necessary) transfer while still hot to centrifuge bottle containing the alcohol precipitate. Insert the 2-hole stopper equipped with glass tubes as described under "Apparatus," connect with suction and place bottle in boiling water bath. (This device has proved quite effective in removing residual alcohol and dispersing gums. Heat only long enough to accomplish this.) Cool, adjust *p*H if necessary to about 4.0 with acetic acid (or with sodium acetate, if already too acid), add pectinase (about 0.25 g Pectinol. 10-M), and digest overnight at 40°C. (about 16 hours).

SEPARATION OF GUM

After digestion, the centrifuge bottle will be found to contain a clear brown soln with a brownish sediment. Add 1–2 ml Supercel, swirl to mix, centrifuge, and decant liquid carefully into volumetric cylinder. Rinse out centrifuge bottle with hot water as before, without wiping or drying interior. Return the gum soln to the centrifuge bottle, rinsing out cylinder with a few ml of hot water, and precipitate

with 4 volumes of alcohol. Keep the total volume such that the centrifuge bottle will contain it. Let stand until well coagulated, then centrifuge and decant liquid.

Continue as in official method for gums in soft curd cheeses, *This Journal*, **34** (1951) the last paragraph bottom of page 71, beginning: "Add to the residue in bottle about 50 ml 70% alcohol, ..."

DETECTION OF GUM

Same as above method. In addition, test with Bial's Orcinol Reagent, using 5 drops of hydrolyzed gum soln, Phloroglucinol reagent, and benzidine reagent. In making this latter test neutralize 1 ml of hydrolyzed gum soln, dilute to 5 ml with water, and use 0.25 ml for the test. Heat to boiling with 2 ml benzidine reagent about 2 min. in test tube, then add a few drops of water to clear. Beware violent bumping and spattering!

Add 10 ml 0.2% anthrone soln to 0.2 ml hydrolyzed gum soln made to 5 ml with water. Results obtained by the Associate Referee using the above tests are as follows:

Test	*Catsup containing 0.1% Tragacanth*	*Catsup Blank*
Benedict's	Red p.p.t. medium	Slight or no reduction
Bial's orcinol	Vivid, rich green soln.	Yellow soln.
Phloroglucinol	Intense red	Shades of yellow to brown
Benzidine	Rich red soln.	Shades of tan to brown
Anthrone	Vivid green soln.	Faintly greenish yellow

This method will be subjected to further study and if satisfactory results appear likely it will be submitted for collaborative study.

ACKNOWLEDGMENTS

The Associate Referee gratefully acknowledges the helpful information and advice given by F. Leslie Hart, former Referee on Gums in Foods, U. S. Food & Drug Administration, Los Angeles; E. W. Coulter, U. S. Food & Drug Administration, Chicago, Ill.; Glenn H. Joseph, Calif. Fruit Growers Exchange, Research Dept., Corona, Calif.; A. K. Balls, U. S. Dept. of Agriculture, Agricultural Research Administration, Albany, Calif.; Frank C. Lamb, National Canners Assoc., Research Labs., San Francisco, Calif.; and R. W. Gormly, Rohm & Haas Co, Special Products Dept., Philadelphia, Pa. Mr. Gormly also kindly supplied the pectinase used in this investigation.

It is recommended* that the method described for the detection of gums in catsup and related products be submitted to further collaborative study.

* For report of Subcommittee C and action of the Association, see *This Journal*, **35**, 52 (1952).

REPORT ON GUMS IN STARCHY SALAD DRESSINGS

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

The last work done by the A.O.A.C. on the detection of gums in starchy salad dressings was performed by Redfern in 1946 (*This Journal*, **29**, 250). He dissolved out both the starch and the gums with calcium chloride solution and then separated the starch from the gum by precipitating out the starch with iodine. He then confirmed the presence of gums by use of basic lead acetate reagent, which he found to be the best universal test. The work of Redfern and all seven of his collaborators showed that 0.2 per cent of gum acacia, gum karaya, or gum tragacanth was easily detected. However, only one of the seven collaborators detected the presence of 0.1 per cent of locust bean gum, and one collaborator reported the presence of gum when no added gum was present. The Associate Referee at the time did not recommend adoption of the method but suggested (1) that additional confirmatory tests for gums be investigated, and (2) that the method be again submitted to collaborative study when the proper confirmatory tests have been developed.

The present Associate Referee was a collaborator on the method in 1946. Since at the time he failed to find 0.1 per cent of locust bean gum in the unknown sample, but could find it in the same unknown sample after he had added 0.1 per cent of such gum to the sample, he decided (1) to rewrite the method to make operations more specific and (2) to insure that 0.1 per cent of any gum was present by weighing it out on an analytical balance and incorporating it in the 50 g sample to be analyzed, rather than trusting that a 50 g portion taken from a large lot mixed at one time contained the required amount of gum.

EXPERIMENTAL WORK

In preliminary work the present Associate Referee was able to detect the presence of 0.1 per cent of gum acacia, gum karaya, gum tragacanth locust bean gum, and guar gum by using the basic lead acetate test. When he used Benedict's solution, he secured positive tests upon the same samples containing the above-mentioned gums. He also secured negative tests with both the basic lead acetate reagent and the Benedict's solution when he tested samples of the salad dressing without any added gum. The Associate Referee then decided to send out collaborative samples containing 0.1 per cent of the previously mentioned gums, except guar gum.

DESCRIPTION OF COLLABORATIVE SAMPLES

The Associate Referee prepared a salad dressing which contained cottonseed oil, 37.7 per cent; egg yolks (10% salt), 5.3 per cent; 100 grain white vinegar, 0.9 per cent; 50 grain cider vinegar, 0.3 per cent; spice mix (salt,

sugar, mustard, garlic powder, and pepper) 1.3 per cent; water 3.1 per cent and paste, 51.4 per cent. The paste contained the following: sugar, 18.0 per cent; 100 gr. white vinegar, 12.1 per cent; 50 gr. cider vinegar, 5.4 per cent; salt, 2.9 per cent; cornstarch, 10.9 per cent; and water 50.7 per cent. The spice mix contained mustard and pepper, each equivalent to 0.3 per cent of the salad dressing, and garlic powder equivalent to 0.1 per cent of the salad dressing.

50 g portions were weighed into two-ounce glass jars. These were separated into five groups. 0.05 g of gum acacia was added to each jar of sample No. 1; no gum was added to jars of sample No. 2; 0.05 g of locust bean gum was added to each jar of sample No. 3; 0.05 g of gum karaya was added to each jar of sample No. 4; and 0.05 g of gum tragacanth was added to each jar of sample No. 5. The gum was weighed out on an analytical balance, placed in a 10 ml beaker, 5 ml of water added, the mixture stirred and the gums allowed to dissolve or gel. Then the portions were transferred to proper sample bottles, using 3–4 ml of water, and thoroughly mixed with the salad dressing in the jars.

Two jars from each of the five samples were sent out to each collaborator but the collaborator neither knew which jar contained the gum nor the quantity of gum. In addition, another jar of salad dressing, marked "Authentic Salad Dressing with no added gum," was sent to each collaborator. He was asked to examine the authentic with no added gum first and then the five samples in numerical order. The duplicate jars were to be used in case a repeat determination was necessary.

METHOD

The method sent out was essentially the same as the one here given. There were a few minor changes in technic suggested by the collaborators or a rewording to secure better clarification. The suggested method follows:

GUMS IN STARCHY SALAD DRESSINGS

REAGENTS

(a) *Calcium chloride soln.*—Density 1.2 at 20 C. If cloudy, allow soln to stand so insoluble matter may precipitate out and then filter.

(b) *Iodine soln.*—Dissolve 2 g of iodine and 6 g of KI in distilled H_2O and make to 100 ml.

(c) *Ethanol.*—95%.

(d) *Ethanol.*—70%.

(e) *Basic lead acetate.*—

(f) *Celite Filter Aid* or equivalent.

SEPARATION OF GUMS FROM STARCH

Defat 50 g of salad dressing by heating on steam bath in 250 ml beaker until fat separates, cool and extract with petroleum ether until last ether extract is colorless. Make alkaline with $MgCO_3$ (2–2.5 g), testing with Alkacid test paper. Heat mixture

in water bath at 80°C. until residual ether and CO_2 are expelled. Then add 100 ml of $CaCl_2$ soln and heat in boiling water bath for 30 min. with occasional stirring. Pour into 250 ml Pyrex centrifuge bottle, centrifuge and decant as much of supernatant material as possible into 250 ml separatory funnel. Add 10 ml of $CaCl_2$ soln to residue in bottle and shake well. Centrifuge and decant supernatant material as before into separatory funnel. Swirl funnel gently and allow oil to separate. Draw off all material below oil into another 250 ml Pyrex centrifuge bottle. Centrifuge and filter supernatant liquid thru 11 cm Büchner funnel with aid of Celite filter aid. The filter paper, Whatman No. 1 or equivalent, should be precoated with layer of filter aid. Collect filtrate in beaker within bell jar. Add 10 ml of $CaCl_2$ soln to residue in centrifuge bottle, shake well, centrifuge and decant supernatant material upon filter in Büchner funnel. Wash filter with sufficient $CaCl_2$ soln so that total amount of filtrate collected is ca 110 ml.

Add slowly and with stirring 20 ml of iodine soln to clear extract to precipitate starch-iodide and a small quantity of Celite filter aid to help filtration. (Iodine should be present in considerable excess over that amount required to quantitatively react with starch. Considerable amounts of reducing substances are present, which must be satisfied before starch can be quantitatively separated.)

Allow starch-iodide, which separates in finely divided condition to stand ca 1 hr and then filter off by suction thru 11 cm Whatman No. 1 or equivalent filter paper, precoated with adequate layer of Celite filter aid. Use wire screen under filter paper to aid filtration. Do not wash pad. (Test for excess iodine in filtrate should be made with starch iodide paper or starch soln. This test must be positive to insure removal of all starch.) To brown-colored filtrate add four volumes of 95% ethanol and let stand overnight. Centrifuge off precipitated crude gum. Wash twice with 70% ethanol. (If possible, gum should be transferred into centrifuge bottle, but in some cases gum adheres so firmly to wall of beaker that it can only be rinsed until washings are clear.)

Heat at 100°C. until alcohol is removed and dissolve residue in 20 ml of H_2O by heating in water bath until no more material will dissolve. Use rubber policeman to assist solution. (Be sure gum is dissolved or it will be lost here.) Centrifuge to remove any insoluble material. Decant supernatant liquid into another 250 ml centrifuge bottle, add one drop of glacial acetic acid, one drop of $CaCl_2$ soln and reprecipitate with four volumes of 95% ethanol. Allow to stand at least one hour or overnight. Centrifuge and wash precipitate twice with 70% ethanol by shaking well and centrifuging.

Again drive off alcohol with aid of gentle stream of air by heating in hot water bath and dissolve precipitate in 10 ml of hot water, using rubber policeman. (Heed warning in preceding paragraph.) Centrifuge to remove any insoluble matter and decant into 50 ml heavy duty pyrex centrifuge tube. (Short cone type is less liable to break.) Adjust volume to 10 ml, add one drop of glacial acetic acid, one drop of $CaCl_2$ soln, and reprecipitate with 40 ml of ethanol. Allow to stand one hour, centrifuge, and wash with 70% ethanol as before. Heavy flocculent precipitate at this point indicates presence of gums. (Very small quantity of precipitate adhering to walls of centrifuge tube or appearing as mere turbidity is to be disregarded, as spice gums present in most salad dressings will usually give such precipitate.)

DETECTION OF GUM

To confirm presence of gums, remove residual alcohol by gentle heating in hot water bath, dissolve residue in 10 ml of hot water, and centrifuge to remove any insoluble material. Decant supernatant liquid into 10 ml graduated cylinder, make up to 10 ml with distilled water and mix. To one ml of this soln add one or two drops of basic lead acetate reagent (reagent 4), one drop at a time. An immediate floc-

culent, curdy or gelatinous precipitate is confirmation of presence of gums. A precipitate may form on standing but this is to be disregarded.

In an endeavor to ascertain whether all the starch was eliminated by the starch-iodide test, the collaborators were asked to make the following test using Benedict's solutions (not part of method):

Transfer by pipet 5 ml of soln from 10 ml graduated cylinder to 50 ml beaker. Add 5 ml of H_2O and 2 ml of conc HCl. Cover beaker with watch glass and boil gently 5 min. Cool and transfer to 10 ml graduated cylinder. Adjust to 10 ml with H_2O and mix. Place 2 ml aliquot in 30 ml beaker and neutralize with NaOH soln using litmus paper as indicator. Remove litmus paper, add 5 ml of Benedict's soln and boil vigorously 2 min. Allow contents of beaker to cool spontaneously. A precipitate appearing upon cooling, which may be yellow, orange or red, caused by reducing sugars formed by hydrolysis of gums, indicates presence of gums.

RESULTS

The results obtained by the collaborators are shown in Tables 1 and 2.

COMMENT BY COLLABORATORS

David Menschenfreund.—Suggested change in procedure in the separation of gums from starch: After adding $CaCl_2$ soln and heating in boiling water I would suggest: "Pour entire contents in 250 ml centrifuge bottle and centrifuge. Separate oil from aqueous layer (allowing as much emulsion as possible to be included) by pouring into 250 ml separatory funnel. Then centrifuge at higher speed (2400 r.p.m.) the remainder of emulsion and semi-solid material in centrifuge bottle. Add to separatory funnel, including as much of viscous material as possible. Further wash remainder of material in bottle with 15 ml of $CaCl_2$ soln. Centrifuge and add as much of liquid as possible to separatory funnel. Draw off all but fat into new centrifuge bottle and centrifuge. Decant on prepared filter paper. Note volume of filtrate and wash residue in bottle with 10 ml of $CaCl_2$ soln. Shake and centrifuge. Filter and make up to 110 ml." In this way a better separation of oil is obtained and more gum is extracted.

The procedure in the method requires care and accurate manipulation. Little difficulty is involved if it is done so.

S. H. Perlmutter.—No difficulties were encountered. My suggestions are: (1) In preparation of basic lead acetate solution, *Methods of Analysis*, 1950, **29.18 (a)**, state either the long method or the short method. Suggest you specify which; (2) Suggest you state how long Benedict's solution may be stored before fresh solution must be prepared. Suggest, "Keeps indefinitely"; (3) Suggest you state a percentage of $CaCl_2$ solution rather than a density at 20°C.; (4) Suggest rewording of first paragraph in method to include type of vessel in which sample is defatted, like "Defat 50 g of salad dressing by heating on steam bath in 250 ml beaker until fat separates, cool and extract . . . "; (5) In the case of sample 1, overnight was not a sufficient length of time for the gums to precipitate out. Suggest you state "Overnight or until gums precipitate out"; (6) In last paragraph you state "Heavy flocculent precipitate, etc." Seems to me that, if no precipitate occurs at very first, one can infer absence of gums.

J. E. Roe.—No difficulties were encountered. However, when first gum precipitation was made in iodine solution, the volume was quite large. Rather than centrifuge, I siphoned off upper portion of liquid until about 200 ml remained. I then

TABLE 1.—*1951 Collaborative results on determination of presence of various gums in starchy salad dressing*

By use of basic lead acetate test

COLLABORATORS	SAMPLE KNOWN TO CONTAIN		SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
	NO ADDED GUM		0.1% GUM ACACIA ADDED		NO GUM ADDED		0.1% LOCUST BEAN GUM ADDED		0.1% GUM KARAYA ADDED		0.1% GUM TRAG. ADDED	
	LEAD ACETATE TEST	GUM	LEAD ACETATE TEST	GUM	LEAD ACETATE TEST	GUM	LEAD ACETATE TEST	GUM	LEAD ACETATE TEST	GUM	LEAD ACETATE TEST	GUM
C. E. Hynds, New York State Food Lab., Albany, N.Y.	No Precipitate	Absent	Flocculent Precipitate	Present	No Precipitate	Absent	Finely Divided Precipitate	Present	Gelatinous Precipitate	Present	Gelatinous, Gummy Precipitate	Present
David Menschenfreund, Food and Drug Admin. Los Angeles, Calif.	No Precipitate	Absent	Flocculent Precipitate	Present	No Precipitate	Absent	Bluish-gray Turbidity	Present	Flocculent Precipitate	Present	Gelatinous Precipitate	Present
S. H. Perlmutter, Food and Drug Admin. Minneapolis, Minn.	No Precipitate	Absent	Curdy	Present	No Precipitate	Absent	Slightly Flocculent	Present	Flocculent	Present	Slightly flocculent	Present
J. E. Roe, Food and Drug Admin. Denver, Colo.	No Precipitate	Absent	Flocculent Precipitate	Present	No Precipitate; Soln. Slightly Bluish	Absent	Gelatinous Precipitate	Present	Flocculent Precipitate	Present	Gelatinous Precipitate	Present
L. W. Ferris, Food and Drug Admin. Buffalo, N.Y.	No Precipitate	Absent	Heavy flocculent	Present	Very Fine	Absent	Very Fine	Absent*	Heavy flocculent	Present	No Precipitate	Absent
M. J. Gnagy, Associate Referee	No Precipitate	Absent	Curdy precipitate	Present	No Precipitate	Absent	Finely Flocculent	Present	Large, flocculent	Present	Small, flocculent	Present
M. J. Gnagy,† Associate Referee	No Precipitate	Absent	Curdy Precipitate	Present	No Precipitate	Absent	Finely Flocculent	Present	Large, flocculent	Present	Small, flocculent	Present

* Collaborator states "Lead acetate test gave a very fine precipitate which was so near like the blank that I called the test negative. This surprised me very much as the sample up to this point gave all indications of being positive."

† After being defatted, these samples stood in beakers for two weeks at room temperature before analyses were completed.

TABLE 2.—*1951 Collaborative results on determination of presence of various gums in starchy salad dressing*
By Use of Benedict's Test

COLLABORATORS	SAMPLE KNOWN TO CONTAIN		SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
	NO ADDED GUM		0.1% GUM ACACIA ADDED		NO GUM ADDED		0.1% LOCUST BEAN GUM ADDED		0.1% GUM KARAYA ADDED		0.1% GUM TRAG. ADDED	
	BENEDICT'S TEST	GUM	BENEDICT'S TEST	GUM	BENEDICT'S TEST	GUM	BENEDICT'S TEST	GUM	BENEDICT'S TEST	GUM	BENEDICT'S TEST	GUM
C. E. Hynds, New York State Food Lab., Albany, N.Y.	No Precipitate	Absent	Orange Precipitate	Present	No Precipitate	Absent	Orange Precipitate	Present	Small Amount Orange Precipitate	Present	Orange Precipitate	Present
David Menschenfreund, Food and Drug Admin. Los Angeles, Calif.	No Precipitate	Absent	Fairly Large Red Precipitate	Present	No Precipitate	Absent	Slight Red Precipitate	Present	Fair Amount Red Precipitate	Present	Fair Amount Red Precipitate	Present
S. H. Perlmutter, Food and Drug Admin. Minneapolis, Minn.	No Precipitate	Absent	Heavy, Yellow Orange Precipitate	Present	No Precipitate	Absent	Slight, Reddish Precipitate	Present	Slight, Reddish Precipitate	Present	Slight, Red Precipitate	Present
J. E. Roe, Food and Drug Admin. Denver, Colorado	No Precipitate	Absent	Medium Amount of Red Precipitate	Present	Large Amount of Red Precipitate	Present	Large Amount of Red Precipitate	Present	Large Amount of Red Precipitate	Present	Large Amount of Red Precipitate	Present
L. W. Ferris, Food and Drug Admin. Buffalo, N.Y.	Heavy Red Precipitate	Present	Heavy Red Precipitate	Present	Trace Red Precipitate	Absent	Red Precipitate	Present	Red Precipitate	Present	No Precipitate	Absent
M. J. Gnagy, Associate Referee	No Precipitate	Absent	Large, Red Precipitate	Present	No Precipitate	Absent	Medium Red Precipitate	Present	Medium, Red Precipitate	Present	Medium Amount Red Precipitate	Present
M. J. Gnagy,* Associate Referee	No Precipitate	Absent	Large, Red Precipitate	Present	No Precipitate	Absent	Medium Amount of Precipitate	Present	Medium Amount Red Precipitate	Present	Small Amount Amount Red Precipitate	Present

* After being defatted, these samples stood in beakers at room temperature for two weeks before analyses were completed.

centrifuged remaining portion .... One observation is that at every precipitation of gums with alcohol the amount of precipitate was noticeably larger in the five unknown samples than in the authentic sample. I have no suggestions to make.

L. W. Ferris.—Difficulties encountered were (1) very little fat was removed with petroleum ether; (2) could not determine neutral point with *p*H paper and magnesium carbonate.

Suggestions are: (1) that preliminary defatting be omitted; (2) that acidity of sample be determined by titration of small amount of product; (3) that the amount of magnesium carbonate needed to make sample alkaline be calculated; (4) that the magnesium carbonate and calcium chloride solution to dissolve the starch be added in one operation.

DISCUSSION OF RESULTS

In the use of the basic lead acetate to confirm the presence of gums four of the five collaborators secured the same results as the Associate Referee did upon the five unknown samples. They successfully confirmed the presence of gums in the four samples containing added gum and they correctly confirmed the absence of gum from the sample that had no gum added to it. The fifth collaborator, however, confirmed the presence of gum in two unknown samples and confirmed its absence in the unknown with no added gum. However, he called the test negative in the case of locust bean gum, although somewhat doubtfully. He did not confirm the presence of gum tragacanth in the unknown sample. It is difficult to explain the latter finding.

In the use of the Benedict's test three collaborators secured the same results upon the five unknown samples and the known authentic as did the Associate Referee. However, one collaborator secured a heavy precipitate on the unknown without added gum and another secured a heavy precipitate on the authentic without added gum. In the Benedict's test submitted, the sample was so diluted and consequently the natural gums in the spices were so diluted that the aliquot taken for the test produced negative results for gums. From the size of the precipitates obtained by the two collaborators one can conclude that all the starch was not eliminated by precipitation with iodine and the filtering off of the starch-iodide. This may have been due to a variation in technique on the part of the various collaborators and an attempt was made to correct this in the method by allowing for a longer period for the starch-iodide to stand before it was filtered. Since there is a possibility of some starch not being removed, the Benedict's test cannot be used as a confirmatory test.

CONCLUSION

The standards for salad dressing provide for the addition of not more than 0.75 per cent of gum acacia, carab bean gum, gum karaya, gum tragacanth, and guar gum, or a mixture of two or more of these gums. Judging from the results of the collaborative work this year and from results obtained by the Associate Referee upon all five gums including guar gum,

as well as the results obtained by the former Associate Referee in 1946, it would appear that the method submitted this year using basic lead acetate as a confirmatory test has a sensitivity of approximately 0.1 per cent for the five gums mentioned. This sensitivity is well within the standard that has been set.

RECOMMENDATIONS*

It is recommended—

(1) That the method given herein be made first action.

(2) That collaborative study be undertaken on the detection of the other emulsifying agents listed as permitted in the Federal standards for salad dressing.

No report was given on cheese (alginates); frozen desserts; or on cacao products.

REPORT ON ECONOMIC POISONS

By J. J. T. Graham (Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Dept. of Agriculture, Washington, D. C.), *Referee*

This year, for the first time, methods of piperonyl butoxide were studied under the direction of an Associate Referee. The Associate Referee on rodenticides included a study of methods for the new rodenticide, warfarin, and the results obtained are very encouraging.

Other products suggested as subjects for study include aerosol insecticides, allethrin, aldrin, and dieldrin; in view of their importance, the time is urgent for study of methods of analysis for insecticides containing these ingredients. Volunteers to act as Associate Referees will be welcomed.

RECOMMENDATIONS†

It is recommended—

(1) That the Toops and Riddick, and Arceneaux methods for determination of the gamma isomer of benzene hexachloride be studied, and that the Fairing and Phillips, and Schecter and Hornstein colorimetric methods for total benzene hexachloride be studied.

(2) That methods for determination of rotenone be continued.

(3) That the Davidow and Harris methods for determination of chlordane be studied.

(4) That the Elmore method adopted as first action in 1950, be further studied to investigate its applicability for determination of thiocyanate nitrogen in the ester type thiocyanates.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 35, 52 (1952).
† For report of Subcommittee A and action of the Association, see *This Journal*, 35, 44 (1952).

(5) That the ultra-violet absorbance of piperonyl butoxide and its interferences be further studied.

(6) That the partition chromatographic separation of piperonyl butoxide and its subsequent determination by the Jones colorimetric method be studied collaboratively.

(7) That collaborative work on determination of warfarin in concentrates be continued.

(8) That work be continued on the development of a procedure suitable for low percentage warfarin baits.

(9) That methods be studied for determination of physical properties of economic poisons, especially particle size and dispersability in aqueous and dry formulations.

(10) That the method for the determination of potassium cyanate in herbicides be adopted, first action, with the revisions suggested by the Associate Referee.

(11) That the method for the determination of esters of 2,4-dichlorophenoxy acetic acid as revised by the Associate Referee be subjected to further study.

(12) That the method for determination of total chlorine in liquid herbicides containing 2,4-D, 2,4,5-T, or mixtures of both in presence of oils and emulsions by a modified Parr bomb procedure, be subjected to further study.

(13) That the Olsen procedure be studied in connection with recommendations 11 and 12.

(14) The Referee concurs in the recommendations of the Associate Referee on pyrethrins.

(15) The Referee concurs in the recommendations of the Associate Referee on parathion.

(16) That a referee be appointed to study dimethyl dithiocarbamates.

(17) That the work of the other Associate Referees be continued.

REPORT ON BENZENE HEXACHLORIDE

By Irwin Hornstein (Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, Beltsville, Maryland), *Associate Referee*

During the past year two colorimetric methods for the determination of total benzene hexachloride have been reported. In addition a cryoscopic method for the determination of the gamma isomer content of lindane and a microscopic method for the qualitative identification of the several isomers have been described. The latter method may also prove useful for the quantitative determination of lindane.

DETERMINATION OF TOTAL BENZENE HEXACHLORIDE

(1) The colorimetric method developed by Fairing and Phillips (1) involves the refluxing of small amounts of benzene hexachloride (80–120 micrograms) with an excess of aniline. Their work shows that a dichlorodiphenylamine is formed, and it is this material which produces a violet color on oxidation with vanadium pentoxide in 50% sulfuric acid. The absorbancy is determined at 555 mμ. The method is specific for benzene hexachloride and is not subject to any appreciable interference from other chlorinated organic insecticides. In carrying out the analysis, fats, waxes, and oils must be removed before the reaction with aniline. It is also essential that a highly purified grade of aniline be used and that no moisture be present.

(2) The colorimetric method for total benzene hexachloride developed by Schechter and Hornstein (2) involves the dechlorination of benzene hexachloride to benzene by means of zinc in acetic acid. The benzene is then absorbed in a nitrating mixture and is converted to *m*-dinitrobenzene; these reactions are carried out in a specially designed all-glass apparatus which eliminates mechanical losses. The *m*-dinitrobenzene, after extraction with ether and removal of the solvent, is treated with methyl ethyl ketone in the presence of 40% potassium hydroxide. The violet-red color produced is determined photometrically at 565 mμ. As little as 5 micrograms of benzene hexachloride can be determined in this manner.

No other chlorinated organic insecticide has interfered in this method. In many cases the analyses can be run directly on the sample or an extract of the sample. If extraction is necessary, benzene or related aromatics should be avoided; methylene chloride, carbon tetrachloride, and acetic acid have been found to be suitable extraction solvents.

DETERMINATION OF THE GAMMA ISOMER IN LINDANE

The increased commercial production of lindane (at least 99% of the gamma isomer of benzene hexachloride) has emphasized the need for an accurate method for the determination of the gamma isomer in lindane.

(1) A melting-point depression method has been developed by Toops and Riddick (3) for the determination of the gamma isomer. They redetermined the freezing point of the gamma isomer, using a platinum resistance thermometer. Time-resistance readings were made at 1°C. intervals while a melt of the gamma isomer was cooling. With the onset of crystallization, these measurements were recorded at intervals of one minute. The time-resistance observations were plotted, and the equilibrium portion of the curve was extrapolated back to the zero time line to obtain the resistance corresponding to the thermodynamic freezing point. From these data a calculated freezing point of 112.86°C. was obtained for 100.00 mole per cent of the gamma isomer. From the freezing point of synthetic samples of the gamma isomer and the alpha and beta isomers, using the proper

thermodynamic equation, the cryoscopic constant was calculated to be 0.0148 mole fraction per 1° lowering of the freezing point for gamma-isomer concentrations of 99 per cent or higher.

Having obtained these values the gamma-isomer content of lindane was determined by a similar method. However, the freezing points were measured with a mercury thermometer constructed to the authors' specifications and calibrated for 100 mm immersion. The thermometer could be read to ± 0.01°C. The results showed an accuracy of about ± 0.05 mole per cent.

(2) A method of analysis combining a fusion technique and chemical microscopy has been developed by Arceneaux (4). A few milligrams of the material are placed between a slide and cover glass and gradually heated with a microflame until the material melts. The cover glass is pressed down to obtain a thin film of the melt and held until the material begins to crystallize. Observations are made between crossed Nicols in a polarizing microscope at low magnification. The fusion pattern differs for the isomers examined. The gamma isomer crystallizes in three different polymorphic forms. However, in preparations containing over 99 per cent of the gamma isomer a characteristic herringbone effect is observed. With concentrations lower than 99 per cent gamma the pattern is considerably more fibrous. The principal quantitative value of the fusion pattern, according to Arcenaux, is in determining whether a sample contains 99 per cent or more of the gamma isomer. This may provide a rapid control method for lindane.

It is proposed that these methods be examined and the more promising be studied on a collaborative basis.

REFERENCES

(1) FAIRING, J. D., and PHILLIPS, W. F. Paper given at the April 1951 Meeting of the American Chemical Society at Boston, Mass.
(2) SCHECHTER, M. S., and HORNSTEIN, I. *Anal. Chem.* **24,** 544 (1952).
(3) TOOPS, E. E., JR., and RIDDICK, J. A., *Ibid.* **23,** 1106 (1951).
(4) ARCENEAUX, C. J., *Ibid.*, **23,** 906 (1951).

REPORT ON PYRETHRINS

BY DAVID KELSEY (Insecticide Division, Livestock Branch, Production and Marketing Administration, Department of Agriculture, Washington 25, D. C.), *Associate Referee*

It has been found (1) that filtration for the removal of the barium sulfate precipitate, called for in the Official Mercury Reduction Method for determination of pyrethrins, has been responsible for low results for the amounts of Pyrethrin I present. It has been stated (2) that a definite possibility of loss exists, in this determination, by adsorption of monocarboxylic acid (chrysanthemic acid) on the barium sulfate precipitate.

From a barium sulfate precipitate filtered off from a pyrethrin determination, and washed in accordance with the directions given in the official method, Beckley (1) was able to extract an oily, bright yellow material which gave a very strong positive coloration with Denige's reagent after being carried through the normal analytical procedure for Pyrethrin I. It was shown that this material contained chrysanthemic acid, but results of tests to establish the insecticidal activity of the material have not yet been published.

In the world-wide collaborative analysis of pyrethrum flowers, carried out by the Standing Sub-Committee on Methods of Analysis of Vegetable Insecticides during 1948–49, and published in 1950, (3), one of the methods of analysis used was the mercury reduction method, with however, two modifications (4):

1. In the determination of Pyrethrin I, hydrochloric acid was used for neutralization of the saponifying alkali, instead of the sulfuric acid called for in the official method, thereby eliminating filtration of the barium sulfate precipitate.

2. In the determination of Pyrethrin II, neutralization of the filtrate with sodium bicarbonate, and the subsequent extraction with chloroform, were omitted.

Preliminary work by the Associate Referee confirmed the findings that higher results for Pyrethrin I could be obtained by the use of hydrochloric acid instead of sulfuric, and during 1951 samples of a commercial pyrethrum concentrate containing approximately 200 mg of pyrethrins per 100 ml were submitted to collaborators for analysis by the official mercury reduction method, paragraphs **5.114** and **5.112** of *Methods of Analysis*, 7th Ed., and by the same method with the following modifications, based on the method used by the Standing Sub-Committee:

(*Par.* **5.114**) Beginning with "To aq. soln in 250 ml flask, . . . " delete thru ". . . Filter thru 7 cm filter paper.", and substitute the following:

"To aq. soln in 250 ml flask, add 1 g of Filter Cel and 10 ml of the $BaCl_2$ soln. Swirl gently, allow the precipitate to settle, and test the supernatant liquid with a little more $BaCl_2$ soln to see if sufficient has been added to obtain a clear soln. Do not shake before making to vol. Make to vol., mix thoroly, and filter off 200 ml thru a fluted 12.5 cm filter paper, and transfer to a 500 ml separator. Add 2–3 drops of phenolphthalein indicator, **2.11 (d)**, neutralize with HCl (conc) and and 1 ml in excess. Add 5 ml of satd NaCl soln and ext. with two 50 ml and one 25 ml portions of petr. ether. Then proceed as directed under **5.111**, beginning "Wash exts with 2 or 3 10 ml portions of H_2O."

(*Par.* **5.112**) Beginning with "conc. filtrate to ca 50 ml, . . . " delete thru ". . . and dry residue at 100° 10 min.," and substitute the following:

"Conc. filtrate to ca 50 ml, transfer to 500 ml separator. Acidify with 10 ml HCl (conc) and sat. with NaCl. (Care must be taken that the acidified aq. layer is satd with NaCl thruout following extns.) Ext. with 50 ml of ether, draw off aq. layer into 2nd separator and ext. again with 50 ml of ether. Continue extn and drawing off of aq. layer, using 35 ml for 3rd and 4th extns. Combine 4 ether exts, drain, and wash with 3 10-ml portions of satd NaCl soln. Filter ether exts thru plug of cotton into 500 ml Erlenmeyer flask, and wash cotton with addnl 10 ml of ether. Evap ether on H_2O bath, and remove any fumes of HCl with current of air and continued heating. Dry in an oven at 100° for 10 min."

The results of this collaborative study are shown in Table 1.

TABLE 1.—*Comparative percentages of pyrethrin content*

(Commercial pyrethrum concentrate as determined by official A.O.A.C. method and by proposed modification)

COLLABORATOR	METHOD USED	PERCENTAGE PYRETHRINS (BY WEIGHT)[1]		TOTAL PYRETHRINS PERCENTAGE BY WEIGHT	MG PYRETHRINS PER 100 ML
		PYRETHRIN I	PYRETHRIN II		
		per cent	*per cent*	*per cent*	*milligrams*
A	Official	1.46	1.17	2.63	209
	Modified	1.64	1.23	2.87	228
B	Official	1.37	1.09	2.46	195
	Modified	1.63	1.08	2.71	215
C	Official	1.48	0.98	2.46	195
	Modified	1.46	0.97	2.43	193
D	Official	1.53	1.14	2.67	212
	Modified	1.87	1.15	3.02	240
E	Official	1.49	1.02	2.51	200
	Modified	1.64	1.12	2.76	219
Average values	Official	1.44	1.08	2.54	202
	Modified	1.65	1.11	2.76	219

[1] Average of two determinations.

These results show that the percentage of Pyrethrin I, as determined by Par. **5.114** of the official method, is substantially lower than the percentage obtained by using hydrochloric acid as proposed in the modification, which eliminates the filtration of the barium sulfate that is formed when sulfuric acid is used. They also show that the percentage of Pyrethrin II, as determined by Par **5.112** of the official method, is not changed by omission of the bicarbonate neutralization and chloroform extraction from the prescribed procedure. The time required for the determinations was reduced, by the proposed modifications, by 10 to 15 minutes in the case of Pyrethrin I, and up to 20 minutes for the Pyrethrin II determination.

RECOMMENDATIONS*

It is recommended—

(1) That the modifications of Par. **5.112** and Par. **5.114** of the Official Method, proposed in this report, be adopted, first action.

(2) That Par. **5.111** of the Official Method be modified as follows, and that the modifications proposed be adopted, first action:

* For report of Subcommittee A and action of the Association, see *This Journal*, 35, 44 (1952).

Beginning with "Do not shake before making to vol.," delete thru ". . . two 50 ml portions of pet. ether.," and substitute the following:

"Swirl gently, allow the precipitate to settle, and test the supernatant liquid with a little more $BaCl_2$ soln to see if sufficient has been added to obtain a clear soln. Do not shake before making to vol. Make to vol., mix thoroly, and filter off 200 ml thru a fluted 12.5 cm filter paper and transfer to a 500 ml separator. Add 2–3 drops of phenolphthalein indicator, **2.11 (d)**, neutralize with HCl (conc) and add 1 ml in excess. Add 5 ml of satd NaCl soln and ext. with two 50 ml and one 25 ml portions of pet. ether."

REFERENCES

(1) BECKLEY, V. A., Pyrethrum Board of Kenya, Kenya Colony, Africa. (Private communication to J. J. T. Graham).
(2) MARTIN and BRIGHTWELL, *J. Soc. Chem. Ind.*, 65, 397 (1946).
(3) Report of the Standing Sub-Committee on Methods of Analysis of Vegetable Insecticides on the World-Wide Collaborative Analysis of Pyrethrum Flowers, Consultative Committee on Insecticide Materials of Vegetable Origin, Colonial Products Advisory Bureau, (Plant and Animals), London, 1950.
(4) *Ibid.*, page 34.

COLLABORATORS

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REPORT ON ROTENONE

BY R. PAYFER (Plant Products Division, Department of Agriculture, Ottawa, Canada), *Associate Referee*

The question of the determination of rotenone is not a simple one, because of the complexity of the structure and the lack of information in the chemical literature.

As a preliminary to the determination of rotenone, a survey was made of the literature and references noted. U.S.D.A. Bulletin E-563, prepared by H. A. Jones, was studied and projects planned to conduct work this fall and winter.

It is a well known fact that the accuracy of analyses performed by the present official method, **5.106** (*Methods of Analysis*), depends on the experience of the analyst and the type of sample. There is no official method for liquid preparations.

It is recommended* that this work be continued along two lines:

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* For report of Subcommittee A and action of the Association, see *This Journal*, 35, 44 (1952).

(1) The use of chromatography on liquid preparations to recover the rotenone, which could then be determined by **5.106.**

(2) Continued study to attempt to find a new approach to the determination of rotenone.

REPORT ON RODENTICIDES

By J. B. La Clair (California State Department of Agriculture Bureau of Chemistry, Sacramento, Calif.), *Associate Referee*

With the release of warfarin (3-acetonyl benzyl-4-hydroxycoumarin) concentrates and prepared rat and mouse baits for sale to the general public, it is essential that methods of analysis for warfarin be available, so that these products can be checked for compliance with State and Federal laws.

Work done in the laboratories of K. P. Link, at the University of Wisconsin, Madison, demonstrated that warfarin could be quantitatively determined by utilizing the ultraviolet absorption maximum of the sodium salt or free acid at 308 millimicrons.

The Wisconsin Alumni Research Foundation procedure, suitable for warfarin concentrates, is as follows:

PROCEDURE I

Extract sample, containing up to 3 mg of warfarin, with 50 ml of ethyl ether by shaking 10 min. Transfer a portion of the extract to a centrifuge tube and centrifuge 10 min. Add a 2 ml aliquot of the clarified ether to 10 ml of 1% sodium hydroxide soln and 10 ml Skellysolve B and shake for 10 min. After centrifuging until clear, transfer a portion of the aqueous phase to a 1 cm quartz cuvette and determine the optical density at 308 millimicrons in a Beckman Model DU spectrophotometer, using 1% sodium hydroxide for the zero setting. Calculate the percentage of warfarin, using an extinction coefficient of 0.459 per mg warfarin per 100 ml of solution.

A modification of the Wisconsin method, U.S.D.A. Method No. 821.0 (Procedure II), was developed for the analysis of 0.5% warfarin concentrates in cornstarch. This modification is the subject of this year's collaborative rodenticide program.

Samples containing known amounts of warfarin in cornstarch were prepared by E. W. Ligon, Jr., U.S.D.A. Pharmacological and Rodenticide Laboratory, Beltsville, Maryland.

PROCEDURE II

WARFARIN, IN CONCENTRATES

MODIFIED METHOD FOR PRODUCTS CONTAINING ABOUT 0.5% WARFARIN IN CORNSTARCH (BASED ON THE UNIVERSITY OF WISCONSIN METHOD)

REAGENTS

(a) *Ethyl dichloride.*

(b) 1% *sodium hydroxide soln* (0.25 *N*).

DETERMINATION

Weigh 0.6 g sample into a 125 ml glass-stoppered flask, and add 50 ml of ethylene dichloride with a pipet. Shake on a shaking machine for at least 10 min. Transfer to a centrifuge tube, stopper, and centrifuge 5 min. at high speed, or until clear.

Pipet 2 ml of the ethylene dichloride extract into a glass-stoppered cylinder, add 10 ml of 1% NaOH soln **(b)** with a pipet, and shake for one min. by hand. Prepare a blank soln in the same manner, using 2 ml ethylene dichloride in place of the extract.

Decant the sodium hydroxide layer into a centrifuge tube, and centrifuge until clear. Pipet sufficient amount of the alkali layer (ca 3 ml) into a quartz cuvette (1 cm cell) and determine the optical density at 308 mμ and slit opening of about 0.86 mm, using a Beckman spectrophotometer, model DU.

CALCULATION

The optical density of a soln of 1 mg warfarin in 100 ml 1% sodium hydroxide was found to be 0.463, using the above procedure. If D is the optical density obtained

$$\% \text{ Warfarin} = \frac{D(10/100)(100)}{(0.463(1000)(.6)(2/50)} = D(.9000).$$

TABLE 1.—*Results of collaborative analyses of prepared concentrates containing known percentages of warfarin in cornstarch*

SAMPLE	WARFARIN (THEOR.)	WARFARIN FOUND BY ANALYSIS		
		COLLAB. (1)	(2)	(3)
	per cent	*rec.*	*rec.*	*rec.*
C	0.466 ±0.003	0.45 (95.7)	0.41 (87.2)	0.42* (89.4)
D	0.500 ±0.003	0.50 (100.0)	0.51 (102.0)	0.51 (102.0)
E	0.427 ±0.003	0.42 (97.7)	0.43 (100.0)	0.43 (100.0)
G	0.543 ±0.003	0.55 (101.9)	0.54 (100.0)	0.54 (100.0)
H	0.602 ±0.003	0.62 (103.3)	0.60 (100.0)	0.61 (101.7)

* 0.51(1) 0.42(2,3,4).

COLLABORATIVE ANALYSES (VARIATIONS)

(1) After extracting the warfarin from the ethylene dichloride solution with 1% sodium hydroxide, the sodium hydroxide was extracted with petroleum ether which had not previously been purified by treating with sodium hydroxide.

(2) Duplicate of (1), using petroleum ether purified by shaking with 1% sodium hydroxide prior to using.

(3) Sodium pyrophosphate ($Na_4P_2O_7$) 1% solution, substituted for 1% sodium hydroxide in extracting warfarin from the ethylene dichloride solution.

(4) Ethyl ether substituted for ethylene dichloride, and 1% sodium pyrophosphate substituted for the sodium hydroxide. Pyrophosphate solution extracted twice with ethyl ether and then twice with purified petroleum ether.

COLLABORATORS

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Late in 1950, the author modified the Wisconsin Alumni Research Foundation method for warfarin concentrates so as to be applicable for the analysis of commercially prepared warfarin baits. This procedure (Procedure III) has given excellent results on all the commercial samples analyzed to date.

PROCEDURE III

PROCEDURE FOR WARFARIN IN PREPARED BAITS CONTAINING APPROXIMATELY 0.025% WARFARIN

Weigh 6.0 g of sample into a 100 ml centrifuge tube. Add 50 ml of ethyl ether, stopper tightly, and shake on a mechanical shaking machine for about 30 min. Place the tube in a centrifuge and spin until the ether layer is clear. Pipet 10.0 ml of 1% sodium pyrophosphate soln into a glass stoppered 6 by $\frac{3}{4}$ inch test tube. Add 2.0 ml of the centrifuged ether extract to the test tube from a pipet. Stopper and shake vigorously for two min. Place the test tube in a centrifuge and spin at high speed until the lower aqueous layer is clear. Draw off the upper ether layer, including any emulsion that persists at the interface, by means of an aspirator connected to a tube drawn to a fine tip. Add about 2 ml ethyl ether to the test tube, shake vigorously, centrifuge, and completely draw off the ether layer. Repeat with a second ether extraction, and then extract twice with petroleum ether (which has previously been extracted with 1% sodium pyrophosphate soln). Completely draw off the solvent layer and allow air to be drawn over the liquid surface for a few seconds.

Fill a 1 cm quartz cuvette with the aqueous soln and determine the optical density from 305–310 mμ on a Beckman Model DU spectrophotometer, using for the zero setting a 1% sodium pyrophosphate soln which has been extracted with ethyl ether and petroleum ether (previously treated with 1% sodium pyrophosphate soln).

Optical density @308 mμ × 0.09 = __% warfarin.

As a check on the accuracy of Procedure III, a bait was prepared to contain 0.025% warfarin by mixing 2.50 grams of collaborative sample "D" (Table 2) with 47.50 grams of a mixture[1] used as the regular diet of of the albino rats in the bioassay laboratory. This prepared bait is more complex than most commercial baits and contains many substances which could cause serious interference in an ultraviolet spectrophotometric procedure.

DISCUSSION

The importance of the error which can be introduced by the alkali soluble impurities occurring in some commercial petroleum ethers, is shown in Table 1, sample "C" (1) and (2).

[1] Ground wheat meal 50 per cent; Soybean meal 30 per cent; Dry skim milk 5 per cent; Fish meal 5 per cent; Alfalfa leaf meal 4.12 per cent; Waste kitchen fat 3.10 per cent; Salt 1.50 per cent; Calcium carbonate 1.25 per cent; Fish oil (irrad.) 0.03 per cent

The extraction and centrifugal separation from the same glassware used in Procedure III has the advantage of fewer transfers and manipulations, thus reducing the errors.

There seems to be a disagreement as to the correct value for the extinction coefficient of warfarin at 308 millimicrons. The Wisconsin Alumni Research Foundation value is reported as 0.459 per mg./100 ml solution. The U. S. Dept. of Agriculture value is given as 0.463. Though the difference is slight, an optical density of 0.471 when calculated to warfarin

TABLE 2.—*Determination of warfarin in a prepared bait containing 0.025% warfarin*

ANALYSIS NO.	SOLVENT	EXTRACTANT	WARFARIN FOUND
			per cent
1	ethylene dichloride	1% NaOH	0.052
2	ethylene dichloride	1% $Na_4P_2O_7$	0.029
3	ethyl ether	1% NaOH	0.048
4	ethyl ether	1% $Na_4P_2O_7$	0.028, 0.027

would give 0.43% warfarin using the Wisconsin value, and 0.42% warfarin for the U.S.D.A, figure.

When ethylene dichloride was used as the solvent for warfarin in the analysis of the prepared bait (Table 2) it was impossible to obtain a clear solution, turbidity remaining even after centrifuging for an hour. The high density of the solvent caused many small particles of the bait to float on the surface of the solvent.

Sodium hydroxide solution used as an extractant for removing warfarin from the organic solvent phase in the analysis of prepared baits dissolves considerable organic matter, as shown by the results in Table 2, Analyses 1 and 3. The sodium hydroxide layer was yellow colored, and this color was not completely removed by extractions with ethyl and petroleum ether. When 1% sodium pyrophosphate solution was used, this solution remained colorless.

By taking a series of readings from 305 through 310 millimicrons any appreciable interference will be disclosed by a modification of the warfarin curve.

Some interfering substances were apparently retained in the sodium pyrophosphate solution of analysis 4, Table 2, as shown by the recovery results of 112 and 111 per cent. Spectrophotometer readings from 305 through 310 millimicrons showed the absorption maximum to be at 307 instead of 308 millimicrons.

RECOMMENDATION*

It is recommended—

* For report of Subcommittee A and action of the Association, see *This Journal*, 35, 44 (1952).

(1) That further collaborative analyses, with a greater number of collaborators, be undertaken on warfarin concentrates during the coming year.

(2) That work be continued on the development of a procedure suitable for low percentage warfarin baits.

REPORT ON CHLORDANE AND TOXAPHENE

By Thomas H. Harris (Insecticide Division, Livestock Branch, Production and Marketing Administration, U. S. Department of Agriculture, Beltsville, Maryland), *Associate Referee*

The Associate Referee has no report on toxaphene to present at this time. No specific method is yet available for the determination of this insecticide.

In accordance with the recommendation contained in the Associate Referee's report of last year the Davidow and Harris methods for technical chlordane were further studied. Most of this work was devoted to the development and application of the Harris method to petroleum oil formulations of technical chlordane. The purpose of the present report is to describe briefly this method, which has been revised slightly, and to present some data obtained on the above mentioned formulations.

METHOD

The method, mentioned briefly in last year's report, *This Journal*, **34**, 672, was revised by substituting naphthalene for beta naphthol as one of

Table 1.—*Technical chlordane in insecticide formulations*

SAMPLE IDENTIFICATION	PERCENTAGES OF TECHNICAL CHLORDANE		
	LABEL CLAIM	CALC. FROM TOTAL CHLORINE	SPECTROPHOTOMETRIC METHOD
	per cent	*per cent*	*per cent*
ID 23393	0.20	0.25	0.30
20305	0.50	—	0.55
18171	1.00	—	1.2
23757	1.00	—	1.1
23750	2.00	2.05	2.2
21850	—	2.03	2.10
20396	2.00	—	2.0
21401	2.00[1]	0.73	0.73
23039	40.00	40.6	40.0
23000	45.00	47.8	44.4
22919	45.50	—	44.0
21553	—	45.5	44.0
23016	45.00	46.5	40.0

[1] Manufacturer was cited for failure of product to meet label claim.

the reagents. It is based on the production of a violet-colored solution when a dilute benzene solution of technical chlordane is heated with methyl alcoholic potassium hydroxide, pyridine, and naphthalene. The modification resulted in better reagent stability, reproducibility, and sensitivity. This method as modified was presented in detail at a recent meeting of the American Chemical Society and will be published later.

This method was used to obtain the data shown in Table 1, on commercial liquid formulations.

It was mentioned in the Associate Referee's report of last year that both the Davidow and Harris methods gave erratic results on dust formulations. A study is being made at the present time to determine the reasons for failure of the methods on these formulations.

It is recommended* that the Associate Referee prepare and distribute to the collaborators, liquid formulations of technical chlordane similar in composition to those being marketed at the present time, for the purpose of evaluating the Davidow and Harris methods.

REPORT ON HERBICIDES

By A. B. HEAGY (State Inspection and Regulatory Services, College Park, Md.), *Associate Referee*

At the 1950 meeting of the Economic Poisons Section, A.O.A.C., the Associate Referee for Herbicides recommended that four methods for various products or combinations be studied. Subsequently, five samples were prepared and distributed. Encountering difficulty with the mails and other obstacles, some collaborators were late in receiving their samples, and did not complete the work in time to make reports. However, a sufficient number of collaborators were obtained to show the merits or deficiencies of some of the procedures, as reported below.

ALKALIMETRIC, TITRATION METHOD

Determination of Esters of 2,4-Dichlorophenoxyacetic Acid in Presence of Soap, Acids, Alcohols, and Oils

Reflux a sample of weight equivalent to 0.7 g of the ester with ca 1 g of KOH and 90 ml of 95% ethyl alcohol for one hour in a 250 ml S/T Erlenmeyer flask. After saponification and cooling, transfer the alcoholic soln, to a 250 ml separatory funnel with 80 ml water, and extract with 75 ml petroleum ether. Draw off the alcohol-water phase into another 250 ml separatory funnel and extract with 75 ml petroleum ether. Draw off alcohol-water phase, wash ether layer a few times with 10 ml portions of water, add washings to alcohol-water soln, and evaporate down to about 50 ml on a steam bath. Make residue to ca 100 ml with water, cool, and transfer to a 200 ml volumetric flask.

Add a few drops of phenolphthalein soln and a few drops of a 1+1 soln of hydrochloric acid to the disappearance of the pink color, and then add 1+1 ammonium hydroxide soln until slightly alkaline. Add sufficient water to give a volume of

* For the report of Subcommittee A and action of the Association, see *This Journal*, 35, 44 (1952).

about 150 ml. Add slowly 10 ml 10% barium chloride soln to precipitate the fatty acids, and let stand 10 min., make to volume, shake, and filter. Test filtrate to see if additional barium chloride is needed.

Transfer a 100 ml aliquot to a 250 ml separatory funnel, acidify with 1+1 hydrochloric acid and add 10 ml in excess. Extract the aqueous phase twice with 75 ml portions of ether. Wash the combined ether extracts free from mineral acid with 10 ml portions of water (three washings will be adequate).

Transfer the ether soln to a 400 ml beaker and rinse the separatory funnel with ether. Add 25 ml of water, a few boiling chips, and evaporate the ether layer on a steam bath.

Dissolve the aqueous mixture in 100 ml of neutral ethyl alcohol and titrate with 0.1 *N* sodium hydroxide, using phenolphthalein as an indicator.

Each ml of 0.1 *N* sodium hydroxide is equivalent to 0.0221 g of 2,4-Dichlorophenoxyacetic acid.

NOTE: If it is desired to use thymolphthalein in the final titration, it should be used also in part (a) for adjusting *p*H (Methods of Analysis, A.O.A.C., *par.* 5.129).

Results from eight collaborators agreed very well with the exception of a high recovery of 42.65 per cent, which was not included in the general average of 36.97 per cent. The guarantee on the commercial product distributed for analysis was 37 per cent.

Results are as follows:

per cent	*per cent*
1. 36.86	5. 37.96
2. 36.98	6. 36.42
3. 36.40	7. 37.51
4. 36.65	8. 42.65

COLLABORATOR'S COMMENTS

1. Time required is too long.
2. $BaCl_2$ will precipitate all the 2,4-D in alkaline solution.
3. Amount of emulsifying soap must be known to estimate the proper amount of $BaCl_2$ to add.
4. Too much glassware needed.
5. Definite amount of 10% $BaCl_2$ should be prescribed to prevent precipitation of 2,4-D. Check filtrate to determine if sufficient $BaCl_2$ has been added.
6. Make correction of 0.1–0.2% for 2,4-D acid soluble in water extracts.
7. Too much depends upon manipulation and technique throughout.
8. Eliminate adjustment of *p*H before addition of $BaCl_2$.
9. Let sample stand for 10 minutes after addition of $BaCl_2$.
10. Add 10 ml of 1+1 HCl before extracting with ether.
11. Use same indicator in adjusting *p*H as is used in titration.
12. Evaporation should be carried out with care to avoid losses.

Determination of Total Chlorine in Liquid Herbicides Containing 2,4-D, 2,4,5-T or Mixtures of Both in the Presence of Oils and Emulsifiers by a Modified Parr Bomb Procedure

Transfer about a 1 g sample, or a suitable aliquot of a sample diluted with isopropyl alcohol and containing from 0.30–0.034 g of chlorine, into a 100 ml beaker. Add 1 g of C.P. KOH and 25 ml of 95% ethyl alcohol. Cover with crystal, heat on steam bath for ½ hour, remove cover glass and evaporate to dryness.

Place the beaker containing the residue in an oven at 120°C. for two hours or

longer if it is evident that some oils still persist. Remove from the oven, transfer the dried residue, with the aid of a small spatula to a 50 ml Parr bomb cup containing the accelerators, 0.7 g of potassium nitrate, and 0.4 g of powdered cane sugar.

Wash the remaining residue which adheres to the 100 ml beaker into the combustion cup, with a small quantity of water, and evaporate to dryness on an asbestos plate heated over a small flame. Care should be taken near the end of the evaporation to prevent spattering. Allow the combustion cup to cool to room temperature, add the contents from the 50 ml beaker, break up any lumps which may be present, and mix well with a stirring rod.

Add ca 15 g of calorimetric grade sodium peroxide, and *mix thoroly*. (Mixing may be accomplished by stirring with a metal rod if it is done quickly and completely, without excessive exposure of the charge to the atmosphere. However, it is a better practice to mix by shaking, which may be done by covering the fusion cup with the plain cover and either holding this down with the thumb or fastening it tightly in the body by the use of the regular screw cap.) Shake thoroly for at least one min. with frequent tapping to prevent adherence of the mixture to the sides of the cup. When the mixing is complete, tap the cup lightly to shake down all of the material from the upper part. Remove the plain cover and brush all adhering particles into the cup, close with the regular head to which the fuse wire has been attached, and ignite in the usual manner.

Place about 100 ml of distilled water in a 600 ml beaker and heat nearly to boiling. After cooling of the bomb, dismantle it and dip the cover in the hot water to dissolve any of the fusion which may be adhering to its under side. Wash the cover with a fine jet of distilled water, catching the washings in the beaker. With a pair of tongs lay the fusion cup on its side in the same beaker of hot water, covering it immediately with a watch glass. After the fused material has been dissolved remove the cup and rinse with hot water, cool the soln, add several drops of phenolphthalein indicator, neutralize with concentrated HNO_3, and add 5 ml in excess. From this point, the chlorine may be determined by electrometric titration or by the Volhard procedure.

NOTE: The above method is based on treating the 2,4-D and 2,4,5-T compounds with alcoholic potash and expelling the volatile oils which cause incomplete combustion with low results by the usual Parr bomb procedure. In instances where the oils are not sufficiently volatile at 120°C., the temperature of the oven may be raised to 135°C.

This procedure was studied by six collaborators. Results are as follows:

	per cent		*per cent*
1.	2.88	4.	3.07
2.	2.29	5.	2.55
3.	2.98	6.	2.80
General average			2.76

Good agreement is noted in the figures reported by four workers. The others varied 0.4 per cent to 0.6 per cent from the average.

COMMENTS

(1) The main difficulty was the volatilization of the oil. This was noted by all. Twenty hours at 135°C., and up to 20 hours at 140°C. was necessary to volatilize all oil.

(2) If electrometric titration is used the concentration of nitric acid in the solution should be specified.

(3) Additional amounts of sugar were necessary to get complete combustion.

DETERMINATION OF POTASSIUM CYANATE IN HERBICIDES

REAGENTS

(a) *Semicarbazide hydrochloride*, $NH_2CONHNH_2 \cdot HCl$.

(b) *Wash soln* (water saturated with hydrazodicarbamide).—Prepare by mixing some potassium cyanate and semicarbazide hydrochloride in water, filter, and wash the precipitate with water. Transfer the precipitate to a flask, add small quantity of water, shake vigorously, and filter. (The solubility of the precipitate in water is about one part in 6600.)

DETERMINATION

Transfer a sample containing 0.2–0.5 g potassium cyanate to a 100 ml beaker, add 20 ml wash soln and 1 g semicarbazide hydrochloride ($NH_2CONHNH_2 \cdot HCl$), and allow to stand 24 hours. Filter on a Gooch or Grade F sintered crucible, wash with 10 ml of the wash soln **(b)**, and dry at 100°C. to constant weight. (The factor from the precipitated hydrazodicarbamide ($NH_2CONHNHCONH_2$) to potassium cyanate is 0.6868.)

Three collaobrators submitted results on this procedure:

	per cent
1.	99.17
2.	99.09
3.	99.20

COMMENT

(1) Sintered glass crucibles, Grade F or M could be used in place of Gooch crucibles and asbestos.

(2) The amount of wash solution prescribed is inadequate.

(3) Special wash solution should be used to dissolve the sample and the reagents, as well as to wash the precipitate.

DISCUSSION

The alkalimetric titration method has proved useful in the examination of some types of 2,4-D ester materials. There are apparently many discrepancies to overcome before this procedure can be accepted for the entire class of esters, oil, soap, etc., products.

Clemens Olsen, of Arizona, has recently studied the alkalimetric titration method and has modified the procedure to obtain concordant results which agree with the Parr bomb method. The main variations involved the adjustment of the *p*H to 8.0 and evaporation of the refluxed solution to dryness. The residue is then taken up with water, $BaCl_2$ is added, and the solution passed through an ion exchange column containing Amberlite IR-100. Results varied from 36.5 per cent to 36.7 per cent, with 36.54 per cent as the average.

This method appears to have considerable merit, and would have been studied if time had been available. It has been submitted to the methods committee for suggestions and comments.

Also, Mr. Olsen has studied the Parr bomb procedure for the determination of total chlorine. Difficulty in securing complete combustion by reagents now in use was instrumental in the decision to devote some time

to the subject. Crystalline boric oxide was used with success, and Mr. Olsen reports the method to be rapid and accurate. Results of 8 determinations varied from 36.4 per cent to 36.8 per cent, with the average 36.63 per cent.

(These modifications, and results obtained, are given here with the permission of Mr. Olsen.)

In examining combinations of 2,4-D and potassium cyanate mixtures the following procedure has been found accurate and adequate for 2,4-D:

Dissolve the sample in water, and add $BaCl_2$, filter into 250 separator, make acid and cool. Return the precipitate to beaker, make strongly acid with HCl, and filter into a second 250 ml separator. Extract each separately with two 75 ml portions of ether. Wash and continue as prescribed in official method **5.129**. The only difficulty encountered is the occurrence of emulsions in section (b), which are easily broken by the addition of saturated NaCl soln.

RECOMMENDATIONS*

It is recommended—

(1) That the alkalimetric titration method for the determination of esters of 2,4-Dichlorophenoxyacetic acid (revised) be subjected to further study. Also, that the Olsen procedure be studied in conjunction with this project.

(2) That the determination of total chlorine in liquid herbicides containing 2,4-D, 2,4,5-T, or mixtures of both in the presence of oils and emulsions, by a modified Parr bomb procedure, be subjected to further study. Also, that the Olsen procedure be studied in connection with this project.

(3) That the described method for the determination of potassium cyanate in herbicides be adopted as first action including the following revisions:

1. Add 20 ml of wash solution. 2. Filter on sintered glass crucibles, Grade F, or Gooch crucibles. 3. Wash with 20 ml of the wash solution. 4. Change factor from 0.7172 to 0.6868, as recalculated from the equation.

REPORT ON PARATHION

By FRED I. EDWARDS (Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Beltsville, Md.), *Associate Referee*

A survey of the analytical methods in use at 18 laboratories was reported by your Associate Referee at the 1950 annual meeting and was subsequently published in *This Journal* (1). At that time, it was recommended that, since the polarographic method of Bowen and Edwards (2) and the sodium nitrite titration method of O'Keefe and Averell (3) were receiving the most widespread use and were the most promising, collaborative work be carried out on these two methods. Accordingly, samples of a

* For report of Subcommittee A and action of the Association, see *This Journal*, **35**, 44 (1952).

technical parathion and a 25% dust were sent to 13 laboratories for study. Eight laboratories reported on the titration method, and five laboratories reported on the polarographic method.

The results of these collaborative analyses are given in Tables 1 and 2. Since the polarographic method shows no advantage over the titration

TABLE 1.—*Collaborative results utilizing O'Keefe-Averell method*

ANALYST	TECHNICAL PARATHION		WETTABLE POWDER	
	PARATHION	*p*-NITROPHENOL	PARATHION	*p*-NITROPHENOL
	per cent	*per cent*	*per cent*	*per cent*
1	94.4	—	23.9	—
	94.6	—	24.1	—
	94.3	—	23.8	—
2	95.9	0.37	23.9	1.05
	96.5	0.36	23.9	1.04
	96.6	0.38	23.9	1.08
3	94.0	0.50	25.3	1.17
	96.2		20.5	
4	94.6	0.38	23.6	1.00
	97.3	0.38	23.6	1.01
	96.9	0.38	23.9	1.02
	96.6	0.38	23.8	1.01
5	94.4	—	26.4	—
	96.3	—	26.2	—
	93.1			
6	97.1	0.37	23.7	1.09
	96.3	0.37	23.4	1.08
	96.3	0.37	23.9	1.04
7	92.0	0.75	23.9	1.25
	93.0	0.77	24.5	1.04
	90.0	0.73	27.9	1.35
8	94.5	1.51	23.9	1.07
	91.8	1.52	24.0	1.11
	93.3	1.50	23.7	1.04
	93.8			
	94.0			
Average	94.8	—	24.2	—
Coeff. of variation	±2.0	—	±0.6	—

TABLE 2.—*Collaborative results utilizing polarographic method*

ANALYST	PARATHION FOUND IN TECHNICAL SAMPLE	PARATHION FOUND IN WETTABLE POWDER
	per cent	*per cent*
1	93.9	24.9
	94.8	23.9
	95.5	24.5
2	96.7	23.0
	97.0	24.6
	96.1	23.6
3	93.5	27.4
	92.5	27.1
	92.5	28.0
		26.7
4	96.2	24.9
	96.7	24.2
	96.6	24.7
5	100.0	23.9
	100.0	23.7
	100.0	23.8
	97.7	
	100.0	
	98.0	
	99.9	
Average	96.7	24.9
Coeff. of variation	±2.4	±0.6

method, and utilizes equipment that is prohibitively expensive for smaller laboratories, no further consideration of this method seems warranted at this time. Samples of a 30% emulsifiable concentrate were sent to selected laboratories for study by both methods. The collaborators' reports confirmed the findings of the Associate Referee that solvents in the formulations prevented the excess zinc being destroyed by hydrochloric acid and caused a very milky medium for the titration. Solutions for *p*-nitrophenol determination were also turbid, making results questionable. Those laboratories reporting on the polarographic analysis of emulsifiable concentrates also obtained erratic results, although more favorable comments were received than on the titration procedure. It is believed that the analysis of emulsifiable concentrates should be further investigated before any collaborative work is undertaken.

Comments of collaborators on the titration method were generally satisfactory. The major complaint still is centered around the potassium io-

dide-starch paper end point. It is difficult to decide exactly when an end point is achieved, and day-to-day variation of color taken, even by an individual operator, is a possible source of error. One collaborator, H. A. Thomson,[1] reported on an alternate end-point technique patterned after the work of LaRocca and Waters (4). His comments on this procedure were very favorable. The technique consisted of a potentiometric titration using a maximum rise in potential as an end point. Platinum and calomel electrodes were used. In view of the general dissatisfaction with the potassium iodide-starch paper end point this potentiometric end point is definitely worthy of investigation, and collaborative studies will be undertaken.

The O'Keefe and Averell titration method (3), which was used for this year's collaborative studies, is described below. It should be noted that this method includes a step in which free *p*-nitrophenol content is determined colorimetrically. This is necessary since free *p*-nitrophenol will interfere with the titration of parathion in a later step. This determination of free *p*-nitrophenol is actually advantageous, because high concentrations of the free compound cause plant injury (5).

METHOD

APPARATUS

(1) *Photoelectric colorimeter*, equipped with a filter to give maximum transmission between 400 and 450 millimicrons.

REAGENTS

(1) *Ethyl ether*, C. P.
(2) *Sodium carbonate*, 1%.
(3) *Sodium hydroxide*, 1N.
(4) *Acetic acid*—hydrochloric acid, 9 volumes of glacial acetic acid in 1 volume of concentrated hydrochloric acid.
(5) *Sodium (or potassium) bromide*, C. P.
(6) *Zinc dust*, iron-free.
(7) *Potassium iodide-starch paper.*
(8) *Sulfanilic acid*, Eastman Kodak.
(9) *Sodium nitrite soln*, 0.1 N, standardized against anhydrous recrystallized sulfanilic acid. (Restandardize weekly.)

SEPARATION OF PARATHION AND *p*-NITROPHENOL

Weigh accurately from a weighing pipet 0.6–0.9 g of sample into 100 ml of ethyl ether contained in a 250 ml separatory funnel. Extract the ether soln four times (or until the extract is colorless) with 20 ml portions of chilled, 1% sodium carbonate soln, collecting the combined aqueous layers in a 200 ml volumetric flask. Transfer the ether layer to a 400 ml beaker, using small portions of ether to effect the quantitative transfer.

DETERMINATION OF *p*-NITROPHENOL IN AQUEOUS EXTRACT

Preparation of Standard Curve.—Weigh accurately 100 mg of *p*-nitrophenol,

[1] Naugatuck Chemicals, Elmira, Ontario.

transfer to a 1 liter volumetric flask, and make up to volume with 0.1 *N* sodium hydroxide. Transfer 2, 4, 6, 8, 10, and 20 ml aliquots of this soln to 100 ml volumetric flasks, and make each soln up to volume with 0.1 *N* sodium hydroxide. Read the light transmittancy of each of the standard solns at 400 mμ by means of a photoelectric colorimeter that has been set to give 100% transmittance with water. Plot the light transmittancies as abscissas against the concentrations (milligrams per milliliter) as ordinates on semilogarithmic graph paper.

Determination of p-nitrophenol.—Add 20 ml of 1*N* sodium hydroxide to the combined aqueous extracts contained in the 200 ml volumetric flask and make up to volume with water. Measure the light transmittancy of the soln at 400 mμ with a photoelectric colorimeter, and read from the standard curve the concentration of *p*-nitrophenol in milligrams per milliliter of soln.

$$\% \text{ } p\text{-nitrophenol} = \frac{\text{mg./ml.} \times 200 \times 100}{1000 \times \text{grams of sample}}.$$

A dilution of the sodium *p*-nitrophenoxide soln to 200 ml is suitable for technical parathion containing up to 0.2% *p*-nitrophenol. If the sample being analyzed contains greater amounts of *p*-nitrophenol, the sodium *p*-nitrophenoxide soln must be diluted with 0.1*N* sodium hydroxide to bring the concentration of sodium *p*-nitrophenoxide in the soln to be measured within the limits of 0.003 to 0.010 mg per ml. The dilution necessary will vary with the sample.

DETERMINATION OF PARATHION IN ETHER SOLUTIONS

Standardization of sodium nitrite.—Weigh accurately 0.4–0.45 g of anhydrous recrystallized sulfanilic acid (the purity of which has been checked by a nitrogen determination) into a 400 ml tall-form beaker. Add 80 ml of water, 10 ml of concentrated hydrochloric acid, 30 ml of glacial acetic acid, and 5 g of sodium bromide. Cool the mixture to 0°–10°C. by the addition of clean shaved ice and place under mechanical stirring. Titrate at 0°–10°C. with 0.1*N* sodium nitrite as rapidly as the spot test will permit. Near the end point add the nitrite in 4 drop portions.

Spot test.—Dip a glass rod into the soln to be tested and then touch the rod quickly to a piece of potassium iodide-starch paper. The end point is reached when an intense blue-black color appears immediately and can be obtained repeatedly during a 1-min. period without further addition of the nitrite.

$$\text{Normality of sodium nitrite} = \frac{\text{Grams of sulfanilic acid} \times 1000}{\text{Ml of } NaNO_2 \times 173.2}$$

Analysis of ether solution for parathion.—Add 35 ml of the acetic-hydrochloric acid soln to the ether soln of parathion that has been transferred to a 400 ml beaker. Add 1 g of zinc dust and cover the beaker with a watch glass. Heat the soln gently on a steam bath until most of the ether has evaporated and the soln is colorless. Add 10 ml of conc hydrochloric acid to complete the solution of the zinc dust. Cool the soln, wash down the beaker and watch glass with 100 ml of water, and add 5 g of sodium (or potassium) bromide. Cool the mixture to 0°–10°C. by the addition of clean shaved ice (ca 100 g) and place under mechanical stirring. Titrate at 0°–10°C. with standardized 0.1 *N* sodium nitrite as rapidly as the spot test will permit. (About 20–30 ml will be required.) Near the end point add the nitrite in 4-drop portions.

$$\% \text{ parathion} = \frac{\text{ml of sodium nitrite} \times \text{normality of sodium nitrite} \times 29.13}{\text{grams of sample}}$$

ANALYSIS OF DUST PREPARATIONS

Transfer a weighed sample of dust or wettable powder to an extraction thimble and extract with 150 ml of ethyl ether in a Soxhlet apparatus for 1 hour. Transfer the ether extract to a 250 ml separatory funnel and proceed with the analysis as described above.

The sample size will vary with the concentration of the dust:

10% = 6.75 grams; 15% = 4 to 5 grams; 25% = 2.5 to 3.5 grams.

RECOMMENDATIONS*

It is recommended—

(1) That the method described for technical parathion and dust formulations be adopted, first action.

(2) That the end-point technique of these methods be further investigated, with particular emphasis on the possibility of utilizing a potentiometric end point.

(3) That the investigation of parathion emulsifiable concentrate analysis be continued.

REFERENCES

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(4) LAROCCA, J. P., and WATERS, K. L., *J. Amer. Pharm. Assn.*, **39**, 521 (1950).
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REPORT ON ORGANIC THIOCYANATES

By HERBERT A. ROONEY (California State Department of Agriculture, Bureau of Chemistry, Sacramento, Calif.), *Associate Referee*

Study of the J. W. Elmore procedure for the determination of organic thiocyanate nitrogen in liquid organic thiocyanate preparations, commonly used in livestock and fly sprays, was continued. At the 1950 meeting of the A.O.A.C. the method† was adopted, first action.

COLLABORATORS

California Spray-Chemical Corporation, Richmond, California.
Hercules Powder Co., Inc., Wilmington, Delaware.
Paul E. Irwin, Division of Chemistry, Commonwealth of Virginia.
Rohm and Haas Company, Inc., Philadelphia, Pennsylvania.
Herbert A. Rooney, California State Department of Agriculture, Sacramento 14, California.

KEY TO UNKNOWN SAMPLES

Sample 5. A kerosene solution of a secondary terpene alcohol thiocyanyl acetate.

Sample 6. A kerosene solution of normal butyl thiocyanate.

* For report of Subcommittee A and action of the Association, see *This Journal*, 35, 45 (1952).
† *This Journal*, 34, 677 (1951).

COLLABORATOR'S DIRECTIONS FOR ANALYSIS

Collaborators were instructed to follow the procedure as outlined in the 1950 report.

TABLE 1.—*Results of analysis*

COLLABORATOR	ORGANIC THIOCYANATE NITROGEN		TOTAL NITROGEN BY KJELDAHL	
	SAMPLE NO.—		SAMPLE NO.—	
	5	6	5	6
A	0.21	0.61	0.20	0.59
	0.21	0.61	0.20	0.60
	0.22	0.61	0.21	0.60
B	0.24	0.62	0.21	0.60
	0.21	0.60	0.21	0.60
C	0.21	0.61	0.21	0.61
	0.21	0.61	0.21	0.60
D	0.21	0.60	0.22	0.61
	0.21	0.60	0.21	0.61
E	0.21	0.60	0.21	0.60

RECOMMENDATIONS*

The collaborative work of the past two years shows the Elmore method gives satisfactory recovery of organic thiocyanate nitrogen in liquid organic thiocyanate preparations used as household fly sprays and livestock sprays. Present information indicates a slightly lower recovery of thiocyanate nitrogen on the ester type thiocyanate, $C_nH_{2n-1}COOCH_2CH_2SCN$ (10–18 carbon atoms) than that obtained by a Kjeldahl analysis, which may indicate the presence of nitrogen in forms other than thiocyanate in this type of product.

It is recommended that the Elmore method as published in the 1950 collaborative report be continued as first action, and that study be continued to investigate the applicability of the method for determination of thiocyanate nitrogen in the ester type thiocyanate.

* For the report of Subcommittee A and action of the Association see *This Journal*, 35, 44 (1952).

REPORT ON DITHIOCARBAMATES

By J. D. PATTERSON (Oregon State Department of Agriculture, Salem, Oreg.), *Associate Referee*

In a previous report[1] some of the difficulties encountered in the analysis of dithiocarbamates were reported. Further, it was stated that it was the hope of the Associate Referee to develop a method specific for dithiocarbamates. At the Boston meeting of April 1951 of the American Chemical Society, Clarke, Baum, Stanley and Hester of the Rohm and Haas Co. presented a paper "The Analysis of Dithiocarbamates." This paper has been presented to *Analytical Chemistry* for publication, but as yet it has not appeared.[2] This report briefly summarizes their method and gives some results obtained in the Associate Referee's laboratory.

APPARATUS

Figure 1 illustrates apparatus as used by Clarke, *et al.* This has been modified in the Associate Referee's laboratory by adding a second methanol KOH absorber and, under some experimental conditions, a second lead acetate absorber. The necessity for these modifications is dependent upon the efficiency of the absorbers. In the author's laboratory, absorbers were made from 8 inch side-neck test tubes.

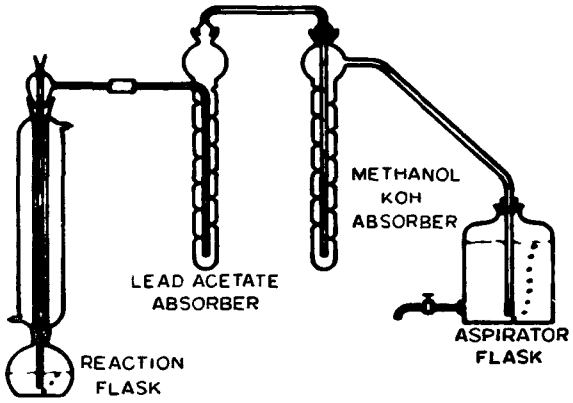

FIG. 1.—Apparatus for dithiocarbamate assay.

PROCEDURE

The apparatus shown in Figure 1 is used. The sample containing about 0.004 equivalents of dithiocarbamate is placed in the reaction flask. (Aliquots of liquid samples are pipetted in, while solid samples are weighed into vials which are then placed in the flask.) The first absorber is charged

[1] Patterson, J. D., *This Journal*, **35**, 788 (1950).
[2] Since this report was presented the paper has appeared in *Analytical Chemistry*, **23**, 1842 (1951).

with 10% lead acetate, and 25 ml of 2 *N* methanolic KOH is pipetted into the second. Aspiration is started (approx. 150 ml/min.) and 50 ml of hot 1.1 *N* H_2SO_4 is poured into the air intake. Mild boiling is maintained and aspiration is continued for 15 minutes. The second absorber is disconnected and its contents rinsed into a beaker with 75–100 ml of water. The solution is just neutralized to phenolphthalein with 30% acetic acid, and then titrated with 0.1 *N* iodine, adding 5 ml of starch indicator and 250 ml of distilled water just before the end point, which is a faint, but definite color change in the white suspension formed.

The blank is run daily with omission of the decomposition step, and is usually 0.2 to 0.3 ml.

The calculation is:

$$\% \text{ dithiocarbamate} = \frac{(\text{Sample titration-blank})(I_2 \text{ Normality})(\text{Eq. Wt. dithiocarbamate})}{\text{Wt. sample (10)}}.$$

Precautions to be observed: (a) Diluted samples must be analyzed immediately because air oxidation destroys certain types of dithiocarbamates in dilute solution. (b) Aspiration must be controlled to prevent foaming of the contents of one part of the system into another. (c) Sufficient time must be allowed for complete decomposition.

Decomposition time: In the paper quoted, most of the work reported was on disodium ethylene bisdithiocarbamates and it was the authors' conclusion that 15 minutes was adequate time for the decomposition of sodium and zinc salts. Work in the Associate Referee's laboratory indicates that some salts such as iron, as well as other commercial samples containing dithiocarbamates, may require up to 2 hours for complete recovery.

ANALYTICAL RESULTS ON VARIOUS DITHIOCARBAMATES

Exp. I.—Determination of sample of Ferbam, twice recrystallized from chloroform, and effects of time and of second methanolic KOH absorber

SAMPLE NO.	SAMPLE WT.	*N* OF I_2	ML I_2 USED 1ST ABSORBER	ML I_2 USED 2ND ABSORBER	DISTILLATION TIME	PERCENTAGE FOUND
					hrs	
1	0.4417	0.08553	34.8	—	1	93.56
2	0.4554	0.08553	36.9	—	1	95.98
3	0.3768	0.08553	30.8	—	1½	97.07
4	0.3561	0.1	24.6	0.8	2	99.51
5	0.3564	0.1	24.65	0.7	2	99.24
6	0.2751	0.1	18.65	0.8	2	98.41
7	blank	0.1	0.35	0.1*	2	—

* Same as blank normally obtained on reagents without aspiration. The larger blank in first absorber tube apparently comes from contaminants in the air being drawn through the apparatus.

Exp. II.—Analysis of a sample of commercial Fermate

SAMPLE NO.	SAMPLE WT.	N OF I_2	TIT.—1ST TUBE	TIT.—2ND TUBE	TOTAL TIT.	TIME	PERCENTAGE
			corrected for blank			*hrs*	
1	0.2196	0.1	11.45	0.4	11.85	1	74.94
2	0.2464	0.1	13.40	0.5	13.9	1	78.32
3	0.2645	0.1	14.25	0.7	14.95	2	78.48
4	0.2393	0.1	12.55	1.0	13.55	2	78.41

Exp. III.—Analysis of a sample of zinc ethylene bisdithiocarbamate

SAMPLE NO.*	SAMPLE WT.	N OF I_2	TIT.—1ST TUBE	TIT.—2ND TUBE	TOTAL TIT.	TIME	PERCENTAGE
			corrected for blank			*hrs*	
1	0.4959	0.1	27.95	1.9	29.4	1½	81.75
2	0.4993	0.1	29.10	1.3	29.95	1½	82.71
3	0.4292	0.1	23.15	1.8	24.95	1½	80.17
4	0.3761	0.1	21.55	0.45	22.0	1½	80.48
5	0.4530	0.1	25.95	0.6	26.55	1½	80.81

* NOTE: Samples 1 and 2 were determined with one lead acetate scrubber. It appeared that there was a possible escape of H_2S from the single scrubber. A second lead acetate scrubber was added in samples 3, 4 and 5.

Exp. IV.—Analyses of a 5% commercial mixture of zinc ethylene bisdithiocarbamate

SAMPLE NO.	SAMPLE WT.	N OF I_2	TIT.—1ST TUBE	TIT.—2ND TUBE	TOTAL TIT.	TIME	PERCENTAGE
			corrected for blank			*hrs*	
1	5.0000	0.1	17.35	0.2	17.55	1	4.84
2	5.0000	0.1	18.00	1.1	19.1	1¼	5.27
3	5.0000	0.1	18.25	0.9	19.15	1½	5.27

Exp. V.—Effect of tetramethylthiouram disulfide. Sample contained 20% purified Ferbam+1% thiouram+1% sulphur

SAMPLE NO.	SAMPLE WT.	N OF I_2	TIT.—1ST TUBE	TIT.—2ND TUBE	TOTAL TIT.	TIME	PERCENTAGE
			corrected for blank			*hrs*	
1	1.0000	0.1	14.45	0.5	14.95	2	20.76
2	1.0000	0.1	14.55	0.55	15.10	2	21.02
			Mixture 20% purified Ferbam+5% thiouram+1% sulphur				
1	1.0000	0.1	16.25	1.4	17.65	2	24.41
2	1.0000	0.1	17.85	0.3	18.15	2	25.20

DISCUSSION

Theoretical problems involved in this analysis are discussed by Clarke, *et al.* and are not repeated here. Volume of aspirated air was not measured

in the above results and may account for some of the variation in time noted for various determinations. Although the experimental work reported here has been limited, it is the Associate Referee's opinion that the results obtained merit further study by this Association, and it is so recommended.*

ACKNOWLEDGMENT

Thanks are here expressed for the work of Roy Shaw of Dairy and Food Laboratories, Oregon State Department of Agriculture, who performed most of the analyses given in this report.

REPORT ON PIPERONYL BUTOXIDE

By Boyd L. Samuel (Div. of Chemistry, Virginia Dept. of Agriculture, Richmond, Va.), *Associate Referee*

Preliminary work has been done by the Associate Referee on the separation of Piperonyl Butoxide from other constituents of commercial insecticides by partition chromatography. Using Harris' (1) procedure it was found that about 80 per cent of technical piperonyl butoxide came through the chromatographic column in a fairly narrow band and traveled at approximately the same rate as the gamma isomer of benzene hexachloride. R. L. Caswell, of the U.S.D.A. Production and Marketing Administration, verified this work and also obtained about 80 per cent recovery. A number of commercial insecticides were analyzed by this method and a factor was used to convert to technical piperonyl butoxide. Excellent agreement with the manufacturers' guarantees were obtained.

However, some samples have such a small amount of piperonyl butoxide present that this method is not sufficiently accurate. Other samples have materials present that come through the column with piperonyl butoxide. These include the gamma isomer of benzene hexachloride, methoxychlor, isobornylthiocyanoacetate, and a small fraction of piperonyl cyclonene. Therefore, a method is needed for the final determination after the chromatographic separation. Caswell found that piperonyl butoxide absorbed strongly in the ultra-violet at 290 mμ and that isobornylthiocyanoacetate did not absorb appreciably at this wave-length. The Associate Referee has verified the absorbance of piperonyl butoxide and has found that the gamma isomer of benzene hexachloride absorbs very little at 290 mμ, but that methoxychlor and piperonyl cyclonene absorb rather strongly. As the methoxychlor can be determined by chlorine analysis and only a small part of the piperonyl cyclonene will be present, it seems probable that a method could be worked out using ultra-violet absorbance which would be satisfactory in many cases, and which would give approximate results when interferences were present.

* For report of Subcommittee A and action of the Association, see *This Journal*, 35, 45 (1952).

The Associate Referee has also been working with the Jones (2) colorimetric method and has obtained some excellent results using the chromatographic separation followed by the Jones (2) procedure in which especially purified tannic acid is used.

The Associate Referee believes that a preliminary separation of piperonyl butoxide in most formulations is necessary before it can be determined accurately by either ultra-violet absorbance or the Jones color method and that partition chromatography offers a satisfactory means of accomplishing this.

RECOMMENDATIONS*

It is recommended—

(1) That the ultra-violet absorbance of piperonyl butoxide and its interferences be further studied.

(2) That the partition chromatographic separation of piperonyl butoxide and its determination by the Jones (2) color method be studied collaboratively.

ACKNOWLEDGMENT

The Associate Referee expresses his appreciation to R. L. Caswell for his assistance and suggestions.

REFERENCES

(1) HARRIS, THOMAS H., "The determination of Gamma Benzene Hexachloride in insecticide products," *This Journal*, **32**, 684 (1949)

(2) JONES, HOWARD A., "Revised Tentative Procedure for the Determination of Piperonyl Butoxide." Mimeographed method distributed to many laboratories and dated Nov. 7, 1949. U. S. Industrial Chemicals, Inc., Research and Development Laboratories, Baltimore, Md. (Will appear in *This Journal* **35**, No. 3.)

No report was given for DDT, alethrin, quaternary ammonium compounds, phenolic disinfectants, or test methods for economic poisons.

The contributed paper entitled "A Comparison of Analytical Methods on Tobacco Containing Non-Nicotine Alkaloids," by R. N. Jeffrey, was published in *This Journal*, November, 1951, p. 843.

REPORT ON DISINFECTANTS

By L. S. STUART (Insecticide Division, Livestock Branch, Production and Marketing Administration, Department of Agriculture, Washington 25, D.C.), *Referee*

An extensive collaborative testing program was initiated to secure additional data which might be used to establish an official "use-dilution"

* For report of Subcommittee A and action of the Association, see *This Journal*, **35**, 44 (1952).

method for evaluating disinfectants. PMA laboratory workers, with the assistance of the Disinfectant Scientific Committee of the Chemical Specialties Manufacturers Association, forwarded outlines of suggested procedures, unknown samples, and selected test cultures to 18 collaborating laboratories late in 1950, and in the first two months of 1951. However, as of this date, only three laboratories have completed all the studies outlined. The results from two of the three were in good agreement, but those from the third were considerably different. Reports from 8 of the laboratories indicated difficulty in maintaining the selected test cultures at a uniform resistance level to the control chemical, phenol.

This particular study was initiated to follow up a similar study conducted during the summer and fall of 1950. Complete results from 9 collaborating laboratories in the earlier study showed poor agreement on all results among all laboratories reporting, but excellent agreement on two results among two groups; that is, 4 laboratories were in absolute agreement as to one result and 5 as to a different result. It was felt that the technique in the method used for contaminating the carriers must have been responsible for failure to secure more consistent agreement between the two groups, and the 1951 studies were changed to correct for this. It now appears that the differences noted may have been the result of variations in the resistance of the selected test cultures as maintained in the different laboratories. It would seem, therefore, that if a test strain of the desired species can be secured which can be maintained satisfactorily by all laboratories, it may be possible to develop a satisfactory method of this type within the next year. Work will be continued to this end.

Some evidence has been submitted to indicate that a nutrient broth, congo red subculture medium would be more efficient than letheen broth in the presently official phenol coefficient procedure. Also, evidence has been submitted indicating that the propagation of the fungus *T. interdigitale* in plates poured with seeded agar will provide spore suspensions of more uniform resistance than propagation in a giant colony, as prescribed in the present fungicidal test.[1] A subcommittee will be set up to investigate collaboratively the desirability of making changes on these points in the existing methods.

It is recommended* that the work being conducted by the subcommittee on media for disinfectant testing be continued. This group should collaborate wherever possible with the existing committees on media standardization of both the Society of American Bacteriologists and the American Public Health Association.

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[1] *Methods of Analysis*, 7th Ed., 1950, page 91.
* For report of Subcommittee A and action of the Association, see *This Journal*, **35**, 45 (1952).

REPORT ON INGREDIENTS FOR DISINFECTANT TESTING

STANDARDIZATION OF BACTERIOLOGICAL CULTURAL MEDIA

By MICHAEL J. PELCZAR (Department of Bacteriology, University of Maryland, College Park, Md.), *Associate Referee*

The standardization of cultural media—from the standpoint of providing reproducible media containing ingredients which can be characterized—presents a formidable task to bacteriologists, and it is a problem which must be resolved. Bacteriologists are guilty of having used for too long, various extracts, concentrates, and hydrolysates of plant and animal tissues for growth of microorganisms without any concerted effort toward standardization of these ingredients.

Two approaches may be followed in an attempt to improve this situation. These are:

1. *Development of chemically defined media.*

The development of synthetic media for each test organism used in an assay procedure would be the ideal solution. However, despite the tremendous advances made in the field of bacterial nutrition, it is not always possible to construct a formula from pure chemical compounds that will support optimum growth of the test organism, and, even where this is possible, the number and cost of the individual ingredients required may sometimes be so great as to rule against routine use.

For example, much work has been done on the nutrition of *Micrococcus pyogenes* var. *aureus*, and it has been established that a mixture of amino acids, salts, glucose, thiamine, and nicotinic acid will suport growth of this organism. Several trial experiments with strain No. 209 (F.D.A.) have been carried out, using chemically defined media containing the ingredients listed above. Although the organism grows, no combination of chemicals used by us, as yet, supports what would be considered *good* growth. Further experimentation with this organism using chemically defined media with some additional ingredients appears justifiable.

The recent program carried out by the U. S. Pharmacopeia Panel on Vitamin B_{12} Assay is illustrative of the amount of work and complications encountered in designing a chemically defined medium for microbiological assay. However, this group has done an outstanding job in standardization of this type of laboratory procedure, both from the standpoint of technique and composition of the test medium.

2. *Standardization of complex culture media ingredients.*

Since it may not be possible to provide chemically defined routine media for the test organism, the alternative would be to standardize the complex nutrients employed, such as peptones, extracts, etc.

This has been accomplished with the peptone, trypticase. The method

of preparation and characteristics of the final product are set forth in the U.S.P. under the heading Pancreatic Digest of Casein (a bacteriological peptone). This form could serve as a model for standardization of other peptones and perhaps other ingredients.

It would seem that any attempt toward standardization of ingredients should cover the following points:

1. Description of raw material from which product is to be made.
2. Method of preparation.
3. Chemical analysis of final product.
4. Performance tests on the final product; *i.e.*, tests for ability to support growth or produce biochemical change desired using a selected set of test organisms.

In 1950, the British Society of General Microbiologists established a committee to work on the standardization of culture media ingredients. They have issued a preliminary report relative to the status of peptones.

In May 1951, the Society of American Bacteriologists, Committee on Bacteriological Technic, established a subcommittee for the same purpose. (The Associate Referee is a member of this committee.) These recent developments are in line with the work initiated in the subcommittee on Standard Methods for the Examination of Dairy Products of the American Public Health Association to develop a synthetic reference agar in 1950 and that initiated by the A.O.A.C. in 1949 to develop standard media for disinfectant testing. The establishment of all these committees is evidence of the general recognition that the cultural media situation must be improved.

It is believed that the immediate practical solution of the problem of developing a standard medium for propagating test cultures, to be used in testing disinfectants, may lie in replacement of both the presently accepted meat peptone and beef extracts by the U.S.P. Pancreatic Digest Casein, or in the single replacement of the peptone and the standardization of a beef extract by analogous procedures for use therewith. These possibilities will be explored in future work.*

REPORT ON PLANTS

By E. J. MILLER (Michigan Agricultural Experiment Station, East Lansing, Michigan), *Referee*

A number of the Associate Referees have been active during the past year in their studies of methods for the analysis of plants.

The Associate Referee on cobalt and copper in plants conducted a collaborative study on the orthonitrocresol method for cobalt and the diethyldithiocarbamate method for analyzing plant materials for these respective constituents, and the results of his studies are included in his report.

* For report of Subcommittee A and action of the Association see *This Journal*, 35, 45 (1952).

The Associate Referee on starch in plants has continued the study of his assignment and has attempted to revise certain procedures for determining starch in plant materials to overcome some of their shortcomings. The results of his studies are included in his report on the subject.

The Associate Referee on sugar in plants has reported the results of further studies on means of effectively clarifying plant extracts to be used for the determination of sugar.

The Associate Referee on zinc and carotene in plants, has reported the results of further study on the dithizone method for determining zinc in plants. No report on carotene in plants was submitted.

No report was received from L. K. Wood, Associate Referee on iodine and boron in plants.

Because of a change in positions R. L. Shirley asked to be relieved of his duties as Associate Referee on sodium in plants. Dr. Shirley's resignation was accepted with regret, and it is hoped that a successor to him can be found soon.

RECOMMENDATIONS*

It is recommended—

(1) That the Associate Referees continue with their respective assignments.

(2) That the following recommendations of the Associate Referees be accepted:

That the nitroso-R-salt method for cobalt in plants be further investigated collaboratively, but that further work with the nitrosocresol method be delayed at present.

That the collaborative work on the carbamate method for copper in plants be continued.

That a collaborative study of the ion-exchange resin technique for clarifying plant extracts for the determination of sugar be undertaken.

That the study of methods for the determination of zinc in plant materials be continued, and that the modified dithizone procedure be submitted to collaborative study, if collaborators can be found.

(3) That the study of methods for determining starch in plants be continued.

(4) That the study on methods for sodium in plants be continued as soon as an Associate Referee for this assignment can be secured.

(5) That the study of methods for determining carotene in plant materials be continued.

(6) That the study of methods for determining iodine and boron constituents of plant materials be continued.

(7) That the study of sampling plant materials for analysis be continued.

* For report of Subcommittee A and the action of the Association, see *This Journal*, **35**, 45 (1952).

ACKNOWLEDGMENT

The General Referee wishes to commend and thank the Associate Referees for their accomplishments during the past year.

REPORT ON ZINC IN PLANTS

By EUNICE J. HEINEN and ERWIN J. BENNE, *Associate Referee* (Michigan Agricultural Experiment Station, East Lansing, Michigan)

The present A.O.A.C. method for determining zinc in plants involves the photometric evaluation of zinc in the form of the dithizonate. At the meeting of the Association last year the authors described a modification of this method which requires less equipment and is less time consuming than the original procedure. The details of the revised procedure are included in the published report, *This Journal*, **34**, 692, (1951); hence they will not be repeated here.

During the past year the authors and their colleagues have continued to use the revised procedure successfully. With this procedure, duplication of results from separately ashed analytical portions is usually good, as illustrated by the following data:

	p.p.m. of zinc in alfalfa hays			
Sample No.	*1*	*2*	*3*	*4*
Duplicate results	30.6, 30.8	23.3, 22.2	27.3, 27.3	13.2, 14.2

On one occasion the authors were requested to analyze several samples of cracked corn which had been impregnated with zinc phosphide for use as a rodenticide. Zinc in these samples was determined by the modification of the above-mentioned method for zinc in plants, and also by the official mercury-thiocyanate method[1] for zinc in insecticides and fungicides. The results obtained for zinc in separately ashed portions of these samples by the two methods are given below:

Sample No.	*Per cent zinc*	
	modified dithizone method	*mercury-thiocyanate method*
1	1.34	1.62
2	1.16	1.05
3	.88	.82

The agreement in values by the two methods was considered to be very good, especially since the zinc phosphide was not distributed entirely uniformly throughout the grain, a fact which made it difficult to secure exactly similar analytical portions.

It was hoped that the revised procedure could be studied collaboratively during the past year, but no collaborators were secured to participate in this study. However, if collaborators can be found a collaborative study

[1] *Methods of Analysis*, 7th Ed., p. 54.

of it will be made next year. Consequently, it is recommended*—(1) that the study of methods for the determination of zinc in plants be continued; and (2) that the modified dithizone procedure be submitted to collaborative study.

REPORT ON STARCH IN PLANTS

THE USE OF THE ANTHRONE REACTION

By CARROLL L. HOFFPAUIR (Southern Regional Research Laboratory,[1] New Orleans, Louisiana), *Associate Referee*

Several recent publications (6, 8) have described the quantitative application of Dreywood's (1) anthrone reaction to the determination of small amounts of pure dry starch. McCready *et al.* (5) applied the reaction to the determination of starch in peas. This procedure involved removal of sugars from the peas with 80% alcohol, extraction of the starch with perchloric acid followed by colorimetric determination of starch in an aliquot of the extract using the anthrone reaction. These analysts suggested that modification of the method along the line of the procedure of Pucher *et al.* (7) should extend the scope of the method to other starchy vegetables and fruits.

In a collaborative study (2) of a modification of the Pucher procedure, several collaborators reported difficulty with the final steps in the method involving acid hydrolysis of the starch to glucose and determination of the reducing sugars produced. Consequently, the substitution of the colorimetric anthrone reaction for this part of the procedure was investigated. A modification of the McCready procedure in which calcium chloride extraction was substituted for the perchloric acid extraction was also tried. Details of the anthrone modified Pucher procedure follow.

METHOD

REAGENTS

Calcium chloride soln.—Dissolve 2 parts of crystalline calcium chloride hexahydrate in 1 part of water. Adjust to a density of 1.30. Make very faintly pink to phenolphthalein with 0.1 *N* sodium hydroxide and filter. Add 6 ml of 0.8% acetic acid to each 100 ml of soln.

Iodine-potassium iodide soln.—Grind 7.5 g of iodine and 7.5 g of potassium iodide with 150 ml of water. Dilute to 250 ml and filter.

Uranyl acetate.—5% aqueous soln.

Sodium chloride.—20% aqueous soln.

Alcoholic sodium chloride.—Mix 350 ml of ethanol, 80 ml of water, and 50 ml of 20% aqueous sodium chloride, and dilute to 500 ml with water.

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* For report of Subcommittee A and action of the Association, see *This Journal*, 35, 45 (1952).

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Alcoholic sodium hydroxide, 0.25 N.—Mix 350 ml of ethanol, 100 ml of water, and 25 ml of 5 *N* sodium hydroxide, and dilute to 500 ml with water.

Glucose standard.—Dissolve 0.100 g of anhydrous glucose in 100 ml of water. Dilute 10 ml of the stock soln to 500 ml and use aliquots of this soln for development of the colorimetric standards.

Anthrone-sulfuric acid.—Dissolve 1 g of anthrone in 500 ml of cold 95% sulfuric acid. Store at ca 0°C. and prepare fresh every 2 days. (This reagent is unstable and gives high blanks and variable results when too old.)

PROCEDURE

Modification of collaboratively investigated method (2).—Prepare sample as directed in method **6.2(b)**. Dry and grind to smaller than 80 mesh. Weigh accurately 50–250 mg of dry powdered tissue into a 100 ml heavy Pyrex contrifuge tube 30×170 mm. Add 5 ml of calcium chloride soln. Place the tube in an oil bath maintained at 120°C. and boil for 15 min. Stir the suspension frequently during the boiling period. Remove from the bath, cool, and dilute with 20 ml of water. Centrifuge for 10 min. at 2000 r.p.m. and decant the supernatant liquid into a 100 ml volumetric flask. Add a second 5 ml portion of calcium chloride soln to the residue, replace in the oil bath, and again boil for 15 min., with frequent stirring. Remove from the bath, cool, and transfer to the volumetric flask with distilled water. Add 3 ml of uranyl acetate soln to precipitate protein, make to volume with water, mix well, and centrifuge a portion. Pipet a 10 ml aliquot of the clear supernatant into a 25×150 mm Pyrex test tube. Add 5 ml of 20% sodium chloride soln and 2 ml of iodine-potassium iodide reagent, and mix well. Allow to stand overnight, centrifuge, and decant the supernatant liquid.

Wash the starch iodide precipitate obtained above by suspending it in 5 ml of alcoholic sodium chloride soln, centrifuge and decant the supernatant fluid. Add 2 ml of alcoholic sodium hydroxide soln to the packed precipitate. Gently shake and tap the tube until all the blue color is discharged. (A stirring rod must not be used and ample time must be allowed for the complex to decompose.) Centrifuge the liberated starch and wash with 5 ml of alcoholic sodium chloride as before.

Add 10 ml of water to the precipitate, place in the oil bath, and boil for 10 min. with frequent stirring. Transfer to a 250 ml volumetric flask, make to volume with water, and mix well. Transfer a 5 ml aliquot to a 22×175 mm heat-resistant test tube and cool in a water bath while adding 10 ml of anthrone-sulfuric acid reagent. In the case of multiple determinations, after the anthrone has been added to a series of tubes cooled in water, mix each one thoroly and heat them together for 7.5 min. in a boiling water bath. Cool the tubes rapidly to 25°C. in a water bath and determine color intensities, using light of wave lengths near 6300Å. (An Evelyn photoelectric colorimeter equipped with a No. 635 filter was used in this investigation.[2]) Prepare a standard curve from color standards containing 0–100 micrograms of glucose developed in the same way and at the same time as the starch aliquots. From this calibration curve obtain the yield of glucose from the starch in the aliquot. Multiply the glucose found by 0.90 to convert to starch.

DISCUSSION OF RESULTS

Calibration curves were prepared using both N.B.S. glucose and a sample of sweet potato starch of known purity. Over the concentration range investigated (0 to 100 micrograms in the 5 ml aliquot used for color

[2] The mention of trade products does not imply that they are endorsed or recommended by the Department of Agriculture over similar products not mentioned.

development), when the logarithms of the transmittancies were plotted against the concentration, the points fell on a straight line passing through the origin. Conversion of the data for glucose to its starch equivalent by use of the factor 0.90 gave a calibration curve identical with that obtained for starch under the same conditions. This is in agreement with the findings of previous investigators (5) and shows that 0.90 is the appropriate factor for the anthrone colorimetric method. Since the slope of the calibration curve varies with the age of the reagent, it is necessary to prepare color standards along with each series of determinations.

Several samples used in previous reports (2, 3, 4) were analyzed by the method outlined above. Values were also obtained by a modification of the McCready (5) procedure in which calcium chloride solution was used instead of perchloric acid for extracting the starch. The color standards used to obtain these values contained the same concentration of calcium

TABLE 1.—*Comparison of methods for determination of starch*

METHOD	STARCH FOUND		
	ALFALFA	PEANUT MEAL	BUCKWHEAT LEAVES
	per cent	*per cent*	*per cent*
Anthrone modified, Pucher method	.20	6.50	9.32
	.18	7.28	8.36
	.24	7.92	7.36
	.16	8.44	8.38
	.20	6.26	7.21
		5.96	7.41
		6.45	7.40
		6.30	6.92
		7.18	
		6.79	
		6.48	
		6.82	
Average	0.20	6.86	7.80
Modified, McCready method	0.76	7.00	10.62
	0.73	8.58	10.62
	1.05	9.72	11.90
	1.30	8.95	11.70
	1.24	8.50	8.90
	1.10	7.90	7.30
Average	1.03	8.44	10.18
Results from 1950 collaborative study (6) Avg.	0.35	6.87	9.34
Iodine colorimetric (6) Avg.	0.14	6.68	9.70

chloride as the sample aliquots since the presence of salts increases the intensity of the color developed. The values obtained, together with previously reported values on these samples, are shown in Table 1. It is apparent that poor agreement was obtained among replicate analyses by both procedures so that neither could be considered satisfactory. However, the average value for the alfalfa sample by the modified Pucher method approximates the values previously obtained for this sample, while the values by the modified McCready method are high probably on account of interfering substances which are not removed by the alcohol extraction. The average values for the peanut meal by all methods, except those obtained by the modified McCready procedure, are in reasonable agreement, while those for buckwheat leaves are low by the modified Pucher method.

It seems probable that the unsatisfactory results with the modified McCready method are due to interference by constituents from the samples such as non-starch polysaccharides, protein, and plant pigments. The poor agreement by the modified Pucher method may be due to losses in starch in the purification steps or to degradation of starch in the extraction. Further investigation is in progress* in an attempt to devise means of overcoming such difficulties.

CONCLUSIONS

A preliminary investigation of the application of the anthrone-sulfuric acid reagent to the determination of starch in plants indicates that all interfering materials are not removed by alcohol extraction. Attempts to apply the reaction to starch isolated by the Pucher procedure gave poor reproducibility.

ACKNOWLEDGMENT

The Associate Referee wishes to thank Miss E. R. McCall for some of the analyses reported.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **35**, 45 (1952).

REPORT ON SUGARS IN PLANTS

By KENNETH T. WILLIAMS (Western Regional Research Laboratory,[1] Albany, Calif.), *Associate Referee*

The study of clarification has been continued during the past year with the emphasis on leafy material (1).

Three clarification procedures were compared on aliquots of extracts of plant leaves: (A) No clarification other than filtration through Celite Analytical Filter-Aid,[2] (B) neutral lead acetate, and (C) the use of selected ion-exchange resins in a batch procedure (2).

The batch ion-exchange resin technique was equal or superior to neutral lead acetate for clarification in the analysis of the leaf extracts. A few plant leaf extracts required no clarification other than filtration through Celite for the determination of sugar.

While color may not contribute to the reducing value of the solution, it can tend to obscure the end point. This is especially true when sugar is determined by certain of the ferricyanide methods. The selected ion-exchange resins gave water-white solutions in all tests made on leafy materials. In some instances light straw-colored solutions were obtained when neutral lead acetate was used for clarification.

After fermentation, a few of the leaf extracts contained reducing substances. Non-fermentable reducing sugars were tenatatively identified in such extracts by paper chromatographic techniques.

RECOMMENDATION*

It is recommended that collaborative study of the ion-exchange resin technique for clarification of plant extracts be undertaken.

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REPORT ON COPPER AND COBALT IN PLANTS

By KENNETH C. BEESON (Plant, Soil, and Nutrition Laboratory, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Ithaca, N. Y.), *Associate Referee*

At the 1949 meeting of the Association it was recommended that a collaborative study of the method for cobalt and copper in plants be con-

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.
[2] The mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others not mentioned.
* For report of Subcommittee A and action of the Association, see *This Journal*, **34**, 45 (1952).

tinued.[1] In preparation for this the orthonitrosocresol method for cobalt was carefully revised to include several modifications and improvements in techniques adopted since the method was first published.[2] The revised method recommended for collaborative study at the 1950 meeting of the Association,[3] was used in this study.

COLLABORATORS

Samples and instructions were sent to ten analysts who had agreed to do collaborative work. Only five completed the analyses in time for this report. Those collaborators reporting are as follows:

1. Prof. I. Motzok, Department of Nutrition, Ontario Agricultural College, Guelph, Ontario.
2. Dr. F. B. Johnston, Chemist, Division of Chemistry, Department of Agriculture, Ottawa, Ontario.
3. Maurice M. Phillippe, Associate Agronomist, Clemson Agricultural College, Clemson, South Carolina.
4. Richard L. Gregory, U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, New York.
5. W. R. Flach, Laboratory Director, Eastern States Farmers' Exchange, Buffalo, New York.

DESCRIPTION AND PREPARATION OF SAMPLES

Three materials, buckwheat flour, timothy, and alfalfa, were selected for collaborative study of the cobalt method. The buckwheat flour required no grinding nor other preparation except further mixing by rolling the material on glazed paper. Portions of the material were reduced by quartering and subsequent mixing until a sample of about 50 grams was obtained.

The timothy was obtained from a low-cobalt area and was known to be deficient in that element. The dried material was ground in a Wiley mill through a 20-mesh screen and was carefully mixed and sampled in the same manner as the buckwheat flour. The alfalfa, a forage of fairly high cobalt content, was also ground and sampled in this manner.

DIRECTIONS FOR ANALYSIS

The collaborators were asked to ash each sample, prepare a solution from the ash, and to analyze the solution for cobalt in eactly the manner outlined in the paper by Gregory, Morris, and Ellis.[4] Prior to weighing out the sample the material was to be dried at 72°C. overnight and mixed before the portion required was removed. Duplicate samples of each weight were taken, as follows:

Sample No. 1, alfalfa (A) 4 grams, (B) 8 grams.
Sample No. 2, timothy (A) 6 grams, (B) 12 grams.

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[1] *This Journal*, **33**, 41 (1950).
[2] ELLIS, G. H., and THOMPSON, J. R., *Ind. Eng. Chem., Anal. Ed.*, **17**, 254 (1945).
[3] *This Journal*, **34**, 43 (1951).
[4] *This Journal*, **34**, 710 (1951).

Sample No. 3, buckwheat flour (A) 6 grams, (B) 12 grams.

The quantities indicated were ashed and 100 ml of solution prepared. Duplicate 40 ml aliquots were taken from each solution.

Where time and facilities permitted, each collaborator was requested to determine copper according to the method presented at the 1949 meeting of the Association.[5]

RESULTS OF THE COLLABORATIVE STUDY

In Table 1 there are presented the results obtained for cobalt by the collaborators. Each datum is presumably the average of duplicate determinations on each solution of the ash and of duplicate ashings. In addition, two weights of the material were taken for analysis. Thus, eight determinations should have been made on each sample. However, since only two collaborators submitted their data as individual determinations on duplicate ashings, and since one submitted one value only for each weight taken for ashing, it was necessary to average all the results in the same manner.

TABLE 1.—*Cobalt content of referee samples*
Moisture-free basis

REFEREE	SAMPLE NO. 1 ALFALFA		SAMPLE NO. 2 TIMOTHY		SAMPLE NO. 3 BUCKWHEAT FLOUR	
	A[1]	B[1]	A[2]	B[2]	A[2]	B[2]
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	0.06	0.24	0.04	0.07	0.06	0.07
2	.132	.149	.048	.050	—	.036
3	.146	.158	.049	.048	.052	.055
4	.13	.13	.05	.05	.06	.06
5	.172	.125	.050	.030	.055	.053
Average	.132	.160	.047	.050	.056	.055

[1] For A, a 1.6 g aliquot was taken from a total of 4.0 g of sample; for B, a 3.2 g aliquot was taken from an 8 g sample.
[2] For A, a 2.4 g aliquot was taken from a total of 6 g of sample; for B, a 4.8 g aliquot was taken from a 12 g sample.

The agreement obtained on Samples 2 and 3 is quite satisfactory. Results closer than 0.01 p.p.m. could hardly be expected, and with one or two exceptions the data fall within a range of that magnitude. The results obtained on the alfalfa are not acceptable. Variability in this sample has been a puzzling factor in the Associate Referee's laboratory, although consistent results are now being obtained (Collaborator No. 4). No explanation has been forthcoming, although the problem has been investigated with the radioisotope of cobalt. Recent findings[6] show that

[5] *This Journal*, **33**, 819 (1950).
[6] Beeson, K. C., and MacDonald, H. A., *Agron. Jour.*, **43**, 589, (1951).

there is little difference between the cobalt content of the leaves and stems of alfalfa or other legumes and grasses. Hence stratification of the material should not be important. Grinding the material to a finer mesh and resampling has not altered the conditions for obtaining erratic results. There was some indication[7] that more consistent results were obtained by collaborators on this sample using the nitroso-R-salt method than the nitrosocresol method for cobalt.

Only two collaborators reported on copper in the three samples (Table 2). The agreement, however, is very good at all ranges of copper content. As in the case of the cobalt data, the copper data are averages of the duplicate aliquots and ashings.

TABLE 2.—*Copper content of referee samples—moisture-free basis*

REFEREE	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 3
	p.p.m.	*p.p.m.*	*p.p.m.*
3	9.7	6.4	3.3
4	10.7	5.5	4.0

COMMENTS OF THE COLLABORATORS

No. 1. This collaborator felt that the erratic results obtained on Sample No. 1 might be due to a lack of experience with the method. His further comments were:

A. Adjustment of the *p*H solution before extraction with dithizone was found to be difficult, because the yellow color of ferric citrate interfered when phenolphthalein indicator was used. More indicator was required than the specified amount.

B. Difficulty was experienced in preventing leakage when vaseline was used as a stopcock lubricant.

C. Formation of the cobalt-nitrosocresol complexes and their extraction with Skellysolve B was complicated by the formation of an interphase scum which was removed only with frequent washing of the Skellysolve layer with distilled water. Losses of Skellysolve during the extraction and washing procedures amounted to as much as 0.25 ml.

D. The petroleum ether solution of cobalt-nitrosocresol was removed with a pipet having a finely-drawn tip. It was found that some water adhered to the Skellysolve and interfered with the photometric readings by fogging the cuvettes. Permitting the solutions to stand in the separatory funnels for two hours did not appear to correct the trouble.

E. A wave length of 370 mμ gave a straight line for the standard curve on semilogarithmic paper.

No. 2. This collaborator observed that checks were not good even on aliquots of the same solution. He also suggested that:

A. In the preparation of dithizone the directions should state a definite period for mechanical agitation before filtration, and

B. The last traces of perchloric acid used to destroy the dithizone should be removed by heating for a brief period over a burner.

No. 5. Considerable difficulty was experienced by this collaborator in keeping the orthonitrosocresol reagent longer than two weeks. Within this time it decom-

[7] *This Journal*, 33, 819–27 (1950).

posed and it was necessary to prepare a new reagent. He observed, further, that the method is cumbersome and subject to considerable variation. He has been very successful in using the nitroso-R-salt method for cobalt.

Comment of Collaborator No. 1 should be noted as a possible source of difficulty in his analyses. The directions specifically called for removal of the aqueous phase. To remove the Skellysolve phase free of water would be very difficult.

CONCLUSIONS

Collaborative results using the nitrosocresol method for cobalt show good agreement on samples containing small quantities of this element, but erratic results on alfalfa containing larger quantities of cobalt. The experience of collaborators, as well as previous collaborative results, suggest that somewhat better agreement would be obtained with the nitroso-R-salt method. It is apparent that while the nitrosocresol method is a desirable one for research problems involving small quantities of cobalt it may not be satisfactory for the routine determination of cobalt, particularly in relatively large quantities. Results on copper, although limited in number, were satisfactory.

RECOMMENDATIONS*

It is recommended—

(1) That the nitroso-R-salt method for cobalt in plants be further investigated collaboratively, but that further work with the nitrosocresol method be delayed at present.

(2) That the collaborative work on the carbamate method for copper in plants be continued.

No report was given on sampling plant materials, iodine and boron, carotene, or sodium.

REPORT ON SPECTROGRAPHIC METHODS

By W. T. MATHIS (Agricultural Experiment Station, New Haven, Conn.), *Referee*

The object of this year's collaborative work on spectrographic methods was to compare actual levels and consistency of results obtained by chemical, spectrographic, and flame photometer methods as standardized and run in different laboratories. This is a slight deviation from the recommendation presented in the Referee's last report, viz., that values be unified between laboratories through the use of reference samples. In view of the results obtained it seems fortunate that the approach was changed at this time because the study has brought to light the fact that

* For report of Subcommittee A and action of the Association, see *This Journal*, 34, 45 (1952).

chemical methods which depend upon comparative standardization are just as fallible as instrumental procedures.

Four different samples of alfalfa were submitted to each of twenty laboratories which had indicated a willingness to participate. It was requested that the samples be analyzed by their usual procedures, either chemical or instrumental, for elements included in their usual routine. On this basis, procedures unfamiliar to the analysts were not involved. Expected percentage ranges of the major elements were given as a guide for selection of control standards. It was further requested that general details of the procedures used be given in the reports as a matter of record for possible future consideration.

Three of the collaborative samples were prepared from commercial brands of alfalfa meal from mills in Ohio, Missouri, and Nebraska, and one sample was from experimental plots in Connecticut. It was hoped that the varying sources might contribute toward minor differences between samples.

Bulk samples were prepared, mixed by ball mill technique and subdivided. The subsamples were spot-checked spectrographically for uniformity. Some results reported on the "as received" basis were calculated to "oven dry basis" using moisture values obtained in our laboratory.

Unfortunately, only about half of the laboratories reported. This seriously handicaps the study in general and permits no comparison at all for variations within respective methods for some of the elements. There was, however, a tremendous amount of excellent work performed by the reporting laboratories, as is evidenced by the following figures: Number of determinations 1,184, sample means 468, and average of 2.44 determinations per sample mean. These means are the values tabulated and used for the statistical analyses. Twenty-five different chemical methods or modifications were used, in spite of the fact that several of the more unusual elements were not run chemically. Instrumental methods involving three main techniques, namely, spark, arc, and flame excitation, were used. Details of individual techniques within each group necessarily conform to some extent to the equipment combinations used.

Interpretation of these data is difficult, because of the uneven relationship between the number of determinations run by different laboratories, the number of laboratories within comparative method groups, the number of methods for respective elements, etc. However, in the study there is one common numerical factor, *i.e.* the four different samples. The sample relationships offer a valid basis for comparison of consistency within laboratories, and consequently of groups of laboratories within a method.

Upon the recommendation and under the guidance of Dr. C. I. Bliss, biometrician at the Conneecticut Experiment Station, an analysis of variance was made. The results are shown in Table 14. (Text resumed on page 414.)

TABLE 1.—*Potassium, per cent*

SAMPLE NO.	CHEMICAL					SPARK SPEC.				FLAME PHOTOMETER									SAMPLES	
	LABORATORY NUMBER																		MEAN	MEAN
	3	5	6	7	11	M	1	1a	M	3	4	6	7	8	9	10	12	M	*Methods*	*Labs.*
1	2.04	1.99	1.96	2.03	2.28		1.88	1.95		1.95	1.90	1.92	2.08	1.34*	1.85	2.17*	1.90			1.98
2	0.98	0.87	0.92	1.04	1.06		0.92	0.91		0.92	0.87	0.88	0.94	0.82	0.87	0.98	0.90			0.93
3	2.26	2.27	2.25	2.15	2.30		2.10	2.15		2.16	2.14	2.16	2.09	1.93	2.20	2.51	2.40			2.20
4	3.27	3.35	3.23	3.71	3.26		3.19	3.16		3.17	3.00	3.25	3.14	2.64	3.10	3.86	3.40			3.25
Mean Lab.	2.14	2.12	2.09	2.23	2.22	2.16	2.02	2.04	2.03	2.05	1.98	2.05	2.06	1.68	2.00	2.38	2.15	2.05	2.08	2.09
Line 1. Methods						+.08			−.05	* Labs. 8–10 not used in means.								−.03		
Line 2. Std. Dev.																			.07	
Line 3. Labs. Within Method	−.02	−.04	−.07	+.07	+.06		−.01	+.01		0.00	−.07	0.00	+.01	−.37	−.05	+.33	+.10			
Line 4. Std. Dev.						.06			.01									.19	(.06 without Labs. 8–10)	
Line 5. Samples Within Lab.—Std. Dev.	.037	.060	.033	.171	.148		.035	.017		.014	.065	.042	.097	.220	.065	.094	.115			
Line 6. Av. Std. Dev. for Method						.090			.026									.089		

Explanation of Terms Used in Tables

Line 1. Methods.—± dif. of method means from their average.

Line 2. Std. Dev.—of method means around their average.

Line 3. Labs. within Method—± dif. of lab. means from mean for method.

Line 4. Std. Dev.—of lab. means around mean for method.

Line 5. Samples within Lab.—Std. Dev. of ind. samples within lab. around the average sample pattern, determined as follows:

The sample means for each lab. is brought to unity with the value of lab. mean average by all methods. The ind. sample values within each lab. are then adjusted proportionally. Calibration differences between labs. and methods having been thus removed, it is now possible to directly compare ind. sample values within each lab. with the respective sample averages by all labs. (column "Samples—Mean of Labs"). The ± difs. within each lab. are expressed as Std. Dev. and represent the variance of the ind. technique around the average sample relationship pattern.

Line 6. Av. Std. Dev. for Method—is the average of the Std. Devs. in line 5 within the method, and indicates the precision of the method.

TABLE 2.—*Calcium, per cent*

SAMPLE NO.	CHEMICAL						SPARK SPEC.				ARC SPEC.		FLAME PHOTOMETER					SAMPLES	
	LABORATORY NUMBER																	MEAN	MEAN
	3	5	6	7	9	11	M	1	1a	M	12	M	3	4	9	10	M	*Methods*	*Labs.*
1	1.56	1.70	1.49	1.72	1.64	1.68		1.54	1.62		1.70		1.52	1.71	1.50	1.24			1.59
2	1.67	1.75	1.60	1.75	1.71	1.62		1.82	1.86		1.82		1.62	1.88	1.63	1.56			1.72
3	1.44	1.56	1.36	1.58	1.48	1.49		1.39	1.50		1.82		1.38	1.48	1.34	1.07			1.45
4	1.07	1.18	1.03	1.17	1.14	1.13		1.01	1.06		0.95		1.04	1.21	1.00	0.84			1.06
Mean Lab.	1.44	1.55	1.37	1.56	1.49	1.48	1.48	1.44	1.51	1.48	1.57	1.57	1.39	1.57	1.37	1.18	1.38	1.48	1.46
Line 1.* Methods Line 2. Std. Dev.							.00			.00		+.09					−.10	.08	
Line 3. Labs. Within Method Line 4. Std. Dev.	−.04	+.07	−.11	+.08	+.01	.00	.07	−.04	+.03	.05	−	−	+.01	+.19	−.01	−.20	.16		
Line 5. Samples Within Lab.—Std. Dev. Line 6. Av. Std. Dev. for Method	.022	.055	.025	.057	.044	.088	.048	.082	.051	.066	.175	.175	.025	.064	.017	.151	.064		

* See explanation, Table 1.

TABLE 3.—*Magnesium, per cent*

SAMPLE NO.	CHEMICAL							SPARK SPEC.			ARC SPEC.			FLAME PHOTOMETER			SAMPLES	
	LABORATORY NUMBER																MEAN OF	MEAN OF
	3	5	6	7	9	10	11	M	1	1a	M	8	12	M	3	4	M	*Methods*	*Labs.*
1	.290	.160	.326	.301	.290	.330	.298		.291	.290		.315	.328		.297	.284			.292
2	.463	.480	.522	.447	.445	.470	.501		.475	.479		.435	.530		.463	.399			.470
3	.220	.200	.367	.232	.225	.260	.293		.231	.207		.300	.278		.207	.210			.248
4	.170	.280	.204	.196	.195	.230	.325		.204	.196		.275	.218		.170	.193			.220
Mean Lab.	.286	.280	.355	.294	.289	.322	.354	.311	.300	.293	.296	.331	.338	.334	.284	.272	.278	.305	.308
Line 1.* Methods Line 2. Std. Dev.								+.006			−.009			+.029			−.027	.024	
Line 3. Labs. Within Method Line 4. Std. Dev.	−.025	−.031	+.044	−.017	−.022	+.011	+.043	.032	+.004	−.003	.005	−.003	+.004	.005	+.006	−.006	.008		
Line 5. Samples Within Lab.—Std. Dev. Line 6. Av. Std. Dev. for Method	.023	.092	.049	.022	.013	.018	.045	.037	.013	.028	.020	.047	.015	.031	.035	.020	.028		

* See explanation, Table 1.

TABLE 4.—*Phosphorus, per cent*

SAMPLE NO.	CHEMICAL										SPARK SPEC.			ARC SPEC.		SAMPLES	
	LABORATORY NUMBER															MEAN OF	MEAN OF
	3	4	5	6	7	8	9	10	11	M	1	1a	M	12	M	*Methods*	*Labs.*
1	.243	.229†	.245	.262	.255	.420*	.225	.250	.253		.222	.221		.240			.242
2	.239	.147	.235	.259	.258	.400	.240	.250	.247		.228	.229		.225			.244
3	.276	.199	.265	.296	.293	.450	.275	.280	.295		.260	.246		.293			.278
4	.243	.186	.250	.270	.258	.410	.250	.260	.262		.235	.235		.245			.251
Mean Lab.	.250	.190	.249	.272	.266	.420	.248	.260	.264	.258	.236	.233	.234	.258	.258	.250	.254
Line 1.* Methods Line 2. Std. Dev.	† Labs. 4–11 not used in means									+.008			−.016		+.008	.014	
Line 3. Labs. Within Method Line 4. Std. Dev.	−.008	−.068*	−.009	+.014	+.008 † Not used.	+.162*	−.010	+.002	+.006	.009	+.002	−.002	.002	—	—		
Line 5. Samples Within Lab.—Std. Dev. Line 6. Av. Std. Dev. for Method	.004	.046	.007	.003	.003	.008	.008	.003	.006	.010	.002	.007	.005	.010	.010		

* See explanation, Table 1.

TABLE 5.—*Manganese, parts per million*

SAMPLE NO.	CHEMICAL						SPARK SPEC.			ARC SPEC.				SAMPLES	
	LABORATORY NUMBER													MEAN OF	MEAN OF
	3	4	6	10	11	M	1	1a	M	7	8	12	M	*Methods*	*Labs.*
1	21.7	13.0	17.0	20.0	11.0		12.7	13.0		31.9	13.6	26.5			18.0
2	50.3	66.0	46.4	51.0	42.0		40.8	43.3		74.0	49.0	58.5			52.1
3	40.0	54.0	35.1	37.0	32.0		32.7	32.0		45.3	48.0	53.0			40.9
4	38.3	45.0	30.9	30.0	43.0		29.0	28.3		26.6	30.1	42.0			34.3
Mean Lab.	37.6	44.5	32.4	34.5	32.0	36.2	28.8	29.2	29.0	44.4	35.2	45.0	41.5	35.6	36.3
Line 1.* Methods Line 2. Std. Dev.						+0.6			−6.6				+5.9	6.3	
Line 3. Labs. Within Method Line 4. Std. Dev.	+1.4	+8.3	−3.8	−1.7	−4.2	5.1	−0.2	+0.2	0.3	+2.9	−6.3	+3.5	5.5		
Line 5. Samples Within Lab.—Std. Dev. Line 6. Av. Std. Dev. for Method	2.8	4.9	1.1	2.8	9.7	4.3	1.8	1.7	1.8	10.1	5.9	3.6	6.5		

* See explanation, Table 1.

TABLE 6.—*Iron, parts per million*

SAMPLE NO.	CHEMICAL				SPARK SPEC.			ARC SPEC.				SAMPLES	
	LABORATORY NUMBER											MEAN OF	MEAN OF
	4	6	10	M	1	1a	M	7	8	12	M	*Methods*	*Labs.*
1	434	437	296		415	433		287	78	440			403
2	74	116	101		82	80		66	56	143			91
3	392	468	397		405	423		250	87	460			417
4	141	170	155		135	140		48	84	205			148
Mean Lab.	260	298	237	265	259	269	264	163	76	312	184	264	265
Line 1.* Methods Line 2. Std. Dev.				1			0	Not used in means—			−81	—	
Line 3. Labs. Within Method Line 4. Std. Dev.	−5	+33	−28	31	−5	+5	7	−21	−108	+128	120		
Line 5. Samples Within Lab.—Std. Dev. Line 6. Av. Std. Dev. for Method	26	11	49	29	14	20	18	56	143	32	77		

* See explanation, Table 1.

TABLE 7.—*Aluminum, parts per million*

SAMPLE NO.	SPEC.			SPEC.			SAMPLES	
	SPARK			ARC			MEAN OF	MEAB OF
	1	1a	M	7	12	M	*Methods*	*Labs.*
1	222	267		101	650			310
2	65	63		71	200			100
3	392	447		128	885			463
4	113	117		53	348			158
Mean Lab.	198	224	211	88	521	305	258	258
Line 1.* Methods Line 2. Std. Dev.			−47			+47	66	
Line 3. Labs. Within Method Line 4. Std. Dev.	−13	+13	18	−217	+217	306		
Line 5. Samples Within Lab.—Std. Dev. Line 6. Av. Std. Dev. for Method	32	34	33	81	18	50		

* See explanation, Table 1.

TABLE 8.—*Sodium, parts per million*

SAMPLE NO.	CHEMICAL		SPEC. SPARK			SPEC. ARC		PHOTOMETER FLAME					SAMPLES MEAN OF	SAMPLES MEAN OF
	6	M	1	1a	M	12	M	4	6	8	9	M	*Methods*	*Labs.*
1	1033		368	437		363		475	776	530	800			598
2	980		362	390		380		615	664	570	1000			620
3	850		362	393		413		375	564	450	800			526
4	755		283	273		220		195	394	275	600			374
Mean Lab.	904	904	344	373	358	344	344	415	600	456	800	568	544	530
Line 1.* Methods Line 2. Std. Dev.		+360			−186		−200					+24	261	
Line 3. Labs. Within Method Line 4. Std. Dev.	—	—	−14	+15	21	—	—	−153	+32	−112	+332	174		
Line 5. Samples Within Lab.—Std. Dev. Line 6. Av. Std. Dev. for Method	50	50	57	45	51	73	73	123	59	43	48	68		

* See explanation, Table 1.

TABLE 9.—*Copper, parts per million*

SAMPLE NO.	CHEMICAL LABORATORY NUMBER			SPARK SPEC. LABORATORY NUMBER			ARC SPEC. LABORATORY NUMBER				SAMPLES MEAN OF	SAMPLES MEAN OF
	6	10	M	1	1a	M	7	8	12	M	*Methods*	*Labs.*
1	3.3	12.9		10.2	12.3		10.6	9.1	8.4			9.5
2	3.6	14.2		11.6	15.0		10.0	8.9	9.6			10.4
3	2.8	10.8		8.2	10.9		5.9	9.0	8.2			8.0
4	2.8	11.8		11.8	13.9		8.0	12.1	7.7			9.7
Mean Lab.	3.1	12.4	7.8	10.4	13.0	11.7	8.6	9.8	8.5	9.0	9.5	9.4
Line 1.* Methods Line 2. Std. Dev.			−1.7			+2.2				−.5	2.0	
Line 3. Labs. Within Method Line 4. Std. Dev.	−4.7	+4.6	6.6	−1.3	+1.3	1.8	−.4	+.8	−.5	.7		
Line 5. Samples Within Lab.—Std. Dev. Line 6. Av. Std. Dev. for Method	.85	.51	.68	.70	.48	.59	1.60	.52	.95	1.02		

* See explanation, Table 1.

TABLE 10.—*Boron, parts per million*

SAMPLE NO.	CHEMICAL				SPARK SPEC.			ARC SPEC.			SAMPLES	
	LABORATORY NUMBER										MEAN OF	MEAN OF
	3	4	10	M	1	1a	M	7	12	M	*Methods*	*Labs.*
1	56.5	23.7	58.0		43.8	44.0		48.0	48.0			46.0
2	14.5	7.2	29.0		13.0	13.3		18.5	12.5			15.4
3	34.0	20.0	46.0		30.5	32.3		53.0	30.3			35.2
4	29.5	16.0	42.0		22.2	22.3		45.5	23.8			28.8
Mean Lab.	33.6	16.7	43.8	31.4	27.4	28.0	27.7	41.2	28.6	34.9	31.3	31.4
Line 1.* Methods				+.1			−3.6			+3.6		
Line 2. Std. Dev.											3.6	
Line 3. Labs. Within Method	+2.2	−14.7	+12.8		−.3	+.3		+6.3	−6.3			
Line 4. Std. Dev.				13.9			.4			8.9		
Line 5. Samples Within Lab.—Std. Dev.	4.6	2.2	4.4		3.1	2.8		5.0	4.3			
Line 6. Av. Std. Dev. for Method				3.7			3.0			4.6		

* See explanation, Table 1.

TABLE 11.—*Miscellaneous elements, parts per million*

SAMPLE NO.	METHOD	ZINC		SPEC.	MOLYBDENUM		SPEC.	LEAD		SPEC.	COBALT
		CHEM.		ARC	CHEM.		ARC	CHEM. A*	CHEM. B*	ARC	CHEM.
	LAB. NO.	6	10	7	6	9	7	6	6	7	6
1		33	44	27	2.8	4.6	5.4	3.2	1.1	3.7	0.22
2		24	31	N.D.	3.8	5.0	4.0	0.0	1.1	1.6	0.12
3		29	35	40	2.1	4.4	4.8	1.1	1.1	2.1	0.18
4		15	30	27	2.2	4.0	7.0	2.1	0.0	1.1	0.06

* A—Wet Ashed. B—Dry Ashed.

The variance values are relative within each element column. For example, line 1 shows that for potassium the methods used differ by a greater degree than do the laboratories within the chemical and spark methods (lines 2 and 3), and by a smaller degree than do the laboratories within the flame method (line 5). In other words, the methods are a more important factor in causing differences in results than are the laboratories

TABLE 12.—*Additional Determinations by Lab. 7 (Parts Per Million)*

	METHOD—SPEC. ARC—(AS RECEIVED BASIS)					
SAMPLE NO.	Ba	Cr	Ni	Sr	Ti	V
1	6.5	1.0	9.0	20	22.5	1.75
2	9.0	0.15	0.2	30	8.5	N.D.
3	19.0	0.5	N.D.	20	25.0	N.D.
4	22.5	0.55	N.D.	30	13.5	N.D.

TABLE 13.—*Non-metals by chemical methods*

ELEMENT		N%		S%	Cl%	F P.P.M.
SAMPLE	LAB. NO.	4	10	9	9	9
1		3.07	2.84	0.42	0.32	14.4
2		2.77	2.50	0.25	0.48	4.8
3		3.02	2.76	0.26	0.29	10.9
4		2.59	2.51	0.22	0.80	9.7

within the chemical and spark groups, while differences between laboratories within the flame group are greater than the differences between methods. Line 6 shows the comparative variation of the samples, which we know are quite different. Line 7 shows the variation of relative sample pattern between methods. Lines 8, 9, 10, and 11 show the variation of the laboratories within the method from the sample pattern of the method. These last values represent the comparative precision of the respective methods, excluding calibration errors.

Obviously, further breakdown of certain of the values in this table is necessary before respective proportions within a value, and possible reasons for some of the variances, can be deduced.

Rather than to continue in terms of logarithmic values it seemed desirable to follow a procedure of the writer's device in which the differences were expressed in terms more easily related to accustomed tolerances. This second procedure, while disregarding some of the factors involved in the conventional analysis of variance, enables one to visualize the pertinent situation at a glance. These data are shown for each element in its respective table and partially summarized for all elements in Table 15.

TABLE 14.—*Results of analysis of variance of the logs. of the analytical data in tables 1–10*

TABLE NO.	1	2	3	4	5	6	7	8	9	10
ELEMENT	K	Ca	Mg	P	Mn	Fe	Al	Na	Cu	B
1. Methods (Labs. within Method)	.005057	.005378	.012033	.008173	.058712	.149213	.018090	.186090	.148019	.019053
2. Chemical	.000969	.001760	.010476	.032262	.011028	.010202	—	—	.719400	.203701
3. Spec.—Spark	.000036	.000924	.000128	.000072	.000028	.000242	.002484	.001953	.018721	.000190
4. Spec.—Arc	—	—	.000112	—	.022233	.329679	1.063612	—	.004904	.060204
5. Flame Phot.	.006228	.011487	.000066	—	—	—	—	.077101	—	—
6. Samples	.779487	.108585	.283971	.008570	.422839	.677822	.327370	.092925	.016635	.309074
7. Sample×Method	.000313	.001916	.002265	.000481	.007845	.013092	.022981	.004866	.001766	.002339
8. Sample×Labs.—Chem.	.000550	.000069	.006623	.000755	.007962	.004311	—	—	.000150	.005040
9. Sample×Labs.—Spark	.000046	.000057	.000264	.000073	.000145	.000090	.000868	.000679	.000305	.000059
10. Sample×Labs.—Arc	—	—	.003131	—	.011773	.035963	.017413	—	.006523	.007750
11. Sample×Labs.—Flame	.000583	.000389	.001277	—	—	—	—	.003993	—	—

TABLE 15.—*Relative precision of methods as inidcated by the two different statistical treatments*

ELEMENT		K	Ca	Mg	P	Mn	Fe	Al	Na	Cu	B
Av. Std. Dev.	Chem.	.090	.048	.037	.010	4.3	29	—	50	.68	3.7
Lines 6, Tables (1–10)	Spark	.026	.066	.020	.005	1.8	18	33	51	.59	3.0
	Arc	—	.175	.031	.010	6.5	77	50	73	1.02	4.6
	Flame	.089	.064	.028	—	—	—	—	68	—	—
Variance of the Logs.	Chem.	.000550	.000069	.006623	.000755	.007962	.004311	—	—	.000150	.005040
(Lines 8, 9, 10, 11.	Spark	.000046	.000057	.000264	.000073	.000145	.000090	.000868	.000679	.000305	.000059
Table 14)	Arc	—	—	.003131	—	.011773	.035963	.017413	—	.006523	.007750
	Flame	.000583	.000389	.001277	—	—	—	—	.003993	—	—

For comparative purposes the various factors have been expressed in terms of the standard deviation and average standard deviation.

To use Table 1 as an example, line 1 (methods) shows that the mean of the total amount of potassium found by each method deviated from the mean of all methods by the amounts indicated. It is, of course, understood that instrumental methods may be brought into agreement with any desired standard levels through the use of appropriate and uniform standardization procedure.

Lines 3 and 5 are the key to evaluation of individual laboratory performance: (1), if the ± difference in line 3 and the standard deviation in line 5 are both small, accurate standardization and good technique are indicated; (2), if line 3 figures are small and line 5 figures large, either poor technique or, in instrumental analysis, a combination of standardization level error and interference within sample pattern is indicated; (3), if line 3 is large and line 5 is small, standardization level is in error in the degree and direction indicated; (4), if both lines 3 and 5 are large, the entire procedure requires improvement.

On this basis reference to Table 1 shows that within the chemical method laboratories 3, 5, and 6 are much better in technique than laboratories 7 and 11.

In the spark method, it might be said at this point that "Labs. 1 and 1a" refer to complete sets of results by two different operators using the same equipment. Although the work was spaced some weeks apart, agreement between laboratories 1 and 1a is therefore favorably biased and should be so considered throughout the study.

The flame method is shown to be approximately equal to the chemical method for potassium by both the analysis of variance (Table 14, lines 8 and 11) and in Table 1, line 6. It is apparent, however, from lines 3 and 5, Table 1, that laboratory 10 is off in standardization level and that laboratory 8 is in need of improvement in over-all procedure.

The same reasoning and evaluation procedure may be applied to the data for the other elements as given in their respective tables.

To pass on to the final comparative evaluation of methods, reference is again made to a summary (Table 15) showing a comparison of results by the two statistical treatments. If these two sets of more or less comparative data are "mentally merged" as to pattern, while keeping in mind the factors within individual laboratories which have influenced these results, a mental picture is formed of the actual potential of each method. Such an impression is more valid than any actual figure arrived at from the limited data in this study. The following conclusions are made:

Potassium.—Spark and flame methods are equal to, or better than, the chemical methods.

Calcium.—Spark and flame methods are about equal to the chemical

method but require very careful standardization for potassium influence. The d. c. arc is too erratic.

Magnesium.—Spark, arc, and flame methods are superior to the chemical methods.

Phosphorus.—Spark and arc methods are equal to the chemical method, but careful control of K and Ca influence is required. The colorimetric methods within the chemical group are subject to extreme level error, and if these results are included the spark and arc methods are superior to the chemical methods as a whole.

All Minor Elements.—The spark method shows better control in general than either chemical or arc methods, and although these results are possibly biased as far as comparisons in this study are concerned, it is a fact that a remarkably consistent result pattern is the rule, on all of these elements, by spark procedure. Chemical method rates second, although the differences between laboratories within this group are terrific, particularly for copper and boron. The arc method consistently gives slightly wider results than does the chemical method. For sodium, where the flame method again enters the picture, it would appear that there is little choice between the four methods. The wide differences in sodium values by these methods, and the fact that the chemical and arc methods are represented by only one laboratory each, raises the question of whether the method differences might not simply be laboratory differences.

One fact is glaringly apparent. The practice of reporting ultimate values for these elements to several significant figures, when such agreement between laboratories is not even approached, is not only meaningless but misleading. On the other hand, if a technique is capable of validating such figures within a given experiment, the additional significant figures become important. It, therefore, behooves the A.O.A.C., through appropriate referees, to try to achieve more uniformity in results by colorimetric chemical methods as well as instrumental methods in the hands of different laboratories.

RECOMMENDATION*

It is recommended—

That the detailed standardization procedure of each of the laboratories which participated in this work be obtained and studied in relation to the respective results reported, with a view toward achieving improvement and uniformity in this regard. It may then be possible for the A.O.A.C. to specify, in principle, and in detail, procedures for standardization which will bring all of these methods into the fold, without having to worry about the various and relatively unimportant details regarding equipment and procedures used.

* For report of Subcommittee B and action of the Association, see *This Journal*, 35, 47 (1952).

COLLABORATORS

The Referee wishes to acknowledge and thank the following collaborators for their kind and excellent assistance in this study:

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REPORT ON COAL-TAR COLORS

By KENNETH A. FREEMAN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

RECOMMENDATIONS*

The Referee concurs in the recommendations of the Associate Referees that the proposed methods for the following determinations be adopted, first action, and that the topics be continued:

Subsidiary Dyes in FD&C Colors—Lower Sulfonated Dyes in FD&C Yellow No. 5.

Sulfonated Amine Intermediates in Coal-Tar Colors—Lake Red C Amine in D&C Red Nos. 8 and 9.

The Referee concurs in the recommendation of the Associate Referee that the topic, Paper Chromatography of Coal-Tar Colors, be submitted to further study and that the topic be continued.

The Referee recommends that the following topics be continued.

Inorganic Salts in Coal-Tar Colors.
Ether Extracts in Coal-Tar Colors.
Halogens in Halogenated Fluoresceins.
Identification of Coal-Tar Colors.
Volatile Amine Intermediates in Coal-Tar Colors.
Unsulfonated Phenolic Intermediates in Coal-Tar Colors.
Non-Volatile Unsulfonated Amine Intermediates in Coal-Tar Colors.
Intermediates Derived From Phthalic Acid.
Subsidiary Dyes in D&C Colors—4-Toluene-azo-2-naphthol in D&C Red No. 35.
Lakes and Pigments.
Spectrophotometric Testing of Coal-Tar Colors.
Determination of Arsenic and Antimony in Coal-Tar Colors.
Boiling Range of Amines Derived from Coal-Tar Colors.
Determination of Heavy Metals in Coal-Tar Colors.
Sulfonated Phenolic Intermediates in Coal-Tar Colors.
Intermediates in Triphenylmethane Dyes.

* For report of Subcommittee B and action of the Association, see *This Journal*, 35, 48 (1952).

REPORT ON SULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS

DETERMINATION OF LAKE RED C AMINE IN D&C RED NOS. 8 & 9

By NATHAN ETTELSTEIN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

Lake Red C Amine (2-chloro-5-aminotoluene-4-sulfonic acid), is one of the intermediates used in the manufacture of D&C Red No. 8 and D&C Red No. 9. In order to be certified, these colors must not contain more than 0.2 per cent of the free amine (1).

Newburger (2) has described a procedure for the determination of Lake Red C Amine in D&C Red Nos. 8 and 9. He precipitated the dye as the barium salt and extracted the Lake Red C Amine with hot water. He then coupled the extracted amine with H-acid and determined the amount colorimetrically. The method proposed by Newburger has been modified by substituting a direct spectrophotometric estimation of the Lake Red C Amine for the colorimetric determination.

METHOD

REAGENTS

Standard soln of Lake Red C Amine (10 mg/l).—Dry a purified sample of sodium salt of Lake Red C Amine for four hours at 105°C. Transfer 100 mg of the amine to a 200 ml volumetric flask and add ca 150 ml of water. When all the amine is dissolved, make to volume with water and mix well. Transfer a 10 ml aliquot to a 500 ml volumetric flask and dilute to ca 450 ml with water and make the standard soln slightly alkaline (*p*H about 8) with (1+1) ammonium hydroxide. Dilute to the mark with water and mix thoroly.

$BaCl_2$.—2% in water.

PROCEDURE

Transfer a 1.0 g sample of the dye to a 500 ml tall-form beaker. Wet the sample with 5 ml of acetone and then add 100 ml of 2% $BaCl_2$ soln. Boil the mixture for 10 min and filter the hot mixture thru a Whatman No. 12 folded filter paper into a 500 ml Pyrex separator. Return the filter paper and the dye slurry it contains to the original beaker, repeat the boiling water extraction and filter as before. Make a third hot water extraction in the same manner. Discard the filter paper and dye slurry.

Cool the combined filtrates, acidify with 5 ml of dilute HCl (1+1) and extract with three 20 ml portions of benzene. Wash the combined benzene extracts with a 20 ml portion of water and add this wash water to the pooled filtrates.

Insert a cotton plug into the stem of the separator and filter the soln into a 500 ml beaker. Add boiling chips to prevent bumping and boil the soln 15–20 min to remove the benzene. Cool the soln and adjust the *p*H to about 8.0 with dilute NH_4OH. Transfer the alkaline soln to a 500 ml volumetric flask, dilute to the mark with water and mix thoroly.

Determine the absorbancy of the sample and the standard at 247 mμ. Calculate the per cent of Lake Red C Amine in the sample by means of the following equation:

$$\% \text{ Lake Red C Amine} = \frac{A_{Un} \times 0.91C \times 100}{A_{Std} \times W},$$

where A_{Un} = Absorbancy of the sample at 247 mμ.
A_{Std} = Absorbancy of the standard at 247 mμ.
C = Concentration of the standard in milligrams per liter.
W = Weight of sample in milligrams.

EXPERIMENTAL

A sample of D&C Red No. 9 free from Lake Red C Amine was prepared by extracting the dye twice with boiling water, drying at 135°C., and powdering. A 20 g portion of the dye and 440 mg of the sodium salt of Lake Red C Amine were thoroly mixed in a ball mill. This mixture was

TABLE 1.—*Analysis of D&C Red No. 9 containing 20 mg per gram of Lake Red C Amine*

SAMPLE NO.	LAKE RED C AMINE FOUND	LAKE RED C AMINE RECOVERED
	mg	*per cent*
1	17.4	87.0
2	17.7	88.5
3	17.2	86.0
4	17.5	87.5
5	17.1	85.5
Average	17.4	86.9

TABLE 2.—*Determination of Lake Red C in D&C Red No. 9*
Collaborative results

COLLABORATOR	LAKE RED C AMINE SAMPLE 1	LAKE RED C AMINE SAMPLE 2
	per cent	*per cent*
1	0.69	1.31
		1.07
2	0.56	1.38
		1.42
3	0.56	1.23
	0.57	1.27
4	0.55	1.07
	0.54	1.07
5	0.64	1.33
	0.63	1.20
Average	0.59	1.23
Lake Red C Amine Added	0.6	1.2

then analyzed for Lake Red C Amine by the proposed method. The results are shown in Table 1.

Two samples of D&C Red No. 9 containing added Lake Red C Amine were prepared and submitted to the collaborators for analysis. The results are given in Table 2.

The Associate Referee wishes to express his thanks to the following organizations for their cooperation:

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Calco Chemical Division, American Cyanamid Company.

RECOMMENDATIONS

It is recommended that the proposed method for the determination of Lake Red C Amine in D&C Red Nos. 8 and 9 be adopted, first action, and that the topic be continued.

REFERENCES

(1) Service and Regulatory Announcements, Food, Drug, and Cosmetics, No. 3. Coal-Tar Color Regulations.
(2) NEWBERGER, S. H., *This Journal*, **24**, 908 (1941).

REPORT ON SUBSIDIARY DYES IN FD&C COLORS—LOWER SULFONATED DYES IN FD&C YELLOW NO. 5

By MEYER DOLINSKY (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

In the Associate Referee's report[1] presented at last year's meeting, it was shown that lower sulfonated dyes in FD&C Yellow No. 5 could be determined accurately by an extraction procedure followed by spectrophotometric determination of the extracted color. It was recommended at that time that the procedure be submitted to collaborative study.

Two mixtures were prepared, each containing purified FD&C Yellow No. 5 to which a known amount of monosulfonated product had been added. These mixtures were then analyzed by the Associate Referee and by the following collaborators:

Calco Chemical Division, American Cyanamid Company—Wm. Seaman reporting.

Food and Drug Administration—Louis C. Weiss and Charles Graichen reporting.

Harmon Color Works, Inc.—Vincent C. Vesce reporting.

National Aniline Division, Allied Chemical and Dye Corporation—A. T. Schramm reporting.

[1] Dolinsky, M., *This Journal*, **34**, 411 (1951).

TABLE 1.—*Determination of lower sulfonated dye in FD&C Yellow No. 5*

	PER CENT SUBSIDIARY ADDED	PER CENT SUBSIDIARY RECOVERED						
		BY ASSOC. REFEREE	BY COLLABORATORS— 1	2	3	4	5	AVERAGE
Sample I	3.9	3.8	3.8	3.7	4.0	3.8	4.0	3.8
			3.9	3.5	3.7	3.8		
					3.8			
					Mean Deviation			0.09
					Maximum Deviation			0.3
Sample II	1.0	1.1	0.8	1.0	0.9	1.2	0.9	1.0
			0.9	1.0	0.9	1.3		
					0.9	1.0		
					Mean Deviation			0.1
					Maximum Deviation			0.3

Results are shown in Table 1.

Since the procedure was not given in detail in the previous report, it is described below.

METHOD

REAGENTS

Isoamyl alcohol.—Reagent grade.
Petroleum ether.—Reagent grade.
Hydrochloric acid.—Conc.
Hydrochloric acid.—Ca 0.25 *N*.

Spectrophotometric Standard

Either (a) the purified disodium salt of 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-phenylazopyrazole, or (b) purified FD&C Yellow No. 5 (recrystallize twice from (1+1) alcohol-water; dry at 135°C.) may be used as the standard.

Dissolve 100 mg of the standard dye in 1000 ml of H_2O. Transfer a 10 ml aliquot of this soln to a 100 ml volumetric flask, add ca 1 g of solid ammonium acetate and dilute to volume with water.

$$\text{For Standard (a)} \quad \% \text{ Subsidiary} = \frac{\text{Absorbancy of sample at 434 m}\mu}{\text{Absorbancy of standard at 434 m}\mu}$$

$$\text{For Standard (b)} \quad \% \text{ Subsidiary} = \frac{\text{Absorbancy of sample at 434 m}\mu}{1.1 \times \text{Absorbancy of standard at 434 m}\mu}$$

APPARATUS

A spectrophotometer capable of measuring absorbancy at 434 mμ.

PROCEDURE

Prepare a soln containing 200 mg of the color in 100 ml of H_2O. (Heat on steam bath if necessary for complete solution.) To 50 ml of this soln add 1 ml of HCl and extract the lower sulfonated dye by shaking the soln successively in 3 separatory funnels, each containing 50 ml of amyl alcohol. Wash the amyl alcohol extracts by shaking successively with 50 ml portions of 0.25 *N* HCl until the washings are practically colorless, passing each acid portion thru the funnels in the order used for

the original amyl alcohol extraction. Dilute the amyl alcohol extracts in each funnel with 1–2 volumes of petroleum ether and remove the lower sulfonated dye by washing with several 10–20 ml portions of water, passing each portion thru the 3 funnels in the reverse order to that previously followed. Transfer the extracted color to a 100 ml volumetric flask, add ca 1 g of solid ammonium acetate, dilute to volume with water, and measure the absorbancy of the unknown soln and the standard soln at 434 mμ using 1 cm cells.

RECOMMENDATIONS*

It is recommended—

(1) That the proposed method for the determination of lower sulfonated dyes in FD&C Yellow No. 5 be adopted, first action.

(2) That study of the topic be continued.

REPORT ON PAPER CHROMATOGRAPHY OF COAL-TAR COLORS

By DORIS H. TILDEN (Food and Drug Administration, Federal Security Agency, San Francisco, Calif.), *Associate Referee*

Among the many applications of paper partition chromatography, there seem to be relatively few involving the separation and isolation of dye materials. Shibata *et al.* (5) separated and isolated some of the anthraquinone pigments; Muller and Clegg (4) made investigations of some dyestuffs on "ascending" paper strips, and Diemar and Janecke (2) have identified the natural pigments carotene and annatto in the presence of dimethylaminoazobenzene. There apparently has been, however, no systematic investigation of the application of this technique to the one hundred and seventeen permitted colors for which regulations and specifications have been set forth in Service and Regulatory Announcements, Food, Drug, and Cosmetics No. 3 (9).†

The successful application of paper chromatography to the separation and partial identification of certain coal-tar colors found in food samples led to further investigation of the technique. Results of these studies suggested combining this procedure with portions of established schemes in developing a more efficient and complete method for separating the coal-tar dyes from each other and from products in which they may be found. The efforts of the Associate Referee this year were directed toward this end, with special reference to the certifiable FD&C, D&C, and External D&C dyes.

Analysis of the dyestuffs themselves is completely covered elsewhere (9, 11), and paper chromatography probably can add little information except to show the presence of apparently closely allied chemical material

* For report of Subcommittee B and action of the Association, see *This Journal*, **35**, 48 (1952).

† Theories regarding the mechanism of paper chromatography have been advanced by many investigators. Probably the most comprehensive discussions and bibliographies may be found in material published by de Whalley (8) and Strain (6.)

which may or may not be an integral part of a dye which meets all specifications. (In this connection it is interesting to note chromatograms of the halogenated fluorescein dyes in certain solvent systems. Besides the principal constituent they display a panorama of colors which indicate sometimes three or four higher and lower halogenated derivatives.)

In most instances the use of paper chromatography on dyes found in food and drug products follows some type of separation of the chromogenic compounds from a mass of other material. There are numerous procedures outlined in the official methods (11) and more complete, compact and comprehensive schemes are given by Freeman (3). He observes that "when the analyst receives the sample for qualitative determination of color content, he should first find out all he can regarding the product because:

"(A) A knowledge of what else the product contains will considerably narrow down the colors that may reasonably be expected to be encountered.

"(B) A knowledge of the nature of the material will enable a planned approach.

"(C) A knowledge of the intended or customary uses of the product may further narrow the field of expected colors."

Gross separation of a mixture of colors may be accomplished by following Koch's (10) procedure for the separation and identification of certifiable coal-tar colors, which is based on the use of immiscible solvents. His scheme for placing the dyes in one of eight groups is an extremely helpful means to narrow down the coloring matter and eliminate many confusing possibilities. According to Koch, the dyes are divided into eight groups, each containing from three to thirty-four colors. These groups may be further broken down into smaller groups or individual dyes. Group 8 of the scheme comprises benzene soluble colors to which paper chromatography was not applied. The colors of this group, however, respond effectively to column chromatography and a method for their separation and identification has been developed by Weiss (7).

In many instances a mixture of colors falling into one of Koch's groups may be resolved into its individual components by means of paper chromatography. Since the resolved colors are isolated without chemical change, recourse may then be had to further means of identification.

Since Koch's scheme of color separation (10) is based on certain solubility characteristics of the dyestuffs in acid or basic solution, sample solutions of authentic certifiable dyes or their mixtures were adjusted to the condition in which they would occur in their appropriate shake-out fraction and then chromatographed.

In approaching these studies the selection of suitable solvent systems was, of necessity, empirical, for although the dyes in a given group have similar solubility characteristics in certain solvents, their chemical and

structural characteristics are in some instances widely different. These differences of dyes within sub-groups perhaps may account for any separability that is evidenced in paper chromatography. Taken as a whole many of the dyes show such similar Rf values in the same solvent system that, although the spots may be satisfactorily compact and the dyes may be distinguished by their different hues, there is a great deal of overlapping in the course of migration. Two-dimensional partitioning has not been found satisfactory for separating the dyes in a chemically unrelated group. It is difficult to get two solvent systems in which some of the dyes do not spread, "comet," or show subsidiary and confusing spots, and the presence of subsidiary material in many of the colors may produce confusing secondary colored areas in the normal position taken by another dye. Since it is highly unlikely that all colors in each group would be encountered in the same solution, a working separation of the dyes within the smaller groups may be accomplished. At least, the presence of more than one of the colors usually is indicated. The choice of another solvent system or change of *p*H may then effect further separation.

A considerable number of solvent systems have been tried and a list is given in Table 1.

TABLE 1.—*Solvent systems used in experimental work with paper partition chromatography of coal tar colors*

1. Phenol 150 g, H_2O 48 g, glacial acetic acid 2 g (acetic acid atmosphere)
2. Phenol—80%; H_2O 20%
3. Phenol—80%; H_2O 20% (acetic acid atmosphere)
4. Phenol solvent: Phenol 100 g saturated with an aqueous soln containing 6.3% sodium citrate and 3.7% sodium (or potassium) dihydrogen phosphate (1)
5. Phenol solvent (#4) +2 ml glacial acetic acid (1)
6. 1% ammonium hydroxide
7. *n*-butyl alcohol saturated with water 95%; conc. ammonium hydroxide 5%
8. *n*-butyl alcohol 40 ml; conc. ammonium hydroxide 10 ml, then made up to 100 ml with water
9. *n*-butyl alcohol saturated with water 97%; glacial acetic acid 3% (acetic acid atmosphere)
10. *n*-butyl alcohol saturated with water 95%; acetic anhydride 5%
11. *n*-butyl alcohol 200 ml; conc. ammonium hydroxide 2 ml; water 88 ml; ethyl alcohol 40 ml
12. Methyl alcohol 95%; water 2%; formic acid 3%
13. Phenol (#2) plus 10 ml ethyl acetate
14. *n*-butyl alcohol saturated with water 95%; amyl acetate 5%
15. *n*-butyl alcohol saturated with water 98%; ethyl acetate 2%
16. 1% ammonium hydroxide saturated with *n*-butyl alcohol
17. 1% ammonium hydroxide 90%; methyl alcohol 10%
18. 1% ammonium hydroxide saturated with amyl alcohol
19. Methyl alcohol (#12) with acetic acid atmosphere
20. Formic acid 95%; ethyl acetate 5%
21. Acetic acid 95%; ethyl acetate 5%
22. Collodine 90%; water 10%
23. Pyridine 90%; water 10%
24. 2–4 lutidine 90%; water 10%
25. Ethyl acetate 98%; acetic acid 2%
26. Toluene 90%; water 10%
27. Amyl acetate; acetic acid 2%

The solvent systems found to produce the best separation, type of migration, or spot, for each dye are given in Table 2. The dyes in groups 3, 4, 5, and 6 are here arranged according to Freeman's (3) solubility sub-grouping.

TABLE 2.—*Certifiable Coal-Tar Dyes arranged according to Freeman's solubility sub-grouping, giving some chromatographic solvent systems for migration and/or separations*

KOCH'S GROUP	FREEMAN'S SUB GROUP	COLOR	COLOR INDEX NO.	SPOTTED FROM:	DEVELOPING SOLVENT SYSTEM (TABLE 1)	RF VALUE
1		D&C Red 19	749	Neutral	9	.83
				HCl dil.	24	.78
3	A	FD&C Green 1	666	Na_2CO_3	12	.83
				Na_2CO_3	18	Solvent front
				HCl dil.	18	.83
		D&C Green 7	667	Na_2CO_3	12	.82
				Na_2CO_3	18	.88
				HCl	9	.54
		D&C Blue 8	714	Na_2CO_3	12	.90
		D&C Violet 1	697	Na_2CO_3	12	.83
		Ext. D&C Blue 1	922	Na_2CO_3	12	.33
		Ext. D&C Blue 3	673	Na_2CO_3	12	.92
	B	D&C Black 1	246	Na_2CO_3	2	.36
		Ext. D&C Red 7	1034	Na_2CO_3	2	.53
	C	D&C Green 5	1078	Na_2CO_3	18	.80
				Na_2CO_3	12	.52
				Na_2CO_3	1	.76
		D&C Yellow 10	801	Na_2CO_3	12	.45
				NaOH	1	.60
	D	FD&C Orange 1	150	HCl	10	.63
				NaOH	10	.43
				HCl	12	.46
				HCl	1	.78
		Ext. D&C Yellow 3	636	NaOH	10	.57
		D&C Brown 1	234	Na_2CO_3	12	.66
				HCl	1	.78
		D&C Red 7 D&C Red 6	163	Na_2CO_3	12	.75

TABLE 2.—(*continued*)

KOCH'S GROUP	FREEMAN'S SUB GROUP	COLOR	COLOR INDEX NO.	SPOTTED FROM:	DEVELOPING SOLVENT SYSTEM (TABLE 1)	RF VALUE
3	D	D&C Orange 4	151	HCl	10	.44
				Na_2CO_3	12	.85
				HCl	1	.78
	Also 7	D&C Red 34	190	Na_2CO_3	12	.53
		Ext. D&C Violet 1	1080	Na_2CO_3	12	.63
		Ext. D&C Violet 2	1073	Na_2CO_3	2	.87
		Ext. D&C Red 12		Na_2CO_3	2	.73
		Ext. D&C Black 1	(Shows subsidiary spots of color in all solvent systems)			
		Ext. D&C Yellow 1	138	Na_2CO_3	2	.74
		D&C Blue 5	1075	Na_2CO_3	2	.91
	E	Ext. D&C Red 3	758	Na_2CO_3	2	.94
		Ext. D&C Red 9	84	Na_2CO_3	2	.70
		Ext. D&C Yellow 4	642	Na_2CO_3	2	.86
4		FD&C Blue 1		Na_2CO_3	2	.76
		FD&C Green 2	67	Na_2CO_3	2	.64
		FD&C Green 3		Na_2CO_3	2	.62
5	A2	Ext. D&C Red 13	252	HCl	11	.20
	5B	FD&C Yellow 1	10	HCl	11	.43
	5C	FD&C Red 4		HCl	11	.21
				HCl	2	.30
				HCl	1	.41
		Ext. D&C Red 10	179	HCl	11	.52
				HCl	2	.33
				HCl	1	.36
				HCl 1+8	10	.40
	5D	FD&C Red 1	80	HCl	2	.25
				HCl	1	.37
				HCl dil.	10	.57
		D&C Red 5	79	HCl	2	.24
				HCl dil.	10	.45
		FD&C Yellow 2	10			

TABLE 2.—(*continued*)

KOCH'S GROUP	FREEMAN'S SUB GROUP	COLOR	COLOR INDEX NO.	SPOTTED FROM:	DEVELOPING SOLVENT SYSTEM (TABLE 1)	RF VALUE
6		FD&C Blue 2	1180	HCl	1	.36
				HCl	7	.46
				HCl	18	.52
		FD&C Red 2	184	HCl	1	.35
				HCl	13	.63
		FD&C Yellow 5	640	NaOH	2	.15
				HCl	1	.29
				HCl	7	.88
				HCl	18	.76
		FD&C Yellow 6		NaOH	2	.30
				HCl	1	.37
				HCl	7	.71
		D&C Green 8		NaOH	7	.92
				HCl	2	nil
		D&C Orange 3	27	HCl	1	.52
				HCl	7	.77
		D&C Red 33	30	HCl	2	.47
				HCl	1	.44
		Ext. D&C Green 1	5	NaOH	7	Solvent front
				HCl	18	.90
		Ext. D&C Red 1	57	HCl	2	.60
				HCl	1	.41
		Ext. D&C Red 2	216	HCl	2	.41
				HCl	1	.39
		Ext. D&C Red 11	31	HCl	2	.52
				HCl	1	.38
		Ext. D&C Blue 4	1054	HCl	2	.33
				HCl	1	.46

Whatman No. 1 filter paper was used throughout the investigation. Other papers were tested but found to be inferior. The length and width of the paper strips were chosen to suit the type and intensity of the dye, the solvent system, and the developing tank. All tanks were of glass with tightly fitting lids, tops, or stoppers.

Ascending chromatography was employed and the dye solution was applied by means of a slender glass rod to a spot on the paper strip about an inch from the bottom. The initial spot of color to be chromatographed should be compact and of small diameter (about 1 cm). In building up a spot of weak color by repeated evaporation care should be taken that the accompanying build-up of acid, alkali, sugars, or gums does not become great enough to affect the migration and partition of the dye material, either chemically, physically, or mechanically. Two or more strips spotted with coloring matter can be run simultaneously in order to furnish material which has been spotted at different *p*H levels, or developed in different solvent systems.

Concentration of color or quantity of dye material on an initial spot does not seem to influence migration except mechanically, as there may be a limit to the "load" that can ascend a paper strip and be effectively resolved. The presence of water or moisture in the system is mandatory for controlled chromatography. Acid or ammoniacal atmosphere in a chromatographic chamber also may affect migration rate, pattern, and even the appearance of the coloring matter present. Exposure of finished chromatograms to hydrochloric or ammonia fumes is sometimes helpful in locating and identifying a dye by its change of color.

In choosing a solvent system and a *p*H range for chromatographing a color, either of two objectives may be considered: It may be desirable to cause the dye to migrate intact, to assume its relative Rf value and to separate completely from other coloring material present; or a system may be employed by which the analyst may wish to demonstrate subsidiary material in the dyestuff. The first objective is generally considered in the analysis of food and drug products where the identity of dyes present is sought. Solvents or *p*H range which tend to disperse a dysetuff into its subsidiaries may lead to confusing results with mixtures but may be highly desirable when studying the composition of a particular dye.

In regulatory work, except for the chance of losing the triphenylmethane dyes, many artificially colored food and drug products may be treated according to the short procedure of the Official Methods (**34.6** and **34.7**), which gives some evidence of the type of dye present, according to its place in a series of washouts from acid amyl alcohol. In using this system, the separation of fractions is not always clear cut, and the fact that coloring matter appears in several fractions may indicate the presence of more than one closely allied dye. It has been found that an informative chromatogram will be produced by combining all of the

washed out fractions, concentrating them, spotting on paper, and developing in the solvent system No. 1.

There are instances where sample material may be acidified and spotted directly on paper for chromatographing. This procedure has been effective with some highly colored beverages, a chlorophyll product, some condiment sauces, and some confectionary products. In many cases the dyes encountered in regulatory samples are not from the same solubility group and there may even be a combination of water and oil soluble colors (as was found in a medicinal salve).

A consideration of possible color combinations in a sample may give a valuable clue to further treatment in order to obtain the most informative chromatogram. In food products, greens are apt to be composed of yellow and blue, or green and yellow or blue; orange-colored products frequently contain yellow and red with or without an orange dye; red colors may have more than one red dye present, and purples are usually a combination of red and blue. Also, most of the green and blue dyes with which we are here concerned belong to a different chemical group than the majority of the other dyes and this difference may suggest a manner of approach for chromatography as well as a separation by immiscible solvents.

The following examples serve to illustrate the application of chromatographic separation to certain coal-tar combinations:

1. FD&C Red 2 and FD&C Yellow 5. Solvent system No. 1, run overnight. Respective Rf values: 0.13; 0.34.
2. FD&C Yellow 5 and FD&C Yellow 6. Solvent System No. 1, 6 hour run. Respective Rf values: 0.2; 0.55.
3. FD&C Red 2, FD&C Yellow 5, FD&C Orange I, and Brilliant Scarlet (C.I. No. 185) Solvent system No. 2, overnight run. Respective Rf values: 0.27; 0.34; 0.81; 0.29.

Some of these separations are shown in Figure 1.

The Official Methods of Analysis (11) includes in the chapter on color a tabulation of color reactions produced on dyed fibers by various reagents (Table 1, p. 658). In regulatory work a sort-out procedure such as is offered by these tests is frequently of great assistance in color identification. Swatches of paper from the appropriate sections of completed chromatograms of authentic dyes have been found to respond to these spot tests with as clean-cut color reactions as wool or silk swatches. For efficient color analyses, chromatograms of the expected colors should be run along with the sample in order to make comprehensive tests on the resolved spots.

The A.O.A.C. table of color reactions is not complete for all the dyes that are at present certifiable. Reactions of the dyes in groups 3, 4, 5, and 6 obtained from chromatograms of authentic coloring material in the course of experimental work are shown in Table 3. The spot tests for the dyes are arranged according to Koch's groups and include those in

FIG. 1.—Paper chromatograms showing the separation of some coal-tar dyes. 1. FD&C Red 2 and FD&C Blue 1. Solvent System No. 1. 2. FD&C Red 2 and FD&C Yellow 5. Solvent System No. 1. 3. FD&C Yellow 5 and FD&C Yellow 6. Solvent System No. 1 (4 hours). 4. FD&C Red 2, FD&C Yellow 2, FD&C Orange 1 and Brilliant Scarlet (C.I. 185). Solvent System No. 2. 5. FD&C Red 2, FD&C Yellow 5, FD&C Yellow 6, FD&C Orange 1. Solvent System No. 1. 6. FD&C Red 2, and Brilliant Scarlet (C.I. 185). Solvent System No. 13.

the table, and in addition the available D&C and Ext. D&C dyes not listed there.

DISCUSSION

There are three aspects to be considered in connection with the examination, separation, and identification of coloring material to which paper partition chromatography may be applied:

1. The detection of closely allied chemicals which may be present as impurities or contaminants in the dye.

2. The behavior of a dye or combination of dyes as associated within a group having similar solubility characteristics, and (text resumed on page 433)

TABLE 3.—*Color reactions produced by various reagents on paper swatches from chromatograms*

DYE	CONC. HYDROCHLORIC ACID	CONC. SULFURIC ACID	10% SODIUM HYDROXIDE	CONC. AMMONIUM HYDROXIDE
		Group 3		
D&C Red 6, D&C Red 7	Pink	Deeper Pink	Light pinkish orange	Yellowish orange
D&C Red 34	Bluish Pink	Bluish Pink	Sl. yellowish pink	Pale Pink
Ext. D&C Red 3	Lilac	Orange	Little change	Paler
Ext. D&C Red 7	Yellow	Orange	Violet	Violet
Ext. D&C Red 9	Purple	Blue	Pink	Pink
FD&C Orange 1	Violet	Violet	Dark red	Dark red
D&C Orange 4	Red	Red	Dull red	No change
D&C Yellow 10	Slightly darker	Brownish yellow	Slightly paler	Little change
Ext. D&C Yellow 1	Violet red	Violet	No change	No change
Ext. D&C Yellow 3	No change	No change	Paler	Paler
FD&C Green 1	Pale Orange-Yellow	Yellowish brown	Decolorized	Decolorized
D&C Green 5	Fades	Little change	Bluish	Fades bluish
D&C Green 7	Yellow	Yellow-green	Blue	Decolorized
D&C Blue 5	Pink	Blue	Sl. deeper blue	Deeper blue
D&C Blue 8	Yellow	Fades	Blue	Blue
D&C Violet 1	Decolorized	Decolorized	Fades	Fades
Ext. D&C Violet 1	Sl. deeper	Bluish	Blue	Blue
D&C Brown 1	Red-brown	Red-brown	Red-brown	Yellow-brown
D&C Black 1	Blue	Blue	Blue	Blue
Ext. D&C Black 1	Blue-green	Bluish	Lilac	Lilac-blue
Ext. D&C Blue 1	Fades	Fades	Lilac	Blue
Ext. D&C Blue 3	Yellow	Fades	Blue	Blue
		Group 4		
FD&C Blue 1	Yellow	Yellow	No change	No change
FD&C Green 1	Pale orange-yellow	Yellowish brown	Decolorized	Decolorized
FD&C Green 3	Orange	Green to brown	Blue	Blue
		Group 5		
FD&C Red 1	Little change	Little change	Dull orange	Little change
FD&C Red 4	Deeper red	Deeper red	Orange-yellow	Orange yellow
D&C Red 5	Little change	Little change	Brownish-yellow	No change
Ext. D&C Red 10	Little change	Lavender-purple	Deep pink	Deep pink

TABLE 3.—(*continued*)

DYE	CONC. HYDROCHLORIC ACID	CONC. SULFURIC ACID	10% SODIUM HYDROXIDE	CONC. AMMONIUM HYDROXIDE
Ext. D&C Red 13	Blue	Blue-purple	Red-brown	Pink
FD&C Yellow 1	Almost decolorized	Very pale, dull	No change	No change
		Group 6		
FD&C Red 2	Slightly darker	Violet to brownish	Dull brownish to orange red	Little change
D&C Red 33	Orange	Orange-pink	Brownish-pink	Bright pink
Ext. D&C Red 1	Bluish-pink	Bluish-pink	Yellow	Yellowish pink
Ext. D&C Red 2	Orange-yellow	Orange	Orange-yellow	Yellow
Ext. D&C Red 11	Little change	Deeper pink	Orange-yellow	Orange-yellow
D&C Orange 3	Orange	Orange	Fades to pinkish orange	Yellow-orange
FD&C Yellow 5	Slightly darker	Slightly darker	Little change	Little change
FD&C Yellow 6	Slightly redder	Slightly redder	Browner	No change
D&C Green 8	(Fluoresces yellow-green under U.V. light)			
Ext. D&C Green 1	Light yellow	Green-yellow	Green	Green
FD&C Blue 2	Slightly darker	Darker	Greenish-yellow	Greenish-blue
Ext. D&C Blue 4	Yellow	Blue green-yellow	Bright blue	Bright blue

3. The isolation of coloring matter from food products in which they may be found alone or as mixtures of colors designed to produce a desired hue.

Most of the regulatory work, of the field analyst especially, will not be greatly concerned with the first category as such, except perhaps with the appearance of subsidiary spots or colored areas which may affect the "diagnosis" of dye material present.

The use of paper chromatography for separating all the dyes in a major group seems to be of more theoretical than practical interest, as in all probability a limited number of them will appear together in any regulatory sample. The separation of many of the dyes within solubility groups may be accomplished, however, with a fair degree of success if the groups are broken down to contain dyes of dissimilar chemical characteristics. A study of the chemical structure of the certifiable coal-tar dyes leads to

the conclusion that there are some stable properties which will react in certain solvent systems in a predictable and reproducible manner, for in general dyes of similar chemical structure have reacted satisfactorily to the same partitioning conditions.

The triphenylmethane dyes have so far shown the best migration and separability from many other dyes, in methyl alcohol-formic acid (No. 12). The azo dyes, apparently and logically enough, respond to solvent systems containing phenol in appreciable concentrations. There is also a definite connection between the acidity or basicity of a dye, and its migratory tendency, but except in some instances, there appears to be little connection between molecular weight and migration. Concentration of the dye, *p*H, temperature, humidity, associations, salts, and doubtless other factors affect the Rf values to the extent that this figure alone probably cannot be used as a reliable landmark.

SUMMARY

Experiments are reported on the use of paper partition chromatography for the separation and partial identification of some coal-tar colors. The work indicates that many of the dyes for which regulations have been promulgated can be effectively separated from each other by using certain solubility characteristics, *p*H levels, and chromatographic solvent systems. The separation of dyes of dissimilar chemical structures can usually be accomplished in better order than is possible with those of closely allied chemical characteristics. In most instances, the dyes encountered in routine samples of foods and drugs must be separated to some degree from the material in which they appear before satisfactory partitioning on paper will occur. The choice of an effective chromatographic solvent system for a regulatory sample is aided by a consideration of the type, nature, uses, and hue of the material under examination.

RECOMMENDATION*

It is recommended—

(1) That the study of the application of paper partition chromatography techniques to the separation and identification of coal-tar colors be continued.

(2) That collaborative work be undertaken.

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No reports were given on intermediates in triphenyl-methane dyes; ether extract in coal-tar colors; halogens in halogenated fluoresceins; identification of coal-tar colors; volatile amine intermediates in coal-tar colors; non-volatile unsulfonated amine intermediates in coal-tar colors; unsulfonated phenolic intermediates in coal-tar colors; sulfonated phenolic intermediates in coal-tar colors; intermediates derived from phthalic acid; lakes and pigments; spectrophotometric testing of coal-tar colors; subsidiary dyes in D&C colors; heavy metals in coal-tar colors; arsenic and antimony in coal-tar colors; boiling range of amines derived from coal-tar colors; inorganic salts in coal-tar colors.

The following contributed papers were published in *This Journal,* November, 1951:

"Ethylenediamine Tetra-Acetic Acid as an Aid in the Analysis of Certain Coal-Tar Color Lakes," By N. Ettelstein;

"Paper Chromatography of Fluorescein and the Halogenated Fluoresceins," by C. Graichen;

"Determination of Dimethylaniline in Ext. D&C Blue Nos. 1 and 2," by K. S. Heine, Jr.;

"Determination of Uncombined p-Aminoacetanilide in Ext. D&C Red No. 1," by K. S. Heine, Jr., and William J. Sheppard;

"Studies in Coal-Tar Colors, X; Ext. D&C Green No. 1," by Charles Stein;

"Identification of Azo Dyes by Spectrophotometric Identification of Their Reduction Products. I. Compounds Which Give Simple Amines or Diamines on Reduction," by J. H. Jones and L. S. Harrow.

The contributed paper entitled "Determination of Diphenylamine in Ext. D&C Yellow No. 1 and Ext. D&C Yellow No. 2." by John E. Clements and Lee S. Harrow, was published in *This Journal,* February, 1952.

ANNOUNCEMENTS

Referee Assignments, Changes, Appointments

TOBACCO:

R. N. Jeffrey, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Beltsville, Maryland, has been appointed Referee on Tobacco.

SYNTHETIC DRUGS:

George E. Keppel, Food and Drug Administration, Minneapolis 1, Minn., has been appointed Associate Referee on Tuinal ®.

Gordon Smith, Food and Drug Administration, New York 14, N. Y., has been appointed Associate Referee on Acetophenetidin and Caffeine (chromatographic separation).

ECONOMIC POISONS:

Paul A. Giang, Bureau of Entomology and Plant Quarantine, Beltsville, Maryland, has been appointed Associate Referee on Parathion to succeed Fred I. Edwards.

FISH AND OTHER MARINE PRODUCTS:

John P. Traynor, Food and Drug Administration, Baltimore 2, Md., has been appointed Associate Referee on Solids in Oysters.

CORRECTIONS IN FEBRUARY JOURNAL

In the method for rot fragments in tomato products, page 96, 2nd and third lines of last paragraph, change "gentian" to "crystal."

On pages 97 and 98 remove "(before reduction)," in both cases, from the titles of Plates 1 and 2.

CONTRIBUTED PAPERS

RAPID DETERMINATION OF MOISTURE IN DEHYDRATED VEGETABLES WITH KARL FISCHER REAGENT BY USE OF FORMAMIDE AS AN EXTRACTION SOLVENT*

By ELIZABETH A. MCCOMB and R. M. MCCREADY (Western Regional Research Laboratory,[1] Albany, Calif.)

The necessity for a rapid and accurate method for the determination of moisture in dehydrated vegetables is again being emphasized because of the increased production of these foodstuffs for the armed forces. Various methods for the measurement of the moisture content of dry food materials are available. The vacuum oven method, which is probably the most widely used, is slow and, in general, neither specific nor quantitative. The 6-hour vacuum oven method (1), as shown by Makower, Chastain, and Nielsen (7), does not give a satisfactory measure of the true moisture content of dehydrated vegetables. To obtain a reliable value by vacuum oven methods requires approximately 40 to 100 hours, an excessively long time for routine control work (6, 8).

The apparent inadequacy of oven methods has led to the application of the Karl Fischer reagent to dry food materials. Mitchell and Smith (9) have included in their study of the chemistry of the Fischer reagent and its application, a discussion of the use of the reagent as compared with other methods for the determination of moisture in foodstuffs. Fosnot and Haman (3), Richter (10), Johnson (5), and Schroeder and Nair (11) have reported on the use of Fischer reagent for moisture analysis of dehydrated foods. The methods proposed for such materials as dehydrated vegetables required heating or refluxing in dry methanol for varying lengths of time before titration with the reagent. Johnson found that in the case of sweet and white potatoes a soaking period of 4 to 6 hours at 60°C. was required to give results comparable to a reference vacuum oven method. The conditions required for extraction as described above show that there is a slow equilibrium distribution of moisture between certain dehydrated vegetables and the methanol extractant.

The difficulty in obtaining a rapid and quantitative moisture value on these materials by use of methanol led to the search for a more favorable and selective solvent. The utility of formamide as a solvent for both water and plant carbohydrates such as pectin and starch (2) suggested its use in the titrimetric procedure. When formamide, instead of dry methanol, was used as the extraction solvent, the moisture content of ground dehydrated potatoes, sweet potatoes, carrots, and peas was determined in approximately 15 minutes.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 1–3. 1951.

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

METHOD

APPARATUS

The qualitative unit of the Senior Model Fisher[2] titrimeter was used for the determination of the electrometric end point. Two platinum wires approximately 0.5 cm apart were sealed in a glass tube (0.9 cm in outside diameter) and connected by leads to the brush post of the titrimeter. The amplifying and "magic eye" system of the titrimeter was used. The magic eye was set so that it was closed until, at the end point, the addition of a slight excess of Fischer reagent caused it to open about 0.6 cm and remain open approximately 30 seconds. A magnetic stirring system was found very adaptable for such titrations, which require the flask to be closed from atmospheric moisture. The titration flask was supported directly over the magnetic stirrer unit and in each flask a 1.5 cm section of a sixpenny nail sealed in Pyrex glass tubing (0.5 cm in outside diameter) served as a stirrer. The stirrer remains with each flask through all operations. The Fischer reagent and formamide were dispensed by means of all-glass automatic buret systems which were protected from atmospheric moisture with drying tubes containing an efficient desiccant. The samples were titrated in 250-ml No. 27 standard-taper, glass-stoppered Erlenmeyer flasks, which had been dried in an oven, stoppered, and allowed to cool. Through all manipulations the flasks were protected as far as possible from atmospheric moisture. For protection during addition of reagents, one-hole cork stoppers fitting the flasks were placed on the buret tips, and the flasks were tightly stoppered between titrations and during stirring.

REAGENTS

Formamide: Practical grade. (The commercial reagent was found to be sufficiently dry.)

Fischer Reagent: Prepare by a method similar to that described by Smith, Bryant, and Mitchell (12). Dissolve 84.7 g resublimed iodine in 269 ml of reagent grade pyridine (<0.1 per cent water) in a 1-liter Pyrex glass-stoppered bottle. Add 667 ml of dried absolute methanol (preferably <0.05 per cent water). To this add 64 g of sulfur dioxide gas. To avoid appreciable heating add the sulfur dioxide slowly at a rate of about 40 g per hour. Stopper the soln tightly and store for 2–3 days before use. (Pyridine of sufficient purity can usually be obtained from several sources. Some commercial methanol is suitably dry for use in the reagent.) If it is found necessary to dry the available supply of methanol add 5 g of magnesium turnings to 1 liter (4). After the initial vigorous reaction subsides, distill off the alcohol. Take care to keep the distillation system free from contamination by atmospheric moisture.

PROCEDURE

Determine the water equivalent of the Fischer reagent and the blank titer of the formamide daily or each time a series of determinations is made, because parasitic side reactions decrease the reagent's effective strength. To determine the blank, titrate 10 ml of formamide in a 250 ml Erlenmeyer flask. To the same flask add, by means of a weight buret, 70–100 mg of water, and titrate. Calculate the water equivalent of the reagent—mg of water per ml of reagent. (It is advisable to average at least three blank and water equivalent values for each standardization. The net titer of the blanks should check within 0.1 ml and the range of the water equivalent values should not exceed 6 parts per thousand.) Carry out all titrations dropwise near the end point until one drop will cause the eye to remain open for about 30

[2] Mention of manufacturers or of trade names does not imply that they are recommended by the Department of Agriculture over others not mentioned.

seconds. Grind the samples of dehydrated vegetables to pass a 40-mesh sieve and store in small tightly sealed containers. Weigh an approximately 500 mg sample of the ground material into a dry, glass-stoppered 250 ml Erlenmeyer flask which contains a stirrer. Add 10 ml of formamide with slight agitation to disperse the sample and to prevent clumping. Heat for 40 sec. on a hot plate held at 150° ± 10°C. Release the stopper slightly and gently rotate the flask during the heating; then cool to room temperature and titrate. (Because of the viscous nature of some of the sample-formamide mixtures, it is sometimes expedient, in addition to using the magnetic stirrer, to rotate or agitate the flask slightly during the titration.)

Calculate the per cent water as follows:

$$\% = \frac{100\ (\text{sample titer}—\text{blank titer})\ (\text{water equivalent})}{(\text{sample weight in milligrams})}$$

DISCUSSION AND RESULTS

In the development of the proposed method it was desired to extract the water from the dried ground vegetable with formamide in the shortest practical time. The factors of greatest importance in quantitative extraction are the characteristics of the particular vegetable, its particle size, and the time and temperature of the formamide treatment. Vegetables ground to pass 40 mesh were used here, although it is recognized that finer materials might be extracted in shorter, and coarser materials in longer, periods of time. The temperature of the vegetable formamide suspensions was maintained at a practical minimum to avoid possible reactions such as lactonization. However, it was necessary to heat the suspensions of some vegetable materials to about 70°C. for a short time in order to extract the water. A hot plate covered with an asbestos pad maintained an aluminum block at 150° ± 10°C. This temperature allowed fairly rapid heat transfer to the vegetable suspensions without serious danger of overheating. The procedure as outlined recommends heating 40 seconds on a metal plate. These conditions are not critical, since no increase in moisture content was obtained from sweet or white potatoes heated from 40 seconds to 2 minutes. Carrots yield the same results with or without heating.

Dried vegetables are different in their chemical composition and physical properties and therefore may require varied conditions in the formamide extractions. Without heating, dried ground carrots, suspended in formamide at 25°C., can be titrated in 2 to 3 minutes or less, while sweet potatoes, peas, and white potatoes require 15 minutes, 1 hour, and more than 1 hour, respectively. The method as outlined may be used for the determination of moisture in dehydrated diced carrots, sweet potatoes and white potatoes and dehydrated peas. If the procedure is modified or if it is to be applied to other dehydrated products, it should be tested by comparison with a standard moisture method.

The results obtained by the titrimetric procedure for different samples of several vegetables are shown in Table 1 and are compared with results

TABLE 1.—*Moisture contents of dehydrated vegetables by volumetric and vacuum-oven methods*

MATERIAL	VOLUMETRIC	VACUUM OVEN	
		40 HOURS, 70°C.	100 HOURS, 70°C.
	per cent	*per cent*	*per cent*
White potato			
1	8.77	8.49	8.69
	8.78	8.48	—
2	9.11	8.72	9.23
	9.23	8.72	9.25
	9.17		
3	5.87	5.73	5.81
	5.88	5.77	5.82
Sweet potato			
1	8.03	7.60	7.98
	8.11	7.59	7.98
2	8.61	8.04	8.43
	8.61	8.03	8.44
3	8.06	7.77	8.09
	8.10	7.78	8.06
	8.14		
Carrot			
1	7.01	6.73	7.01
	6.94	6.73	7.00
2	8.00	7.68	8.24
	8.04	7.67	8.22
3	8.39	8.34	8.69
	8.44	8.35	8.64
Pea			
1	5.68	5.32	5.46
	5.68	5.32	5.48
2	7.79	7.70	—
	7.87	7.71	—
3	6.77	6.34	—
	6.68	6.33	—
	6.70		
4	6.51	6.36	6.49
	6.62	6.38	6.47
	6.63		
5	5.74	5.47	5.66
	5.75	5.47	5.61

obtained by vacuum-oven drying at 70°C. for 40 hours and for 100 hours. These drying times were chosen because they encompass approximately the drying time range recommended by Makower *et al.* (7, 8) for various dehydrated vegetables as obtained with their reference method. Thus, for white potatoes the recommended drying range is 43 to 67 hours. Examination of the results in Table 1 shows that the titrimetric method yields results in agreement with the recommended oven procedure to within about 0.2 per cent in moisture content. Similarly, good agreement is obtained for sweet potatoes where the recommended drying time found by the redrying and the lyophilization (8) procedures is about 100 hours. For carrots (sample 3) the titrimetric results agree within 0.1 to 0.2 per cent with those obtained by the recommended drying method (about 40 hours), but in samples 1 and 2 slightly greater deviations of about 0.3 per cent are noted. No interpretation of the results can be made for peas, inasmuch as there are no vacuum oven reference method calibrations available for this material. The titrimetric results in this case are approximately equivalent to those for 100-hour oven drying as in the case of sweet potatoes.

The good agreement obtained between the two independent techniques (titrimetric and vacuum oven) suggests that the results represent a close approximation to the actual water content of these materials.

ACKNOWLEDGMENT

The authors wish to thank K. T. Williams of this Laboratory for his contribution toward the development of this procedure.

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CRYOSCOPIC DETERMINATION OF ADDED WATER IN MILK*

By HERMANN C. LYTHGOE†

The application of the cryoscopic method to certain milks of known purity from individual cows, following the discovery of unusual high freezing points[1] on many otherwise normal delivery samples, has furnished the Massachusetts Food and Drug Division with much information that is not generally known. Cows giving abnormal milk, the analysis of which might indicate the presence of added water, are more apt to be found by such investigation than by the routine examination of the mixed milk from many herds. Accordingly, after Nussbaum (10) reported that the only determination needed to establish the presence of added water in milk was the freezing point, Lythgoe (8) showed certain interpretive limitations of this application by analyzing the probability distribution of determinations on milks of known purity from one group of 133 individual Holstein-Friesian cows and from another group of 73 cows, breed unidentified. Unfortunately, the subquoted conclusion did not emphasize that it applied to milk from individual cows: "The freezing point of milk is more susceptible to changes by the addition of water than are other constants; but if watering is suspected by this figure, further examination should be made to confirm this by using other methods of analysis. In all cases where the figures are not far from normal, samples of known purity are desirable, if not necessary."

The failure to make the intended interpretation inescapably clear has created the need for this supplementary analysis, based on previous records (1–6, and 8). It intends to show the probable range of freezing points for individual cow's milk, for individual herd milk, and for mixed herd milk.

To emphasize the need for discretional interpretation, an unusual case is cited. About 30 years ago an experienced chemist in the Division recommended that a milk producer be prosecuted for offering for sale certain milk which he alleged, because of an unusually high freezing point, contained added water. Other tests did not indicate that the milk contained added water. (The milk was being retailed from a three or four cow dairy in a small Massachusetts town.) Because opinion differed on whether or

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† Formerly Director, Food and Drug Division, Massachusetts Department of Public Health. Retired June 1, 1946. Residence, 36 Fair Oaks Avenue, Newtonville, Massachusetts.

[1] A distinction is noted herein between the term "freezing point determination," and the erroneously used A.O.A.C. term "freezing point depression," as applied to milk. Although the freezing point of milk can be depressed by the addition of an electrolyte, such as the acid produced by souring, it is always elevated by the addition of water. Reference Table 41.28, in the current edition of the A.O.A.C. Methods of Analysis (9), is captioned "Table for Determining Added Water in Milk by Means of the Freezing-Point Depression (Based on Winter Table)." All of the temperature figures in this table except −0.550C. are of freezing point elevation, and hence the term "depression," as applied to milk, is a misnomer. Adding milk to water will depress the freezing point of the water, but not of the milk. The term "freezing-point depression," as ordinarily used, is applied to the lowering of the freezing point of the solvent by the addition of another substance.

not the dealer should be prosecuted, the inspector who took the original sample proceeded to the farm, observed the entire milking process, and returned with samples of known purity. Although the same chemist who made the previous determination obtained a freezing point determination on one sample among those of known purity which was almost identical with that on the first sample, he insisted that some water must have been surreptitously added to the inspector's "known purity" sample. Subsequently, three chemists and the inspector went to the farm, secured additional samples of known purity, and again confirmed the previous high freezing point determination. This and similar examinations of known purity milks from individual cows, following the discovery of somewhat high freezing points on delivery samples, has been of inestimable value to the Food and Drug Division.

The cryoscopic method is described meticulously in the A.O.A.C. Methods of Analysis (9), with a suggestion for interpreting the results as follows: "Tolerance of 3% may be allowed on results for added H_2O determined on basis of the average freezing point of -0.550°C. Owing to narrow variations found in market milks of genuine character it is not necessary to deduct the tolerance figure from results showing added H_2O in excess of 3%."

The figure -0.550°C. represents an average, and consequently must depend upon some observed freezing points much higher and some much lower than the average. The tolerance is based upon the average, and should be more accurately expressed as "± 3 per cent."

Using the equation $x = 100 + 181.82y$, where x = per cent added water and y = freezing point of the milk, the freezing points are plotted on a straight line against the per cents of added water in Figure 1. The plot line is extended from the fourth quadrant into the third quadrant and the freezing points between -0.01° and -0.44°C. have been omitted. The freezing point figures for unwatered milk (0.00% added water) begin at -0.550°C. but since about one-half of the samples of pure milk may be expected to freeze below -0.550°C. the plot line obviously should extend into the negative percentages of added water equivalent. (In many of the subsequent figures the added water equivalents have been plotted instead of the freezing points.)

During 1945 and early in 1946 the inspectors and chemists of the Westfield Laboratory of the Massachusetts Food and Drug Division collected and examined milk of known purity from each of 52 cows distributed in five herds. The freezing point determinations on these samples are plotted on arithmetic-percentage scales in Figure 2. The chart definitely shows the absence of the unusually high and low freezing points on the samples obtained from the individual cows when the milk so obtained from each herd is mixed separately and the freezing points on the herd milks plotted. Nearly half (49.5%) of the extreme figures obtained from the 52

samples do not appear within the range observed for the freezing point determinations on the herd milks. The added water equivalent of the milk from the five herds varied from +0.18% to −1.82%, averaging −0.55%.

The figures tabulated in the former article (8) have been replotted on

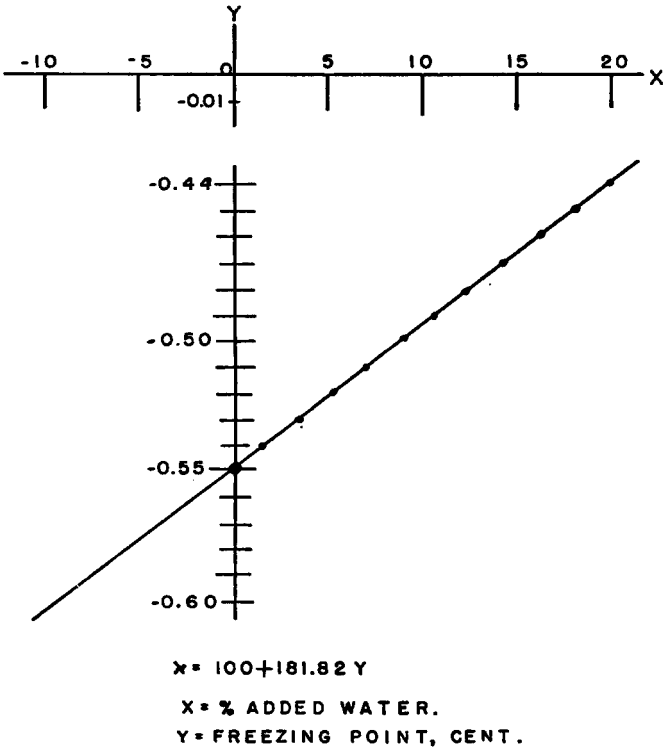

FIG. 1.—Per cent added water *vs.* freezing point of milk.

Hazen and Whipple (11) arithmetic probability paper, and the straight lines of expected probability drawn through the quartiles have been extended to the 0.01% and the 99.99% range of the probability scale. The median of each line differs from the actual median by less than 0.001°C. Extrapolation of the line representing the 133 samples from the individual Holstein-Friesian cows indicates that only one cow in 10,000 is apt to give milk with a freezing point as high as −0.510° or as low as −0.592°C. Extrapolation of the other group of 73 samples indicates a similar probability of finding a freezing point as high as −0.522° or as low as −0.584°C. The figures for these lines of probability, together with similar figures

obtained from the milk of 17 herds, are shown on arithmetic-percentage scales in Figure 3. The freezing points are expressed as added water equivalents. The highest freezing point of these samples from 206 individual cows was −0.510°C., corresponding to a plus 7.27% added water equiva-

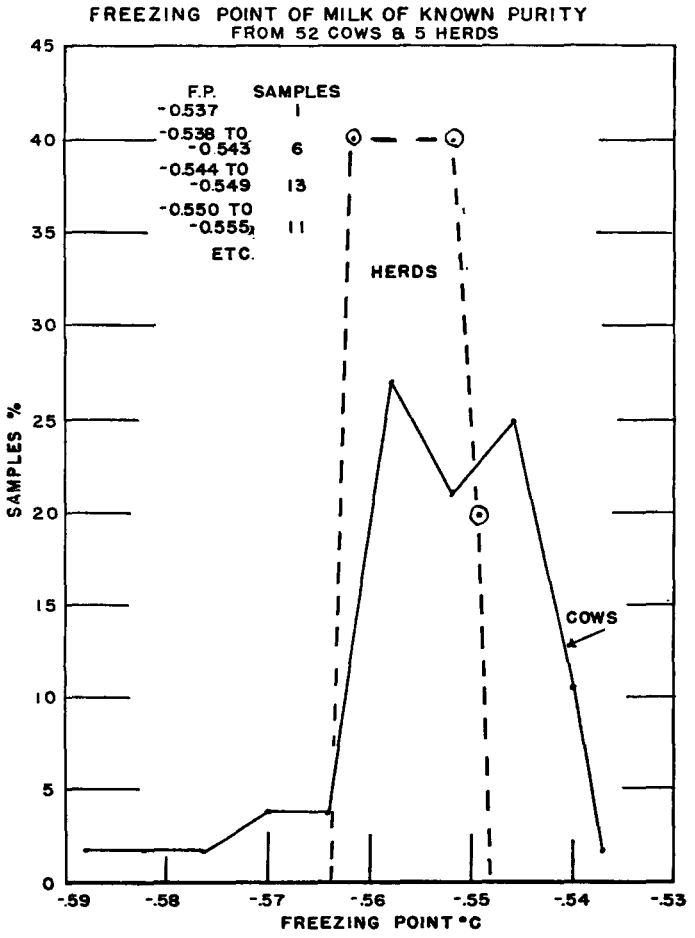

FIG. 2.—Freezing points of milks of known purity from 52 cows and 5 herds.

lent. At the opposite end of the series, the lowest freezing point corresponds to a minus 6.27% added water equivalent. A mixture of equal quantities of these two samples would contain the equivalent of 0.91% of added water, a figure well within the tolerance.

The three important reports in this country on the cryoscopy of milk

are by J. T. Keister (5), J. Hortvet (2), and E. M. Bailey (1). Using ice and salt as the freezing medium for the Beckmann method, Keister reported 30 determinations on milk from 16 cows, mostly afternoon and morning milkings (see summary in Table 1). Hortvet described the cryoscope, now recognized in the A.O.A.C. Methods of Analysis. In his summary of determinations on 60 samples from individual cows, he usually reported the highest, the lowest, and the average freezing points of groups.

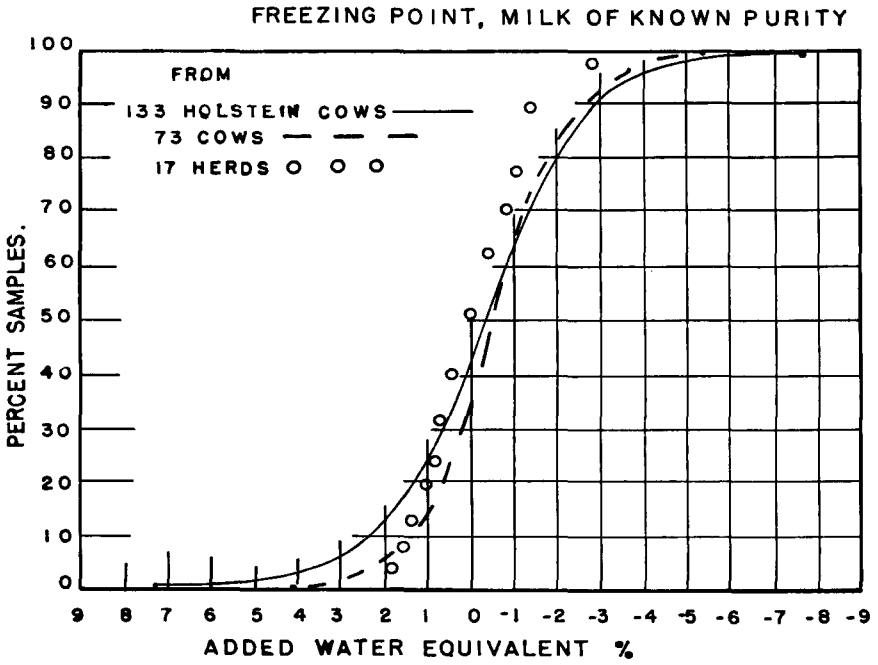

FIG. 3.—Freezing points of pure milks expressed as added water equivalent.

He gave similar data on milk from 15 herds. The lowest freezing point on milk from an individual cow was −0.562°C. Also, an identical figure was quoted as the lowest freezing point observed on herd milk (Table 1). It seems very probable that samples with a freezing point below −0.562°C. in the herd could have been discovered if he had determined the freezing point of the milk from each of the 44 cows in that herd. Bailey, as Associate Referee for the A.O.A.C., included in his determinations the results on 86 samples of milk of known purity obtained from 34 cows in four Connecticut herds. The collaborative work from the Minnesota Dairy and Food Divisions on milk from 17 Minnesota cows, and the data by Libby, McNeill and Libby of Chicago, on 27 samples obtained from 23

cows, are also summarized in Table 1. The Westfield Laboratory examined 148 samples from individual cows between 1935 and 1946, in addition to the 52 samples referred to above. Combined in Table 1 is a summary of the results on 321 samples obtained from 249 cows and included is a summary of the 133 and 73 samples referred to in a previous article (8).

In the absence of a complete record, the author has attempted to estimate the quartiles for Hortvet's reported determinations on arithmetic probability paper. Using the 148 Massachusetts samples, the upper and lower quartiles were plotted upon the 25 and the 75 per cent points of the accumulative probability scale and a straight line drawn through them was extended in both directions. Another line parallel to the one representing the 148 samples was then drawn through the average freezing point of the Hortvet samples plotted at the 50 per cent point. This line is 0.002°C. higher than the former line. The Hortvet quartiles are estimated as −0.540° and −0.554°C. Table 1 gives values for the highest, the upper quartile, the median, the lower quartile and the lowest freezing points of the 321 samples, plus the 133 and the 73 samples previously mentioned.

TABLE 1.—*Freezing point distribution in °C. of individual cow's milk of known purity based upon quartiles, summarized from six documented sources*

SOURCE	LIBBY	HORTVET	CONN.	MINN.	MASSACHUSETTS			KEISTER	WEIGHTED AVERAGE
Number of Samples	27	13	86	17	148	133	73	30	527
Highest	−0.532	−0.534	−0.523	−0.540	−0.527	−0.510	−0.532	−0.541	
Upper Quartile	−0.539	−0.540*	−0.541	−0.541	−0.542	−0.544	−0.547	−0.548	−0.543
Median	−0.547	−0.547	−0.544	−0.546	−0.548	−0.552	−0.553	−0.555	−0.548
Lower Quartile	−0.549	−0.554*	−0.557	−0.554	−0.556	−0.559	−0.558	−0.561	−0.557
Lowest	−0.560	−0.562	−0.580	−0.560	−0.583	−0.585	−0.578	−0.576	

* Estimated.

The freezing point of milk from individual cows is distinctively variable. The highest and lowest freezing points of milk of known purity from 321 individual cows and the percentage of added water needed to raise the lowest freezing point to the highest freezing point are shown in Table 2. This determination has been computed by disregarding the minus signs, subtracting the highest freezing point from the lowest freezing point, dividing the difference by the lowest freezing point, and multiplying the quotient by 100. (If 0.550°C. were used to compute the added water, the results would be slightly higher, *i.e.*, 10.2 instead of 9.6 per cent for the Massachusetts samples.)

Based on an average freezing point of 0.550°C., there are 193 samples in the plus and 128 samples in the minus added water classification (see summary in Table 3).

The Libby samples are characterized by an excessive number in the plus added water classification, while those by Keister are equally unusual in the negative side. Because nearly the same number of samples

TABLE 2.—*Highest and lowest freezing point, range difference and interpolated maximal added water equivalent for the range in °C. of individual cow's milk of known purity, summarized from six sources*

SOURCE	HIGHEST	LOWEST	DIFFERENCE	ADDED WATER NECESSARY TO RAISE THE LOWEST TO THE HIGHEST	NO. OF SAMPLES
				per cent	
Connecticut	−0.523°	−0.580°	0.057	9.8	86
Massachusetts	−0.527°	−0.583°	0.056	9.6	148
Keister	−0.541°	−0.576°	0.035	6.1	30
Libby, McNeill & Libby	−0.532°	−0.560°	0.028	5.0	27
Hortvet	−0.534°	−0.562°	0.028	5.0	13
Minnesota	−0.540°	−0.560°	0.020	3.6	17
Totals	−0.523°	−0.583°	0.060	10.3	321

TABLE 3.—*Percentage distribution of the negative and positive interpolated added water equivalents (−0.550°C. taken as the average freezing point of individual cow's milk of known purity)*

SOURCE	POSITIVE	NEGATIVE	SAMPLES
	per cent	*per cent*	
Libby, McNeill and Libby	77.8	22.2	27
Hortvet	69.2	30.8	13
Connecticut	65.1	34.9	86
Massachusetts	60.8	39.2	148
Minnesota	53.0	47.0	17
Keister	26.7	73.3	30
Totals	60.0	40.0	321

were examined by each, the results on both groups could be used in the final computation without causing erroneous conclusions. Figure 4 on arithmetic probability scales bears three straight lines drawn through the quartiles; one for Libby's figures on 27 samples, another for Keister's figures on 30 samples, and a third for Massachusetts figures on 148 sam-

TABLE 4.—*Probability figures for three groups of data*

	MASS. VALUES °C.		LIBBY VALUES °C.		KEISTER VALUES °C.	
	HIGH	LOW	HIGH	LOW	HIGH	LOW
1 in 10,000	−0.510	−0.586	−0.516	−0.571	−0.518	−0.590
1 in 1,000	−0.517	−0.581	−0.521	−0.567	−0.525	−0.584
1 in 100	−0.525	−0.573	−0.527	−0.562	−0.532	−0.577

ples. The heavy dots from the 321 samples fall notably close to the solid line which characterizes the determinations on the 148 Massachusetts samples. Using these data to determine comparable probabilities in terms of chances of finding one cow in 10,000, one in 1,000, and one in 100, respectively, the range limits for the highest and the lowest freezing points are listed in Table 4.

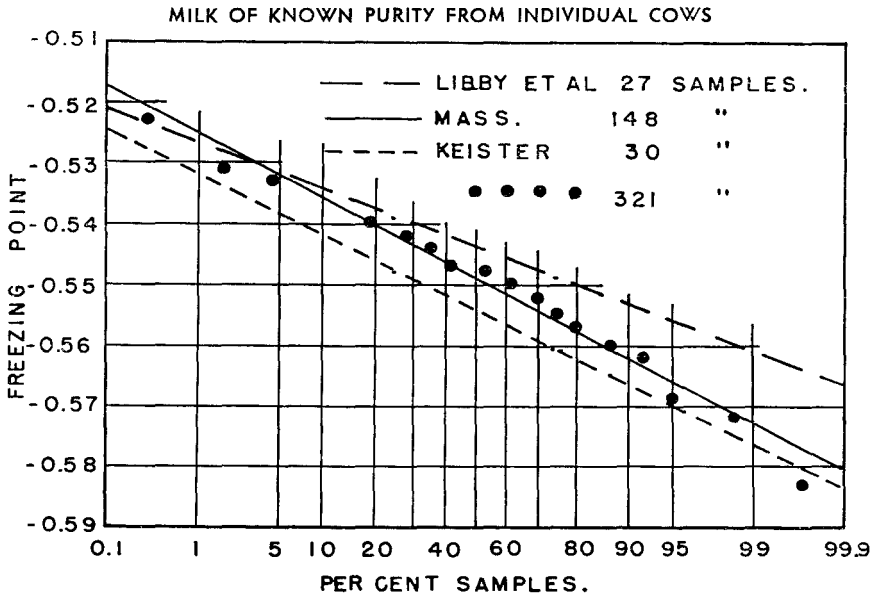

FIG. 4.—Freezing point of milk from individual cows (various data).

The median freezing point of the series of 321 samples is −0.548°C., with 16 samples freezing at this temperature. Based on a "new average" of −0.548°C., 152 samples fall in the plus added water series and 153 in the minus added water series, while the 16 remaining samples fall in the ±0.00% added water equivalent classification. If the 16 samples are divided equally between the plus and minus series, the entire series can be grouped as 160 plus samples and 161 minus samples. These two series are plotted as a curve (somewhat resembling the catenary) in Figure 5. What would happen to the milk from, say, 40 cows when dumped into a pasteurization vat is obvious. The extremely high and extremely low freezing points on milks from individual cows will escape detection when a portion of the mixture is tested.

Experience has shown that unless the proprietor of an establishment for the pasteurization of milk sells at least 500 quarts daily, he cannot operate

profitably. Consequently, a person making chemical or physical examinations of such milk may assume that the sample consists of milk from at least 40 cows and does not need to consider in the interpretation of his results the extremely high or low determinations obtained from the milk of one or two cows in 1,000, or even in 100.

Two series of freezing points of herd milk of known purity are plotted on

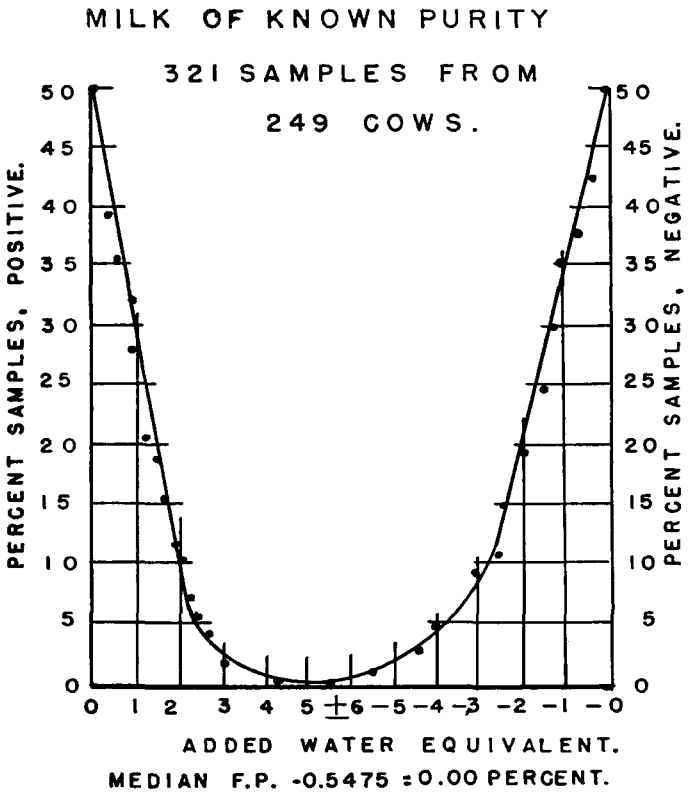

FIG. 5.—Added water equivalent (plus and minus) *vs.* percentage distribution of samples.

arithmetic probability scales in Figure 6. One series contains the determinations from samples obtained from 17 Massachusetts herds as well as the results reported by Hortvet (2), Keister (5), and Bailey (1). The latter's figures include those from four Connecticut herds and those reported to him on the Minnesota herds, thereby making a grand total of 33 samples from 28 herds. It is noted that the determinations reported to Bailey by Libby *et al.* differ from those on the other herd samples. When

plotted on similar scales, they plot not in one line, but in three straight lines. These three probability series vary in freezing points; first, from −0.530° to −0.534°, second, from −0.535° to −0.549°, and third, from −0.550° to −0.557°C. The determinations from four herds reported by Libby *et al.* to Hortvet (3) were not included among those reported to Bailey. The freezing points on these vary from −0.538° to −0.554°C., averaging −0.547°C. These 40 results were obtained from the milk of 30 herds varying in size from 2 to 16 cows each, averaging 7.55 cows per herd.

The determinations on the Libby herds were rearranged in five groups of

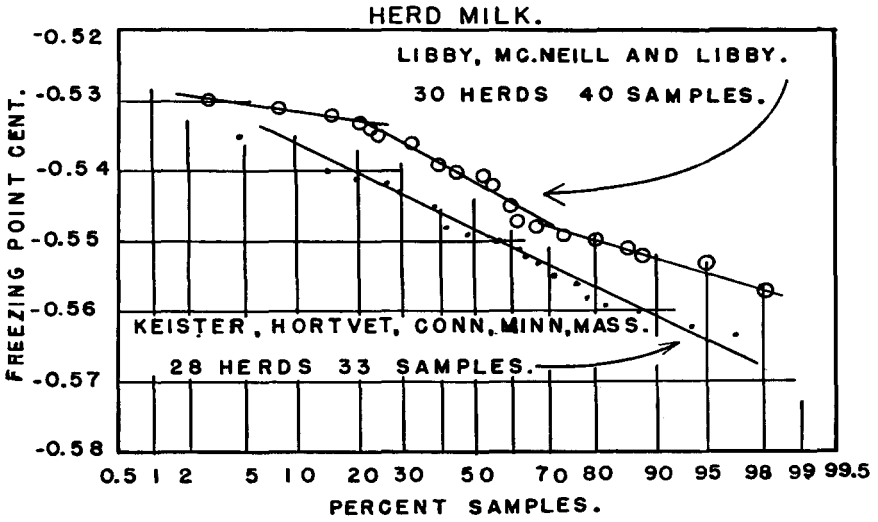

FIG. 6.—Probability distribution of freezing points of herd milks.

50 cows each. Each group of five was started with the lowest number of cows per herd (there were four herds of two cows each) and continued with an increasing number of cows per herd. It was then found that there were more than 50 cows in each of four groups. The highest number of cows per herd of these four groups then made the sixth group of 51 cows. These groups varied from 46 to 53 cows per group, averaging 50.33 cows per group. The weighted average freezing point of each of these groups was −0.537°, −0.540°, −0.542°, −0.542°, −0.543° and −0.546°C., all in the plus added water equivalent classification.

Bailey's four herds consisted of two herds of 7 cows each, one of 11 and one of 17 cows. The average freezing points of the numerous samples from each herd varied from −0.542° to −0.550°C. The freezing points of the 17 Massachusetts herd samples varied from −0.541° to −0.560°C.

In his conclusions as Associate Referee, Bailey (1) places the highest freezing point of milk from normal individual cows as −0.530°C. and gives the same figure for that of milk from normal herds. He gives the lowest freezing point for milk from individual cows and herds as −0.566° and −0.562°C. respectively. These figures were repeated by Hortvet (4), who in addition recommended the 3% tolerance.

It does not seem likely that the highest freezing points of milk from both individual cows and from herds should be identical. The extreme figures for solids, solids not fat, proteins, lactose, serum concentration, and serum ash of milk from individual cows do not appear in the milk from herds. By analogy, a similar variance in the range for the freezing points of the different types of samples may be expected to occur (Fig. 2).

If the sample from an individual cow shows a freezing point determination as high as −0.525°C., the presence of added water should be confirmed by other methods. If a sample with the same freezing point is from a herd of several cows (at least eight) the sample contains added water. Milk produced by 40 or more cows, as is invariably the case with commercially pasteurized milk, should have a freezing point neither higher than −0.540° nor lower than −0.560C. If higher, the sample is probably watered, and if lower and the acidity is normal, the sample probably contains an added electrolyte.

CONCLUSIONS

The freezing point of milk from individual cows is distinctively more variable than has been reported by earlier workers in this country. This variability has been demonstrated in milks of known purity taken from a larger number of individual cows than was used for deducing the average and the tolerance prescribed in the A.O.A.C. Methods of Analysis. Extrapolation of the three lines in Figure 4 indicates that there is about one chance in 1,000 that a sample of milk of unknown origin may be the product of an individual cow if the freezing point is as high as −0.520°C. However, if the analyst has definite evidence that the sample was from the mixed milk of six or more cows, such milk can be definitely declared to contain added water.

Commercially pasteurized milk usually can be assumed to be a mixture from at least 40 cows, and its freezing point should neither be higher than −0.540° nor lower than −0.560°C. If such a sample freezes above −0.540°C., the analyst is probably justified in declaring the presence of added water.

The literature furnishes overwhelming evidence that the extremely low and high freezing points of milk from individual cows are not found in the mixed milk obtained from herds of 15 or more cows. The following quotations are indicative of the above statement: First, in relation to cow's milk: "If the protein-fat ratio is less than 0.7 or the percentage of fat in

the solids is above 35.0, samples may be declared watered by a low refraction of the serum, not necessarily below the minimum for all samples of known purity. This is particularly so when dealing with herd milk." (6) Second, in relation to goat's milk: "In the absence of specific information relative to the source of the milk, the chemist who is making the analysis must assume that the sample may have been obtained from an individual animal and must base his conclusions as to whether the milk is normal or adulterated by comparison with analyses of milk obtained from individual animals rather than from herds. This is more important in dealing with goat's milk than with cow's milk because the goat herds are small, usually from 3 to 5 goats each, and if any abnormality exists in the milk of any one of these goats, it will not be effectively lost in the mixture, as is usually the case of cow's milk when herds are larger" (7).

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ADDENDUM

The summarized report by S. J. Rowland and R. Aschaffenburg, on p. 52 of the 1950 Annual Report of the National Institute of Research in Dairying, University of Reading, England, relating to the freezing points of milk from individual cows, merits consideration.

DETERMINATION OF ALCOHOL IN WINE AND THE EFFECT OF TEMPERATURE ON THE DETERMINATION*

By A. D. ETIENNE (Alcohol and Tobacco Tax Division, Laboratory Branch, U. S. Bureau of Internal Revenue, Washington 25, D. C. *Associate Referee*

Collaborative tests have been conducted on the determination of the percentage of alcohol in wines by means of the Etienne Tube.[1] Four commercial wines, Burgundy, Port, Sauterne, and Sherry were used so as to cover the field of red and white wines both fortified and unfortified. Four-ounce samples of each wine, labeled G, H, I, and K, respectively, and a supply of reagent were sent to each of the Branch Laboratories of the Alcohol Tax Unit and to one State Control Laboratory. Twenty-five reports were made on each wine representing a total of 100 determinations. The alcoholic content of each of the wines as determined by pycnometer was as follows:

G—Burgundy—13.45% by volume
H—Port —20.17% by volume
I —Sauterne —12.14% by volume
K—Sherry —20.47% by volume

Individual results are not listed, but a breakdown showing the accuracy of the determinations is given in Table 1.

TABLE 1.—*Deviation of results from true alcohol content*

WINE	RANGE OF DEVIATION (NUMBER OF RESULTS)			
	0.0–0.15%	0.16–0.30%	0.31–0.50%	over 0.50%
G	9	1	13	2
H	9	9	6	1
I	21	2	2	0
K	4	5	13	3
Total number	43	17	34	6

Results indicate that 43 per cent of the analyses were within 0.15% of the actual amount; 60 per cent were within 0.30%; and 94 per cent were within 0.50%. At present there is no explanation as to why such a large number of results fell in the 0.31–0.50% range.

The over-all averages and deviations from the true results are as follows:

Per cent	*Per cent*
G = 13.24	difference = −0.21
H = 20.12	difference = −0.05
I = 12.10	difference = −0.04
K = 20.17	difference = −0.30

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 1–3, 1951.

[1] *This Journal*, 33, 1016 (1950); 35, 66 (1952).

A study has also been made of the effect of temperature on these determinations. A fortified wine (Port) and an unfortified wine (Sauterne) were used for this purpose. The wines were run in triplicate at 60°F. 78°F. and 95°F. The results are tabulated below in Table 2.

TABLE 2.—*Effect of temperature*

	60°			78°			95°		
	ALC. BY VOL.		DIFF.	ALC. BY VOL.		DIFF.	ALC. BY VOL.		DIFF.
		per cent	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*
Sauterne	10.9			12.3			13.5		
(12.14%)	11.0	10.93	−1.21	12.1	12.13	−0.01	13.5	13.5	+1.36
	10.9			12.0			13.5		
Port	18.8			20.2			21.3		
(20.17%)	18.8	18.8	−1.37	20.2	20.17	0.0	21.5	21.37	+1.2
	18.8			20.1			21.3		
Mean Average			−1.29			0.0			+1.28

The above results indicate that for an accurate determination the temperature at which the tests are made should be taken into consideration. The critical temperature is 78°F. As the temperature falls below the critical temperature the results are low, and as the temperature rises above the critical temperature the results are high. For practical purposes a correction factor of 0.07% alcohol may be added to or subtracted, from the determined percentage for each degree above or below 78°F. If the temperature is above the critical temperature the factor is subtracted and if the temperature is below the critical temperature the factor is added. It follows, that for an accuracy of ±0.5% the tests should be conducted within the temperature range of 70°F. to 85°F.

DETERMINATION OF QUATERNARY AMMONIUM COMPOUNDS AS REINECKATES*

By JOHN B. WILSON (Division of Food,[1] Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

The official ferricyanide method for quaternary ammonium compounds in commercial preservatives[2] was designed especially for use on solutions of these compounds over a concentration range of from 0.5 to 10 per cent. However, there are on the market a number of products used as sanitizing agents by physicians, surgeons and dentists to which the method cannot conveniently be applied because their dilution (1–500, 1–1000, 1–4000,

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 1–3, 1951.

[1] Frank A. Vorhes, Jr., Chief.

[2] *Methods of Analysis, A.O.A.C.*, 7th Ed., p. 463.

or even 1–15,000) requires the use of too large a volume of sample to furnish the 0.5 g of quaternary requisite for a reasonable titer. It was thought possible that ammonium Reineckate, a well-known precipitant for many organic compounds containing nitrogen, might be a suitable reagent for determining quaternary ammonium compounds.

A number of quaternary ammonium compounds were available for study. In the case of those in solid form, 200 mg was weighed, dissolved in water, and diluted to 100 ml. Several aliquots of each were treated with Reineckate reagent and filtered after a time. The precipitate was dissolved in acetone which was allowed to evaporate in a warm place in a tared beaker and weighed. The residue was dissolved by warming in alcohol; this solvent was also allowed to evaporate spontaneously and the residue was dried in a desiccator and weighed.

In the case of the liquid products, di-isobutylphenoxyethoxyethyl-dimethylbenzylammonium chloride, and alkyldimethylbenzylammonium chloride, a suitable dilution was made and quaternary ammonium compounds were determined by the official ferricyanide method. A suitable volume of the analyzed solution was diluted to give a 0.2% solution of the quaternary and subjected to the treatment outlined above.

In the case of the solution of a certain quaternary, said to be a methyl-chlorobenzyllauryl derivative of ammonium chloride, the ferricyanide method returned only a small fraction of the stated strength. The Reineckate method gave higher values, but these were still only about 40 per cent of the expected amount of quaternary. Apparently this compound did not react in the same way as other compounds studied.

The method used is described below.

METHOD

(Applicable to preservatives, tinctures, and isotonic solutions.)

Reineckate reagent: Place 0.75 g of ammonium reineckate in a 125 ml conical flask, add 50 ml of H_2O, stopper and shake for about 2 min, then filter.

Solvents: Reagent grade acetone and alcohol.

PROCEDURE

Place 100 ml of sample containing 10–100 mg of quaternary ammonium compound in a 250 ml beaker; add with stirring 5 ml portions of Reineckate reagent until the liquid has a bright pink color. Let stand 30 min. and add more reagent unless the supernatant liquid has a deep pink color. Stir again for 1–2 min. After several hours filter thru a sintered glass crucible of fine porosity and wash beaker and filter with at least three 15 ml portions of H_2O. (It is unnecessary to transfer all of the precipitate to the crucible.) Wash down the sides of the crucible with water and dry by suction. If the precipitate forms a cake in the filter, mix with the wash water with the stirring rod used before.

Dissolve the Reineckate salt in acetone as follows: Set up a suction apparatus to fit the glass crucible, using as a receiver a test tube provided with a side tube for the application of suction. Using a 5 ml pipet, wash down the sides of the beaker used for precipitation and add this liquid to the crucible. Rinse the beaker a second time and add to the liquid in the crucible. Stir to dissolve and draw the liquid thru

TABLE 1.—*Determination of quaternary ammonium compounds as Reineckate salts*

CHEMICAL NAME	BASIS	MOLECULAR WEIGHT	FACTOR	PRESENT			
				100 MG	50 MG	20 MG	10 MG
				FOUND			
Alkyldimethylbenzylammonium chloride	Anhydrous	357.00	0.5579	100.9	50.5	20.5	10.5
Cetyldimethylbenzylammonium chloride[1]	Anhydrous	396.08	0.5834	92.6	46.3	18.6	9.3
	Hydrated	414.10	0.6099	96.8	48.4	19.4	9.7
Cetyldimethylethylammonium bromide[2]	Anhydrous	378.48	0.6135	97.2	48.8	20.1	9.7
	Hydrated	396.49	0.6427	101.9	51.1	21.0	10.2
Cetylpyridinium chloride[3]	Anhydrous	339.98	0.5458	91.4	47.0	19.1	10.7
	Hydrated	357.99	0.5747	96.2	49.5	20.1	11.3
Cetyltrimethylammonium bromide[4]	Anhydrous	364.44	0.6045	101.9	44.7	20.1	10.3
	Hydrated	382.46	0.6344	106.9	47.0	21.1	10.8
Di-isobutylcresoxyethoxyethyldimethylbenzylammonium chloride[5]	Anhydrous	462.10	0.6028	97.1	49.7	20.2	10.4
	Hydrated	480.11	0.6444	103.8	53.1	21.6	11.1
Di-isobutylphenoxyethoxyethyldimethylbenzylammonium chloride[6]	Anhydrous	448.07	0.6130	95.4	47.4	19.1	9.3
	Hydrated	466.09	0.6376	99.2	49.3	19.9	9.7
Dodecylacetamidyldimethylbenzylammonium chloride[7]	Anhydrous	409.02	0.5911	99.9	51.2	20.9	10.7
	Hydrated	427.04	0.6172	103.3	53.5	21.8	11.2
Lauryldimethylbenzylammonium chloride[8]	Anhydrous	339.98	0.5458	94.4	45.8	14.5	7.1
	Hydrated	357.99	0.5747	99.4	48.3	15.3	7.5
Laurylpyridinium chloride	Anhydrous	295.88	0.5112	91.7	45.4	16.3	8.2
	Hydrated	313.90	0.5423	97.7	48.2	17.3	9.0
Triethylbenzylammonium chloride	Anhydrous	227.77	0.4460	97.4	49.2	17.8	7.9
	Hydrated	245.79	0.4813	105.1	53.1	19.2	8.5

TRADE NAMES: [1] Zettyn ®; [2] EthylCETAB; [3] Ceepryn ®; [4] CETAB; [5] Hyamin 10X ®; [6] Hyamin 1622 ®; also Phemerol ®; [7] Dobenzyl Chloride; [8] DC 12.

with suction. Wash out the beaker a third time, and wash down the sides of the crucible several times with small amounts of acetone. When the liquid passing thru is colorless, disconnect, and wash into the test tube with acetone any pink material which may have dried on the bottom or outside surface of the crucible or on the inside of the funnel.

Discard the small quantity of greenish solid in the crucible which is due to impurities and decomposition products of the reagent. Transfer the acetone soln to a tared beaker (use a 50 ml beaker for 20 mg or less of quaternary ammonium compound and a 100 ml beaker for 25 mg or more) and evaporate on a warm (but not hot) surface. If a few drops of moisture remain, pass a gentle current of air into the beaker till it appears dry. Dissolve the residue by warming in 10 ml (or more if needed) of alcohol; allow the solvent to evaporate spontaneously, dry in a desiccator, weigh and calculate the weight of quaternary ammonium compound corresponding to the weight of crystals.

(To remove the greenish solid from the crucible, add 10–12 ml of HCl (1+1) and stir to dissolve. Draw the liquid thru by suction and wash several times with H_2O. Now reverse the crucible and wash by filling the bottom cavity with solvent. Use two fillings each of water, alcohol, water, acetone, and water in the order given for this purpose.)

Table 1 contains the results of several determinations on each of 11 quaternary ammonium compounds, the molecular weight of each compound, and the theoretical factor for calculating the quaternary ammonium compound from the Reineckate salt. Several of the compounds examined crystallize with one molecule of water of crystallization and in these cases the factors and the corresponding weights of the quaternary in both the anhydrous and hydrated form are given. It will be noted that in several cases the weight of sample taken lies between the recovery values calculated to the hydrated and anhydrous basis. This may indicate either a partial loss of water of crystallization on storage, or nonuniformity of composition. In most cases recoveries are excellent.

When 100 mg quantities were determined recoveries ran from 91.4 to 101.9 mg on the anhydrous basis or from 96.2 to 106.9 on the hydrated basis; with 50 mg, 44.7 to 51.2 mg on the anhydrous basis and 47.0 to 53.5 on the hydrated; with 20 mg, 14.5 to 20.9 mg on the anhydrous basis and 15.3 to 21.8 mg on the hydrated. When 10 mg was present, from 7.1 to 10.7 mg was recovered on the anhydrous basis and 7.5 to 11.2 on the hydrated basis.

IDENTIFICATION OF CERTAIN QUATERNARY AMMONIUM COMPOUNDS AS REINECKATES*

A. H. TILLSON, WM. V. EISENBERG, and JOHN B. WILSON (Division of Microbiology and Division of Food,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

On two occasions during the past few years, it has become necessary to identify with some degree of certainty two quaternary ammonium compounds. In the one case, a preservative containing alkyldimethylbenzylammonium chloride had been added to a beverage, and in the other case, di-isobutylphenoxyethoxyethyldimethylbenzylammonium chloride had been applied to plastic bandages in large amounts.

Ammonium Reineckate was chosen as a possible reagent and a few tests showed that crystalline Reineckates could be prepared which were identifiable by optical crystallographic methods. The Reineckates were crystallized from a number of solvents, and it was found that the crystals obtained from 95% alcohol were better adapted to this means of identification than those from other solvents.

In preparing the crystals a water solution of the quaternary ammonium compound is treated with an excess of ammonium Reineckate and stirred. In most cases, if 20 or more milligrams of the quaternary is present, a precipitate forms at once. With smaller quantities the mixture should be allowed to stand at room temperature for 30 mintues or more and then stirred for one or two minutes. As little as 3 mg of lauryldimethylbenzylammonium chloride in 20 ml of solution gives a visible precipitate under these conditions. After standing for several hours, the precipitate is filtered on a fritted glass crucible of fine porosity and washed several times with water. After drying the precipitate by suction, it is dissolved through the filter with acetone and transferred to a small beaker in which the acetone is carefully evaporated. The dry residue is then dissolved by warming in a minimum quantity of alcohol. If a considerable quantity of precipitate is available, crystals will be deposited on cooling and are best filtered upon a fritted glass crucible and dried by suction. When only a small quantity of precipitate (30 mg or less) is obtained, it is better to dissolve the precipitate in 10 ml of alcohol and permit the solvent to evaporate spontaneously on a warm, but not hot, surface over which a current of air continually passes.

Thirteen of the available quaternary ammonium compounds were chosen and Reineckates were prepared as outlined above to show the applicability of the procedure. The simplest compound chosen was triethylbenzylammonium chloride, which does not give a precipitate with

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* Presented at the Annual Meeting of the Association of Official Agricultural Chemists. held at Washington, D. C., October 1–3, 1951.

† Frank A. Vorhes, Jr., Chief.

the ferricyanide method. This compound also gives only a slight blue color with the bromophenol blue method (equal to about one fiftieth of the intensity of that produced by an identical quantity of lauryldimethylbenzylammonium chloride).*

Lauryldimethylbenzylammonium chloride, cetyldimethylbenzylammonium chloride, and alkyldimethylbenzylammonium chloride were chosen because the latter is a widely used quaternary ammonium compound. The lauryl compound comprises about 60% of it and the cetyl compound a much lesser proportion.

A second pair of closely related compounds are: (1) cetyltrimethylammonium bromide and (2) cetyldimethylethylammonium bromide.

A trio of chosen closely related compounds consists of the following: 1. di-isobutylphenoxyethoxymethyldimethylbenzylammonium chloride, 2. di-isobutylphenoxyethoxyethyldimethylbenzylammonium chloride, and 3. di-isobutylcresoxyethoxyethyldimethylbenzylammonium chloride.

The next compound contains a nitrogenous group in the chain, *viz.:* dodecylacetamidyldimethylbenzylammonium chloride.

The next contains two chlorine atoms on the benzene ring, *viz.:* lauryldimethyldichlorobenzylammonium chloride and the final pair contain the pyridine ring and differ in the matter of the length of the side chain attached to the nitrogen atom: (1) laurylpyridinium chloride and (2) cetylpyridinium chloride.

It is believed that this choice of quaternaries gives a wide variety of composition and that comparisons of the closely related members give an excellent basis for judging the selectivity of the described identifications.

OPTICAL-CRYSTALLOGRAPHIC PROPERTIES OF THE REINECKATES

Material crystallized from 95% alcohol was used for optical-crystallographic studies. Refractive indices were determined by the immersion method using liquids made up of varying amounts of mineral oil, halowax oil, and methylene iodide. All indices were determined within ± 0.003. For several substances n_β could not be determined; however, for some of these an intermediate index, n_i, is given where the latter is of diagnostic value in relation to the crystal habit.

Triethylbenzylammonium Reineckate

When examined in ordinary light under the microscope, this substance is observed to be in the form of large, pink-colored, platy crystals, often rhomboid in outline. In parallel polarized light (crossed Nicol prisms) the extinction is symmetrical, and brilliant interference colors are shown. In convergent polarized light, acute bisectrix figures with a very small axial angle and negative optic sign are frequently seen. The refractive

* *This Journal*, 29, 314 (1946).

indices are $n_\alpha = 1.593$, $n_\beta = 1.687$, and $n_\gamma =$ ca. 1.697; n_α and n_γ are the most common indices.

Lauryldimethylbenzylammonium Reineckate (DC-12)

In ordinary light this substance is seen to be in the form of large platy crystals, often rhomboid or 6-sided in outline. In plane polarized light (crossed Nicol prisms) the extinction is symmetrical or parallel and the elongation is negative. Brilliant polarization colors are shown. In convergent polarized light partial acute bisectrix figures with negative optic sign, are frequently seen. The refractive indices are $n_\alpha = 1.576$, $n_i = 1.669$, and $n_\gamma = 1.678$; n_i and n_γ are the most common indices.

Alkyldimethylbenzylammonium Reineckate

When examined under the microscope in ordinary light this substance is observed to be in the form of plates, often rhomboid in outline. With crossed Nicol prisms the extinction is symmetrical. Brilliant polarization colors are exhibited. In convergent polarized light, partial acute bisectrix figures are often observed with negative optic sign. The refractive indices determined are $n_\alpha = 1.576$ and $n_\gamma = 1.651$.

Cetyldimethylbenzylammonium Reineckate (Zetlyn ®)

When examined in ordinary light under the microscope, this substance is observed to be in the form of large plates and prisms, often rhomboid in outline. With crossed Nicol prisms the crystals show brilliant polarization colors and the extinction is symmetrical or inclined. In convergent polarized light, partial acute bisectrix figures are infrequent and have negative optic sign. The refractive indices are $n_\alpha = 1.572$, $n_\beta = 1.651$, and $n_\gamma = 1.660$.

Cetyltrimethylammonium Reineckate (Cetab)

This substance, when examined in ordinary light under the microscope, is observed to be rod-like in habit. With crossed Nicol prisms the extinction is parallel and the elongation positive. The crystals show first order gray polarization colors. No interference figures are observed with convergent polarized light. The refractive indices are $n_\alpha = 1.591$ shown crosswise, $n_i = 1.609$ shown lengthwise, and $n_\gamma = 1.616$ shown lengthwise; n_α and n_i are the most common indices.

Cetyldimethylethylammonium Reineckate (Ethyl Cetab)

When examined in ordinary light under the microscope this material is observed to be in the form of thin plates and rods. With crossed Nicol prisms, first order gray interference colors are exhibited. The extinction is parallel and elongation positive. No interference figures are observed with convergent polarized light. The refractive indices are $n_\alpha = 1.587$

shown crosswise, $n_\beta = 1.599$ shown crosswise, and $n_\gamma = 1.626$ shown lengthwise; n_β and n_γ are the most common indices.

Di-isobutylphenoxyethoxymethyldimethylbenzylammonium Reineckate (Phemerol ®)

In ordinary light under the microscope, this substance is seen to be in the form of rods and plates, with occasional fibrous aggregates. With crossed Nicol prisms the extinction is parallel and the elongation positive. First order gray and white interference colors are shown, together with occasional red or blue colors. With convergent polarized light, optic axis figures with a small axial angle (estimated to be 15° to 30°) and negative optic sign are frequently seen. The refractive indices are $n_\alpha = 1.577$ shown crosswise, $n_\beta = 1.671$ shown lengthwise, and $n_\gamma = 1.678$ shown lengthwise.

Di-isobutylphenoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 1622 ®)

When examined in ordinary light under the microscope, this substance is observed to be in the form of large, pink-colored, platy crystals. With crossed Nicol prisms the extinction is parallel or inclined, and the elongation is positive. First order gray interference colors are usually shown. In convergent polarized light, optic axis figures with a small axial angle (estimated to be 15° to 30°) and negative optic sign are commonly observed. The refractive indices are $n_\alpha = 1.577$ shown crosswise, $n_\beta = 1.671$ shown crosswise and $n_\gamma = 1.678$ shown lengthwise; n_β is the most common index.

Di-isobutylcresoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 10X ®)

When examined in ordinary light under the microscope, this substance is observed to be in the form of platy crystals. With crossed Nicol prisms the extinction is parallel and the elongation is positive. First order gray interference colors are shown by most of the crystals. In convergent polarized light partial acute bisectrix figures may be seen with negative optic sign. The refractive indices are $n_\alpha = 1.582$, $n_i = 1.638$ shown crosswise, and $n_\gamma = 1.670$ shown lengthwise; n_i is the most common index.

Dodecylacetamidyldimethylbenzylammonium Reineckate (Dobenzyl chloride)

When examined in ordinary light under the microscope, this substance is observed to be in the form of plates and rods. With crossed Nicol prisms the crystals exhibit brilliant polarization colors. The extinction is parallel and the elongation is negative. No interference figures are seen with convergent polarized light. The refractive indices are $n_\alpha = 1.582$ shown lengthwise and $n_\gamma = 1.664$ shown crosswise. Both indices are readily found.

Lauryldimethyldichlorobenzylammonium Reineckate

In ordinary light under the microscope, this substance is seen to be in

the form of large rods and plates. With crossed Nicol prisms the extinction is parallel and the elongation is negative. Brilliant interference colors are exhibited. In convergent polarized light, partial acute bisectrix figures with negative optic sign are observed. The refractive indices are $n_\alpha = 1.582$ shown lengthwise, $n_i = 1.593$ and $n_\gamma = 1.677$ shown crosswise; n_α and n_i are the most common indices.

Laurylpyridinium Reineckate

This substance, when examined in ordinary light under the microscope, is observed to be in the form of platy crystals. With crossed Nicol prisms the extinction is parallel and the elongation is positive. Most of the crystals show gray or white polarization colors, but some show first order yellow or red. In convergent polarized light no interference figures are seen. The refractive indices are $n_\alpha = 1.609$ shown crosswise, $n_i = 1.636$ shown lengthwise, and $n_\gamma = 1.651$ shown lengthwise; n_α and n_i are the most common indices.

Cetylpyridinium Reineckate (Ceepryn ®)

When examined in ordinary light under the microscope, this substance was observed to be in the form of fibrous aggregates, and was unsatisfactory for a complete optical study. With crossed Nicol prisms the extinction of individual fibers is parallel and the elongation is negative. Most of the crystals exhibit first order gray or white polarization colors, and a few show yellow color. No interference figures were seen. The refractive indices could not be determined for this material.

Table 1 is a summary of the optical-crystallographic data for the Reineckates of the thirteen quaternary ammonium compounds studied. The substances are arranged according to ascending value of the lowest index.

Examination of the data reveals that most of the Reineckates can be readily differentiated on the basis of their refractive indices. Three compounds, however, namely lauryldimethylbenzylammonium Reineckate (DC-12), di-isobutylphenoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 1622), and di-isobutylphenoxyethoxymethyldimethylbenzylammonium Reineckate (Phemerol), have almost indentical refractive indices. The first of these Reineckates may be differentiated because of the difference in sign of elongation. The latter two, which are closely related to each other chemically, could not be distinguished from each other by optical methods, but are readily distinguished from a third closely related compound, di-isobutylcresoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 10X).

Members of other groups of closely related compounds named earlier in this paper can be distinguished from each other without difficulty. It is believed that this method furnishes a very useful and practical means of identifying quaternary ammonium compounds.

TABLE 1.—*Reineckates of quaternary ammonium compounds*

	REFRACTIVE INDICES[1]			OPTIC SIGN	EXTINCTION[2]	ELONGATION	HABIT
	α	β	γ				
Cetyldimethylbenzylammonium Reineckate (Zettyn®)	1.572	1.651	1.660	—	i, s		Rhomboid plates
Alkyldimethylbenzylammonium Reineckate	1.576		1.651	—	s		Rhomboid plates
Lauryldimethylbenzylammonium Reineckate (DC-12)	1.576	1.669i	1.678	—	p, s	−	Rhomboid plates
Di-isobutylphenoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 1622®)	1.577	1.671	1.678	—	p, i	+	Plates
Di-isobutylphenoxyethoxymethyldimethylbenzylammonium Reineckate (Phemerol®)	1.577	1.671	1.678	—	p, i	+	Rods, Plates, Fibrous
Cetylpyridinium Reineckate (Ceepryn®)	Unsatisfactory for optical study				p	−	Fibrous
Lauryldimethyldichlorobenzylammonium Reineckate (*Dichloran*)	1.582	1.593i	1.677	—	p	−	Rods and Plates
Di-isobutylcresoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 10×)	1.582	1.638	1.670	—	p	+	Plates
Dodecylacetamidyldimethylbenzylammonium Reineckate (Dobenzyl chloride)	1.582		1.664		p	−	Plates and Rods
Cetyldimethylethylammonium Reineckate (Ethyl Cetab)	1.587	1.599	1.626		p	+	Plates and Rods
Cetyltrimethylammonium Reineckate (Cetab)	1.591	1.609i	1.616		p	+	Rods
Triethylbenzylammonium Reineckate	1.593	1.687	1.697 (ca.)	—	s		Rhomboid plates
Laurylpyridinium Reineckate	1.609	1.636i	1.651		p	+	Plates

[1] Above refractive indices ±0.003, at 24°–26°C; i =intermediate index.
[2] Extinction: p =parallel; i =inclined; s =symmetrical.

SUMMARY

A method is given for the preparation of Reineckate salts of quaternary ammonium compounds of a variety of composition.

The Reineckate salts of these compounds have been prepared, and their optical crystallographic properties have been determined. These are given in a convenient form and may be employed in the identification of the salts.

DETERMINATION OF SUCARYL ® IN SUGAR-FREE BEVERAGES*

By JOHN B. WILSON (Division of Food,† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

Sucaryl (cyclamate sodium or sodium cyclohexylsulfamate) has recently became an article of commerce as an artificial sweetener. It has no food value and one of its chief uses is in special dietary products for use by persons restricted as to intake of sugar. As this substance is said to be unaffected at boiling temperatures it may be used as an ingredient of food products which are cooked or otherwise heated during the process of manufacture.

In comparatively pure solution, sucaryl may be determined from the barium sulfate formed when sucaryl is treated with nitrous acid in the presence of soluble barium salts. (Nitrite reacts with sulfamic acids with the evolution of gaseous nitrogen and the formation of sulfuric acid.)

Among the simplest foods in which sucaryl is used are sugar-free beverages which contain water, fruit acid, artificial sweetener, artificial color and artificial flavor. When such mixtures are treated with nitrous acid and barium chloride, the precipitate of barium sulfate is highly colored and otherwise contaminated.

A number of unsuccessful experiments were carried out in an attempt to extract the cyclohexylsulfamic acid with immiscible solvents from solutions of sucaryl acidified with sulfuric acid. The writer has found, however, that in the dry state cyclohexylsulfamic acid is fairly soluble in chloroform. He has also found that sodium cyclohexylsulfamate can be extracted from a dry mixture of this salt and sodium citrate or tartrate by treating the mixture with warm alcohol.

To obtain the free cyclohexylsulfamic acid, the sodium salt is passed through a cation exchange resin and the solution and washings are evaporated. A method based on these findings has been developed and applied to a number of mixtures prepared in the laboratory as well as to a few samples of commercial sugar-free beverages.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October 1–3, 1951.

† Frank A. Vorhes, Jr., Chief.

METHOD

APPARATUS

Cation column: Assemble a cation exchange resin column using Duolite C-3 and activate it as directed in J.A.O.A.C. **33**: 1000 (1950).

PROCEDURE

Place a measured volume of decarbonated sugar-free beverage expected to contain 50–100 mg of sucaryl in a 250 ml beaker; add sufficient *N* NaOH to neutralize the free acids to phenolphthalein as determined by a separate preliminary titration and evaporate to dryness on the steam bath. Extract the dry residue with 50 ml, 25 ml, 15 ml, 10 ml, and 10 ml portions of alcohol by boiling a few moments on the steam bath and pouring through a small filter paper into a 150 ml beaker. Evaporate to dryness on the steam bath. Dissolve the residue in 100 ml of H_2O, pass through the cation column and collect the effluent in a 400 ml beaker; wash the column with 250 ml of H_2O and evaporate to dryness on the steam bath. Extract the dry residue with 20 ml, 15 ml, 10 ml, 5 ml and 5 ml portions of $CHCl_3$ heating each portion to boiling for a few moments and pouring through a small filter paper into a tared 50 ml beaker. Allow the solvent to evaporate on a warm, but not hot, surface; when visibly dry place in a desiccator for several hours and weigh. The weight of the crystals of cyclohexylsulfamic acid multiplied by 1.123 gives the weight of sucaryl.

Since certain flavoring constituents (vanillin) and other ingredients of samples of unknown composition may also be present in the residue it should be analyzed by the barium sulfate method as a check on the weight of sucaryl. For this check analysis dissolve the residue in small portions of warm water aggregating 100 ml, filter each in rotation into a 250 ml beaker, add 2 ml HCl (1+1), 5 ml of 2.5% $NaNO_2$, 5 ml of 10% $BaCl_2 \cdot H_2O$ and stir. Finally add 5 ml more of 2.5% $NaNO_2$, stir and let stand 2–3 hours. Filter on a Gooch crucible and wash with 2–3 15 ml portions of water, then with alcohol. Dry at 105° for several hours, cool in a desiccator and weigh. Weight of $BaSO_4 \times 0.862$ = weight of sodium cyclohexylsulfamate.

It was found that in some cases sufficient artificial color came through with the crystals of cyclohexylsulfamic acid to give it a slight color.

The procedure was carried out on three types of sample with added known quantities of sucaryl. The results are given in Table 1.

TABLE 1.—*Determination of sucaryl in synthetic samples*

INGREDIENTS	TYPE A		TYPE B			TYPE C	
Sucaryl, mg	111.5	111.5	111.5	111.5	111.5	112.3	112.5
Water, ml	100	100	100	100	100	100	100
Citric acid, mg	none	none	100	100	100	100	100
Artificial color	none	none	none	none	none	present	present
FOUND:							
cyclohexylsulfamic acid, mg	95.0	92.2	99.3	100.6	94.5	95.2	95.7
Sucaryl, mg	107.1	111.8	111.9	113.4	106.5	106.9	107.8

Some samples of commercial sugar-free beverages (one brand; several flavors) labeled as containing 0.25% sucaryl were obtained on the open market. In addition to sucaryl, fruit acid, color, and flavor this particular

brand contains pectin which must be first removed. This was accomplished by diluting 50 ml of sample to 250 ml with alcohol in a volumetric flask. Most of the pectin came down and after filtering through a folded filter the solution was neutralized with NaOH and the analysis was continued as above. Triplicate determinations on a so-called "White Soda" showed the presence of 0.2135, 0.2105, and 0.2077 g of sucaryl per 100 ml. The extracted residue was odorless and appeared to be pure cyclohexylsulfamic acid. The first two of these residues were dissolved in water and subjected to the check analysis by precipitating $BaSO_4$. These gave a content of 0.199 and 0.195 g per 100 ml of sucaryl, respectively.

In making an analysis of two samples of a so-called "Cherry Soda" the residues had a strong odor of vanillin and other flavors. Their weights showed the presence of 0.350 per 100 ml and 0.287 per 100 ml. When the check determination was made, the precipitates of $BaSO_4$ showed the sucaryl content to be 0.185 g and 0.164 g sucaryl per 100 ml.

SUMMARY

A method is described for determination of sucaryl in sugar-free beverages as cyclohexylsulfamic acid, subject to check by precipitation of barium sulfate. A procedure is given for preparation of sample when pectin is present, but because of the varied composition of sugar-free beverages on the market it will probably be necessary to devise other means of eliminating interfering agents before applying the method to all types of samples.

ISOLATION AND MICROSCOPIC IDENTIFICATION OF CYCLAMATE SODIUM (SUCARYL ®) AS CYCLOHEXYLSULFAMIC ACID*

By A. H. TILLSON and JOHN B. WILSON (Divison of Microbiology and Division of Food† Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

While searching for means of identifying sucaryl ®, some crystals of cyclohexylsulfamic acid were examined under the polarizing microscope and found to be suitable for identification by this means. To obtain the crystals, solutions of cyclohexylsulfamate sodium containing about 100 mg in 100 ml were passed through a cation resin exchange column, the effluent and washings were evaporated on the steam bath and the residue recrystallized from chloroform.[1] The properties of the crystals are given below.

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† Frank A. Vorhes, Jr., Chief.

[1] *This Journal*, p. 459.

OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF CYCLOHEXYLSULFAMIC ACID

When examined in ordinary light under the microscope, this substance was observed to be in the form of colorless rods and elongated prisms which often showed a six-sided outline. In parallel polarized light (crossed Nicol prisms) the extinction is parallel or inclined and the elongation is negative. White and first order gray interference colors are shown. The crystals commonly exhibit multiple twinning of the albite type, appearing as multiples made up of numerous very narrow lathlike (or sometimes broad) individuals. In convergent polarized light an acute bisectrix figure with moderate axial angle (ca. 30° to 45°) and positive optic sign is often observed. The refractive indices, determined by the immersion method, are $n_\alpha = 1.500$ shown lengthwise, $n_\beta = 1.513$, and $n_\gamma = 1.541$ shown crosswise (all ± 0.003). All three indices are readily determined.

While the procedure outlined above is suitable for use on comparatively pure cyclamate sodium, such as that in tablet form or in solutions, the sweetener might also be encountered in admixture with citric or tartaric acid and artificial color in dietary sugar-free beverages or mixed with many other food ingredients in other types of products.

Solutions were then prepared to contain cyclamate sodium along with citric or tartaric acid and artificial color. It was found that substantial quantities of these acids and of the artificial colors were dissolved by chloroform, but if the sodium salts were formed, a separation could be made from the acids due to the fact that sodium cyclohexylsulfamate is rather soluble in alcohol, whereas the sodium salts of citric and tartaric acid are quite insoluble. In most cases very little of the artificial color went into solution in alcohol and most of that was eliminated by the ensuing steps in the process of isolation as given below. While the final crystalline residues were slightly colored, insufficient color was present to interfere with the identification.

Isolation from other food ingredients has not been fully studied, but the procedure for use on sugar-free beverages is given below.

ISOLATION OF CYCLOHEXYLSULFAMIC ACID

Place a quantity of sample expected to contain 50–100 mg of cyclamate sodium in a beaker and neutralize to phenolphthalein (if the sample is highly colored, titrate as in 9.55[2] and add the required volume of standard alkali) and evaporate to dryness. Extract the dry residue with three 25 ml portions of alcohol by heating on the steam bath, and after thoro mixing with a stirring rod pour thru a small filter into another beaker. Evaporate to dryness. Dissolve in 100 ml of H_2O. Pass thru a cation exchange resin column (Duolite C-3 was used in these experiments), follow with 200 ml of H_2O, and evaporate effluent and washings to dryness. Extract the dry residue with two 25 ml portions of chloroform in the same manner as the alcohol extraction above. Evaporate the filtered $CHCl_3$ solution in a small beaker on a warm, but not hot, surface under a gentle current of air.

[2] *Methods of Analysis*, A.O.A.C. 7th Ed., 1950.

Examine the crystalline residue under the polarizing microscope.

The crystalline residue obtained by application of the method for determination of cyclamate sodium in sugar-free beverages by John B. Wilson, which was discussed at this meeting, is equally suitable for the identification.

If interfering ingredients are present, it may be necessary to remove them. As an example, a sample of a sugar-free beverage which contained pectin was so viscous as to make it difficult to evaporate or to pass through an ion exchange resin column. To remove the pectin, 50 ml of sample was placed in a 250 ml volumetric flask which was gradually filled to the mark with alcohol while mixing. After standing for 30 minutes the precipitated pectin was filtered off and 100 ml aliquots of the filtrate were treated as above.

Other interfering agents will no doubt require other methods of removal.

SUMMARY

(1) The optical crystallographic properties of cyclohexylsulfamic acid are given.

(2) A method for isolation of cyclohexylsulfamic acid from sugar-free beverages is given.

(3) Further study on the removal of interfering agents is recommended.

A MANOMETRIC ADAPTATION FOR THE RAPID MICROBIOLOGICAL ASSAY OF VITAMINS AND AMINO ACIDS*

By WILLIAM H. STAHL (Pioneering Research Laboratories, U. S. Army Quartermaster Corps, Philadelphia 45, Pa.)

Recently, Siu and Mandels (1, 2) published a manometric method for a rapid assay for growth wherein growth of the organism is measured by determining the respiratory oxygen consumption. They demonstrated the applicability of the technique to the evaluation of the susceptibility of different materials to microbiological degradations and to the assay of fungitoxic substances. They suggested the feasibility of adapting this method to the biological assay for vitamins, amino acids, etc., and this is the object of the present paper. In 1944, Atkins, Williams, and Frey (3) reported on a manometric procedure employing the Warburg apparatus for vitamin estimation with lactic acid bacteria. Unfortunately, Warburg manometers are not well adapted to routine assay, whereas the manometers used herein can be easily manipulated by untrained technicians. Essentially, they are a macro-modification of the Barcroft-

* Presented at the Miniature Meeting of the Philadelphia Section of the American Chemical Society, January 18, 1951.

Haldane differential manometers; 250 ml flasks are used as vessels and are connected to the manometers by means of flexible tubing. Since a closed system is used, the measurements are not affected by fluctuations of atmospheric pressure, and precise temperature control is not mandatory.

Lactic acid bacteria are the organisms most commonly used for the microbiological assay of vitamins and amino acids. In their growth on glucose the metabolism of these organisms is predominantly fermentative as opposed to respiratory, and lactic acid production is much greater than oxygen absorption. Hence, manometric estimation of growth is accomplished more advantageously by determining the carbon dioxide liberated from a bicarbonate buffer by the lactic acid formed than by measuring O_2 consumed (4). Certain growth substances such as choline, inositol, and *p*-aminobenzoic acid can best be determined by the growth response of certain mutant forms of fungi (5, 6). The fungi are then harvested, dried, and weighed. This procedure is both time consuming and tedious. It can be avoided by the use of manometers, and, in this case, oxygen consumption is preferentially measured. With this procedure the time for the determination is shortened from 72 hours to 18 hours.

METHODS

The manometers.—Details of the construction of the manometers have been previously given (1). To measure O_2 consumption, the carbon dioxide evolved in respiration is absorbed by potassium hydroxide in a well. This procedure is applicable to methods where fungi are used. In the determinations using bacteria where CO_2 evolution is measured, the cup containing the alkali is omitted.

Assay procedure for valine.—The determination of valine is chosen for description as one of the several successful applications. The experience with assays for methionine and aspartic acid parallel it in general. *Streptococcus faecalis* 9790 was used with the medium reported by Baumgarten *et al.* (7). The standard curve obtained from measurement of turbidity (as produced in test tubes when incubated 30 hours at 37°C.) was run simultaneously with that of the curve for the response as measured by manometers mounted in incubators maintained at 30°C. The medium in the 250 ml flasks attached to the manometers differed from that in the test tubes (5 ml basal medium plus 5 ml water) in that 5 ml of 0.2 *M* sodium bicarbonate solution was used in place of the water. This amount of bicarbonate was calculated to be twice the maximum amount, based on the titrimetric values, required in a valine assay. The *p*H of the basal medium in the test tubes was 6.8; that in the flasks after addition of the bicarbonate was 8.4. The inoculum used in the standard test tube set-up was the normal amount, viz., one drop per tube, wherein the 24 hour growth in 10 ml of complete medium was centrifuged and washed twice

with 5 ml of sterile saline, and finally made up to approximately 2 ml. In the case of the manometer flasks, the 24 hour cultures of the organism were washed twice and suspended in 30 ml of saline, and 1 ml of this suspension was added to each of the flasks. This represents approximately 130 times the normal amount added to the test tubes and was purposely done to step up the rate of acid production. (Initial turbidity of the solution is unimportant when using the manometric technique.)

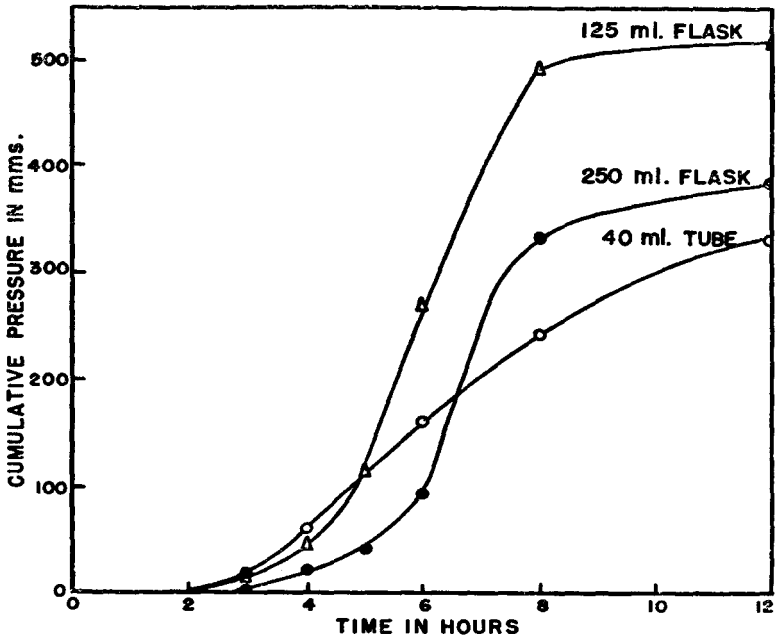

FIG. 1.—Course of lactic acid production by *S. faecalis* grown in 3 different size vessels.

Assay procedure for choline.—The standard curve was obtained by the method of Horowitz and Beadle (5), using the *cholineless* mutant of *Neurospora crassa*. After 72 hours growth at 30°C., the stationary cultures were filtered off on tared filter papers and weighed after drying in a vacuum oven for 2 hours at 50°C. In the manometric method, a small cup containing 1.5 ml of 10% potassium hydroxide and a filter paper wick was suspended in the flasks. This absorbed the evolved carbon dioxide and the manometers recorded O_2 pressure changes. The inoculum was prepared by adding sterile water to a culture of the fungus until the resultant turbidity gave a reading of 200 on the Klett-Summerson photocolorimeter when a red filter having a maximum at 640 mμ was used.

For the standard test, 1 drop of the suspension was added, and in the manometric test, 1 ml was added.

The increase in time consumed in the preparation of the equipment for the manometric procedure as compared to the test tube method is negligible. The additional time is simply that of attaching the inoculated flasks to the manometers by means of their ground-glass joints. Aspetic technics are used throughout in both procedures.

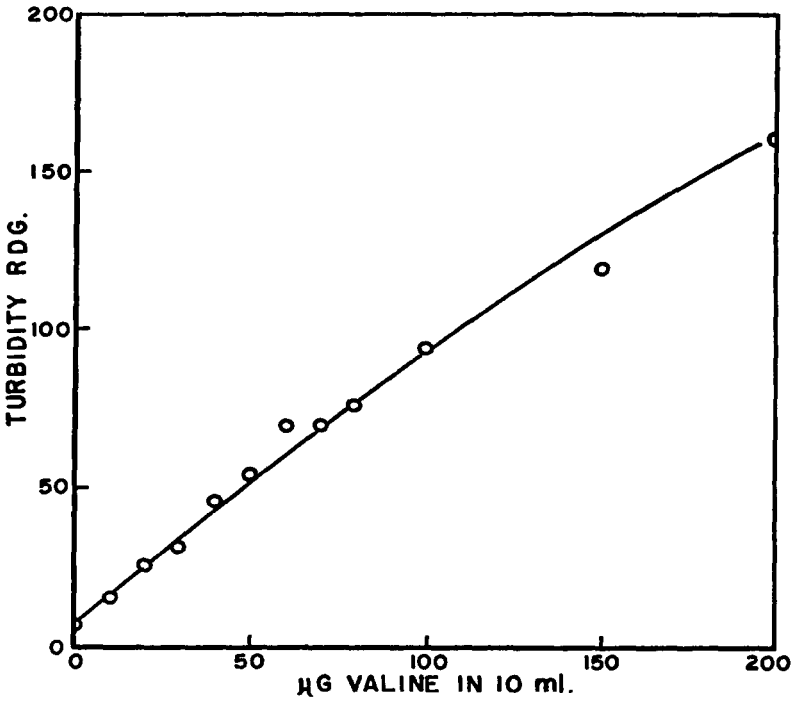

FIG. 2.—Typical standard curve obtained for d-valine. Time = 30 hours.

RESULTS

Choice of vessel size to be used with manometers.—Originally, Siu and Mandels (1, 2) used a 250 ml Erlenmeyer flask in their study of mildew susceptibility of fabrics, etc., mainly because it allowed exposure of a relatively large fabric surface. The manometer was then designed to accommodate the expected pressure differences. Since the manometers are fixed in size, it was of interest to determine whether different sized vessels were necessary for the 10 ml total volume of basal medium for bacterial growth and the 20 ml used for fungal growth. Figure 1 shows the growth

curve obtained with *Streptococcus faecalis* at 37°C. using 40 ml tubes and 125 and 250 ml Erlenmeyer flasks. The 250 ml flask was used thereafter, since (A) it allowed the pressure curve (growth period) to level off before that of the 40 ml tube; (B) the pressure changes were not so great as those in the 125 ml flask and required less resetting of the manometers. As designed, the manometers can register a pressure of approximately 200 mm before it becomes necessary to equalize the pressure in both flasks to avoid forcing over the manometer fluid. Use of a manometer fluid of

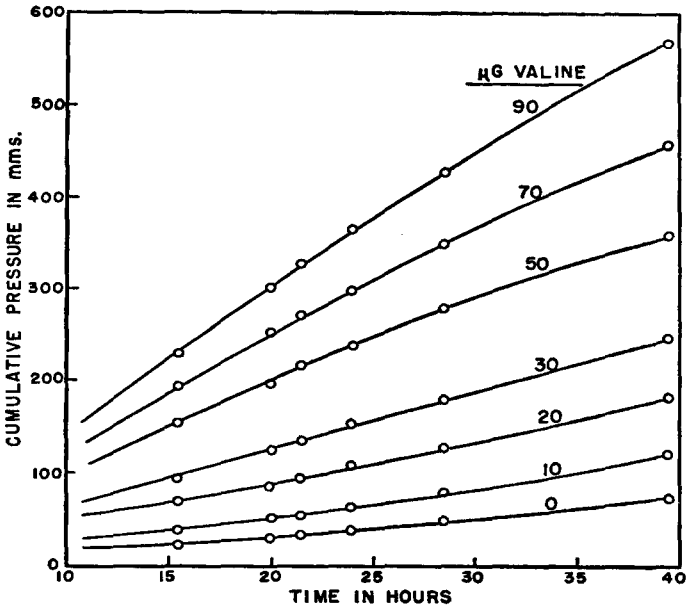

FIG. 3.—Lactic acid production by *S. faecalis* in response to varying amounts of valine.

greater density than the Brodies solution used herein would avoid this difficulty (8).

At the same time that a standard curve for the growth response of *S. faecalis* to varying amounts of valine was determined by turbidimetric measurements, a series of time-pressure curves, with varying amounts of valine, were prepared. These are illustrated in Figures 2 and 3. In all cases they are averages of duplicate readings. Figure 4 is a translation of the values of Figure 3, and show cumulative pressure against micrograms of valine after a certain time of incubation. Simultaneously, a lactalbumin hydrolysate containing 11.2% nitrogen was run at suitable dilutions with both the test tube and manometric techniques. In the latter case,

however, due to lack of sufficient manometers, only 3 dilutions could be run. Using the resultant turbidimetric curve, calculations to a moisture-free basis gave 5.98 per cent of l-valine, This agrees well with a series of other values found in the literature (9) for lactalbumin. An average of 223 micrograms of dl-valine per ml of test lactalbumin was obtained from the turbidimetrically measured curve. The average for the 3 manometric

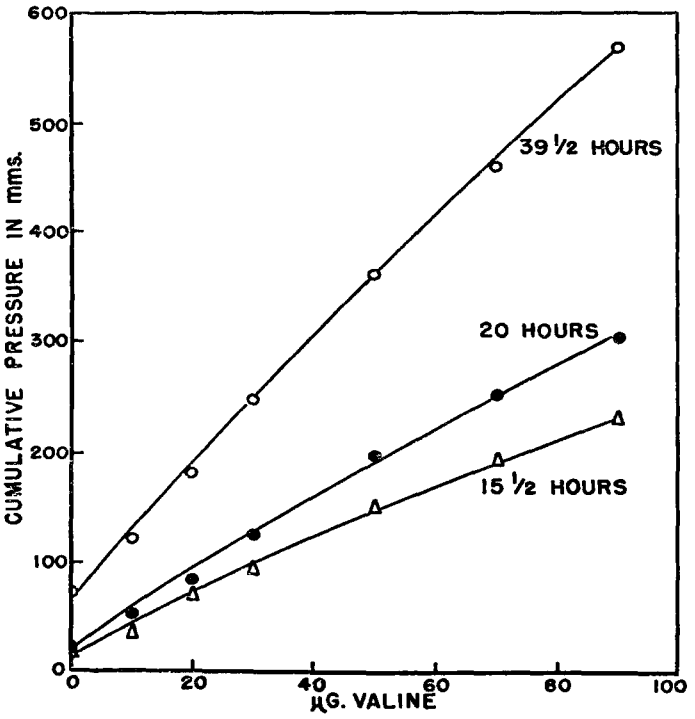

FIG. 4.—Effect of valine on lactic acid production.

values, taken from the curves of Figure 4, are: 15½ hrs., 237.5 micrograms; 20 hrs., 240 micrograms; and 39½ hrs., 204.4 micrograms.

In another series of experiments with valine, a set of curves similar to that in Figure 2 was obtained but with points at 4, 5, 6, 8, 12, and 22 hours. A 10 hour and 11½ hour plot of the cumulative pressure against micrograms of valine as shown in Figure 5 gave smooth curves similar to those in Figure 4.

The effect of choline concentration on growth of the fungus *Neurospora crassa* is given in Figure 6, and was determined by the dry weight of my-

celium at 3 days. The course of respiration as determined on the manometers is given in Figure 7. One hundred and twenty-five ml flasks were used in this experiment. An earlier change in the course of respiration might have been achieved if the spore suspension used as inoculum had been aseptically washed several times with distilled water. This would have prevented possible carry-over when this relatively large volume of inoculum was used. In Figure 8, the data of Figure 7 are replotted, with time constant and the amount of choline varied.

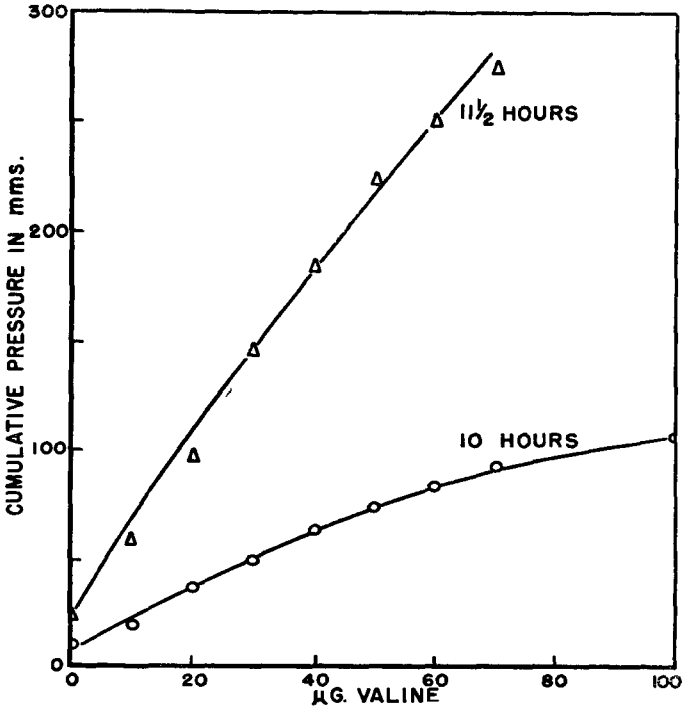

FIG. 5.—Lactic acid production by *S. faecalis* with varying quantities of valine.

DISCUSSION

The primary objective of this study was to determine the feasibility of applying the manometric technique of Siu and Mandels (1, 2) to the microbiological assay of amino acids and vitamins. Limitations of both time and number of available manometers precluded extensive application. However, sufficient data have been accumulated to show that manometers can be used for this purpose. Refinements such as vessel size, size of manometer capillary, specific gravity of manometer fluid, size of inocu-

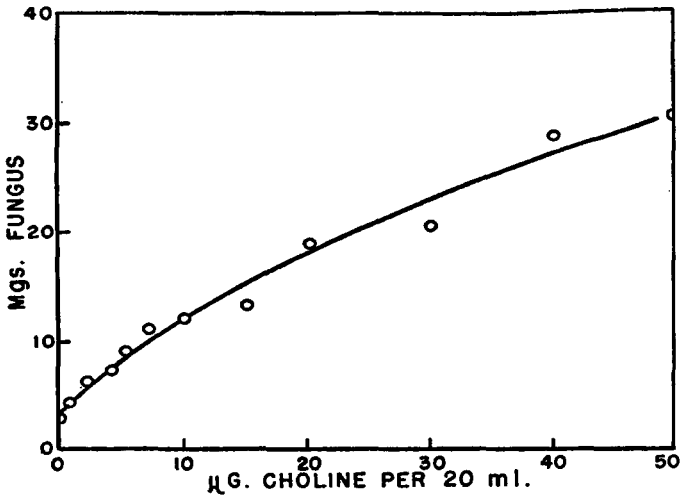

FIG. 6.—Dry weight of cholineless fungus after 3 days as a function of concentration of choline in the medium.

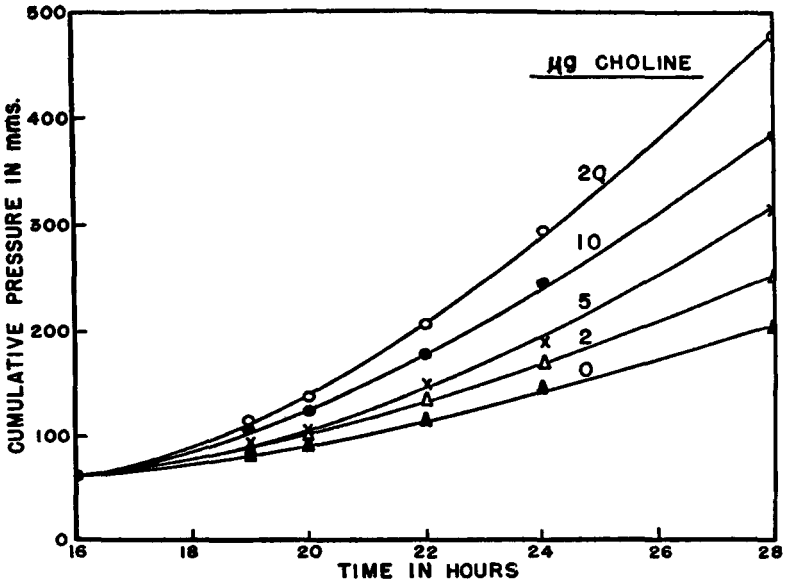

FIG. 7.—Course of respiration of *Neurospora crassa* in response to varying amounts of choline.

lum, volume of basal medium, buffers, temperature and other variable factors would improve the precision.

Preliminary experiments indicate that it is possible to reduce the number of manometers necessary to run an assay on an unknown substance by setting up one series of perhaps 5 manometers and placing a constant amount of the standard on the right side vessel. Then the pressure gener-

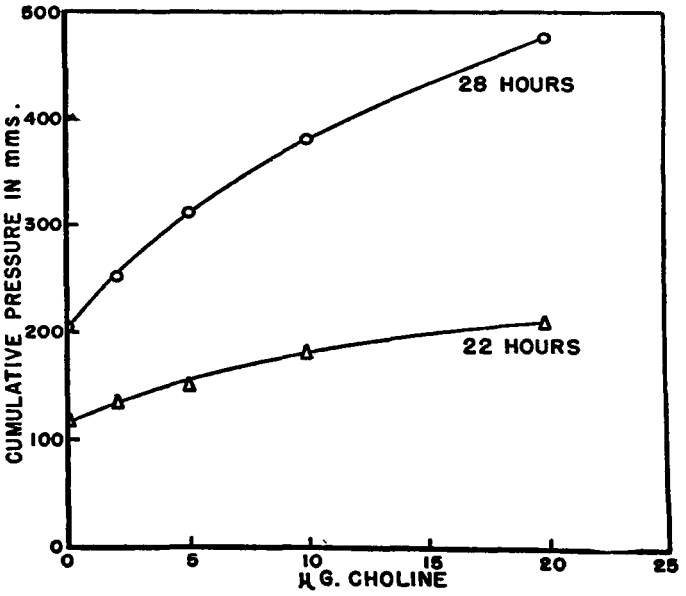

FIG. 8.—Effect of choline on the oxygen consumption.

ated by the presence of this quantity opposes the pressure generated by the effect of the 5 dilutions of the unknown on the left. These latter dilutions should be so made up as to bracket the quantity of standard used. Values should be taken only from the straight line portion of the normal growth curve.

Since respiration or fermentation of the microorganisms used correlates with their growth as measured by turbidity, titration, or direct weighing (3, 4), advantages of the manometric method may be pointed out. When using bacterial growth methods, the manometric adaptation should allow determination of amino acids or vitamins in crude materials, particularly in the case wherein turbidity cannot be avoided in the preparation of the samples for assay. One example would be in the vitamin B_{12} assay of chicken feeds; others in the various vitamin assays of distillery mashes and Army concentrated rations.

A reduction of the time factor for assay is in some cases extremely desirable and can be obtained by the use of manometers. For example, Figure 4 shows that in 15½ hours (approximately half the time needed to produce a good turbidity), a standard curve was derived which allowed a fairly satisfactory determination of valine in lactalbumin. It is possible to cut this down even further with the above suggested refinements. The weighing of mycelium in those methods which use a fungus as the microorganism is a rather tedious procedure. Here too, time may be cut from 3 days to 1 day, as indicated by the curves in Figure 8.

SUMMARY

1. A manometric method has been devised which is applicable to the determination of amino acids, vitamins, and accessory growth factors.

2. The method is based upon a determination of growth as measured by carbon dioxide pressure generated as the result of release of lactic acid by the bacteria; in the case of assays using fungi, it is based upon determination of growth as measured by oxygen consumption.

3. The manometric procedure eliminates the necessity of filtering off, drying, and weighing the fungi which are used for certain assays. Elapsed time in this case may be cut from 3 days to 1 day.

4. The time in the case of bacterial growth responses may be lowered from 30 hours to at least as low as 11 hours.

ACKNOWLEDGMENTS

Acknowledgment is due to Estelle K. Radbill and Abraham Clearfield for their valuable technical assistance; also to Lloyd Colio of Wyeth Institute for Applied Biochemistry and Helen R. Skeggs and L. D. Wright of Sharpe and Dohme, Inc., for their valuable suggestions.

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A NEW CHEMICAL APPROACH TO THE DETERMINATION OF VITAMIN B_6*

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The color reactions used in the chemical determination of pyridoxine are chiefly those which depend upon the phenolic nature of the vitamin. The Folin-Denis reagent, ferric chloride, diazotized sulfanilic acid and 2,6-dichloroquinonecholroimide all react with phenols to produce color. Colorimetric methods for the determination of vitamin B_6 based on the phenolic properties have been reported by Swaminathan (1) and Bina and Thomas (2) and involve coupling of the vitamin with diazotized sulfanilic acid. Scudi (3) and Hochberg, Melnick and Oser (4) have published methods which utilize 2,6-dichloroquinonechloroimide for this purpose. Another color reaction, based on the reactivity of the α-methyl group, has been used in the preparation of cyanine dyes (5). Stiller, Keresztesy and Stevens (6) oxidized pyridoxine to the 4,5-dicarboxylic acid and fused this acid with resorcinol to obtain a fluorescent phthalein. However, neither of these latter reactions is quantitative or specific.

Pyridoxine is a substituted pyridine derivative. It has been demonstrated by König (7) that the pyridine ring can be ruptured with cyanogen bromide and that the resulting product can be coupled with an aromatic amine with production of a colored complex. This reaction is the basis for several chemical methods for the determination of nicotinic acid (pyridine 3-carboxylic acid). Of the pyridine derivatives which we have investigated those substituted either at positions 2 or 6 (the carbon atoms adjacent to the nitrogen of the pyridine ring) give little or no color with the König reagent even after heating for several minutes. This is illustrated by α-picoline, α-aminopyridine, picolinic acid and quinoline, which are all α-substituted compounds and give no color with cyanogen bromide and an aromatic amine (8). Similarly pyridoxine, also a 2-substituted pyridine derivative, does not give the König reaction (9). It appeared probable that it does not give the test because of the presence of the 2-methyl group.

It is possible to convert the methyl groups on a pyridine ring to carboxyl groups by oxidation (10) (11). It has been demonstrated by previous workers (12) that carboxyl groups on the 2, 4, and 6 positions are less stable than those on the 3 and 5 positions. Pyridine-2,3,5,6-carboxylic acid and pyridine-2,3,5-carboxylic acid can be converted to pyridine-3,5-carboxylic acid (with loss of CO_2) by heating to 120–150°C. It occurred to the writers that removal of the α-methyl group from pyridoxine

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by these means might yield a derivative that would react with the König reagent.

To show that this is possible, quinoline was oxidized by heating to 180° in the presence of sulfuric acid with a small amount of mercury as catalyst. The neutralized solution gave a positive test when treated with the König reagent. Pyridoxine was then treated in the same manner and also gave a positive test. When sulfanilic acid was used as the aromatic amine the resulting color had an absorption maximum at 480 mμ,

Although mercury was used as a catalyst in our preliminary tests, previous investigators have reported that selenium is an effective catalyst for oxidation of alkyl groups in substituted benzene or pyridine compounds. Woodward, Badgett and Kaufman (13) used selenium and sulfuric acid for oxidation of nicotine to nicotinic acid. The writers were able to demonstrate that α-methylpyridine gives the König reaction after oxidation with sulfuric acid and selenium.

Since nicotinic acid also gives the König reaction it is necessary to differentiate between pyridoxine and nicotinic acid. The latter vitamin is not affected by the sulfuric acid selenium treatment. Therefore, if the König reaction were carried out on a mixture of nicotinic acid and pyridoxine before and after the oxidation treatment, any increase in color would be due to pyridoxine. Thus it should be possible to determine both nicotinic acid and pyridoxine on the same extract or solution. However, since many vitamin preparations contain several hundred times as much nicotinic acid as pyridoxine, it can readily be seen that the increase in color resulting from the oxidation of pyridoxine could not be measured accurately in the presence of such a large quantity of nicotinic acid. For this reason an attempt was made to separate the two vitamins. It is not feasible to separate them gravimetrically by means of their salts or by extraction with organic solvents.

Scudi (3) has reported that pyridoxine at *p*H 7.0 could be adsorbed quantitatively on zeolite. This suggestion led us to test adsorption techniques as a means of making the separation. We were able to demonstrate with the use of a synthetic zeolite "Decalso", that pyridoxine was adsorbed quantitatively from a solution containing the two vitamins at *p*H 7.0. Niacin and niacinamide were not adsorbed. For this purpose a base exchange tube $\frac{3}{4}$ inch in diameter and 10 inches long that holds approximately 10 grams of decalso (similar to that used in the thiochrome determination of thiamine) was found satisfactory. The tube has a sealed-in capillary tube which regulates the rate of flow of eluate at approximately one ml per minute.

For the elution of pyridoxine from the decalso, hot potassium chloride solution was not completely satisfactory since the salt interfered at a later step in the procedure. Although it was possible to remove the vitamin from the potassium chloride eluent by extraction with alcohol, a more

practical procedure is the use of hot *N* ammonium hydroxide as the eluting agent.

The ammonium hydroxide eluate is evaporated just to dryness and oxidized while heating with sulfuric acid and selenium. After neutralization, the resulting solution is treated with cyanogen bromide and sulfanilic acid. (Of the aromatic amines used in the König reaction, sulfanilic acid has been found most satisfactory in the chemical determination of nicotinic acid (14). It also gives a color with higher absorbancy than other aromatic amines tested and we have used it routinely in the reaction with oxidized pyridoxine.)

Results of recovery experiments are presented in Table 1. Recovery ranged from 90 to 103 per cent for products known to contain pyridoxine hydrochloride, and is regarded as satisfactory for this type of procedure.

TABLE 1.—*Recovery of pyridoxine when added to commercial preparations containing mixtures of the B vitamins*

SAMPLE NO.	PYRIDOXINE ASSAYED	PYRIDOXINE ADDED	TOTAL FOUND	RECOVERY
	γ/ml	*γ/ml*	*γ/ml*	*(per cent)*
1	6.5	6.4	13.0	101
2	8.0	6.4	13.3	92
3	6.4	3.2	8.6	90
4	6.4	6.4	12.8	100
5	6.4	6.4	12.8	100
6	6.4	6.4	13.0	102
7	8.3	5.0	13.7	103
8	4.5	4.0	8.25	97

The biologically active forms of vitamin B_6 are known to be pyridoxine, pyridoxal, and pyridoxamine. These forms were tested to determine whether or not they react quantitatively with the König reagent. Pyridoxine and pyridoxal gave the test after oxidation with sulfuric acid and selenium. With pyridoxamine, however, only a very faint color was produced.

It is apparent that the difference between pyridoxamine and pyridoxine is the presence of a primary amine. Primary amines can be converted to alcohols by nitrous acid. When pyridoxamine was treated with sodium nitrite and hydrochloric acid, then oxidized with sulfuric acid and selenium, it was found to give the König reaction, and the absorbency of the color indicated that the conversion of pyridoxamine was complete. This provides a means not only for the determination of total vitamin B_6, but also for differentiation of pyridoxamine from the other two forms.

Table 2 gives a comparison of results, obtained by the method described here, with data obtained by the chloroimide procedure described by Hochberg *et al.* (4). Results compare favorably, except for brewer's

TABLE 2.—*Comparison of cyanogen bromide-sulfanilic acid and chloroimide methods*

SAMPLE	STATED CONTENT	FOUND	
		CNBr	CHLOROIMIDE
1. Multi Vitamin Capsule	0.75 mg/2 tab	0.84	0.75
2. Multi Vitamin Capsule	0.125 mg/cap	0.156	0.164
3. Multi Vitamin Capsule	0.75 mg/cap	0.75	0.84
4. Multi Vitamin Capsule	0.5 mg/tab	0.46	0.48
5. Multi Vitamin Capsule	125 μ/cap	182	166
6. Multi Vitamin Capsule	1 mg/cap	0.85	0.76
7. Liver, Iron, & B Vitamins	125 mg/cap	162	146
8. Pyridoxine Hydrochloride (injection)	50 mg/ml	49.4	55.0
9. Pyridoxine Hydrochloride (injection)	50 mg/ml	55	54
10. B Vitamins (injection)	10 mg/ml	11	10.9
10. B Vitamins (injection)	2 mg/ml	2.2	2.95
12. B Vitamins	2 mg/cap	0.46	0.84
13. B Vitamins	2 mg/tab	2.3	2.0
14. B Vitamins (injection) (0.5% Phenol)	0.3 mg/2 ml	0.26	0.08
15. B Vitamins (injection) 0.5% Phenol)	0.3 mg/2 ml	0.38	0.018
16. Pyridoxine Hydrochloride (injection) (0.5% Phenol)	20 mg/2 ml	20	21
17. Fortified Yeast Extract	0.5 mg/5 ml	0.48	0.53
18. Brewer's Yeast	4 mg/100 g	4.5	Trace

yeast (sample 18) and for low potency preparations containing phenol (samples 14, 15).

This method has been used chiefly for the determination of B_6 in pharmaceuticals. In order to determine B_6 in more complex materials a preliminary purification of the sample before adsorption on decalso is necessary. Work on this phase of the problem is still in progress.

SUMMARY

A method for the direct measurement of vitamin B_6 has been presented. The basic steps are:

1. Solution of the vitamin.
2. Separation from niacin and other materials by adsorption on decalso at *p*H 7 and elution with ammonium hydroxide.
3. Evaporation of the eluate to dryness, conversion of pyridoxamine when present, and oxidation with sulfuric acid and selenium.
4. Development of color by reaction of the pyridine ring with cyanogen bromide and sulfanilic acid.
5. Photometric measurement of color.

Pyridoxine fails to give the test without special treatment, and a means is offered for its differentiation from the other two forms of the vitamins.

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A STUDY, BY PAPER CHROMATOGRAPHY, OF THE OCCURRENCE OF NONFERMENTABLE SUGARS IN PLANT MATERIALS

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Experimental studies on the removal of non-sugar reducing substances from extracts of several different plant varieties have shown that some of the extracts contain appreciable quantities of reducing materials not fermented by bakers' yeast. Examination of these substances by paper chromatography, to determine the presence of nonfermentable sugars, revealed spots on the chromatograms tentatively identified as melibiose (residue from raffinose), manninotriose (residue from stachyose), galactose, ketoheptoses, arabinose, and xylose (2).

A more satisfactory sugar analysis of plant material can be made if the sugars present have been identified. Therefore, to determine whether nonfermentable sugars are widely distributed in plants, extracts of a wide variety of such materials were prepared and examined by paper chromatography. This is a report on this investigation.

PROCEDURE

A hot 80 per cent ethanol extract of each plant material was prepared and diluted to a definite volume (4). The alcohol was removed from ali-

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quots of these solutions by evaporation on a steam bath; the concentrates were cooled, filtered through a mat of Celite Analytical Filter-Aid[2] and diluted to about 50 ml with distilled water. The solutions were passed through columns of Amberlite IR-100H and Duolite A4 ion-exchange resins, respectively, for deionization and removal of color, followed by fermentation with washed bakers' yeast, as previously described (3). The fermented solutions were filtered through a mat of Celite and the filtrates were again passed through columns of cation and anion exchange resins, respectively. The deionized solutions (*p*H 5.5–6.0) were evaporated nearly to dryness on a steam bath, then placed in a desiccator containing sulfuric acid (sp. gr. 1.84) and evaporated to dryness, under partial vacuum, at room temperature. At the time of preparing the chromatograms the residues were dissolved in 0.05–0.10 ml of distilled water. Each concentrate represented approximately 1.5 g of the original fresh plant material.

The paper chromatographic technique was similar to that used in previous studies (2). A solvent mixture of 1-butanol-ethanol-water (10:1:2) was used for all chromatograms. Tentative identification of the sugars was made by spraying the dried papers with one of the following reagents: Acidic solution of resorcinol, aniline trichloroacetic acid, an alkaline solution of 3,5-dinitrosalicylic acid, or a 1-butanol solution of orcinol and trichloroacetic acid (1, 2). Authentic samples of galactose, melibiose, the pentoses, and the available ketoheptoses were used as control sugars for the paper chromatograms.

RESULTS

The chromatographic data are given in Table 1. The spots identified as pentoses were adjacent to the group of authentic pentoses and gave the characteristic pink color with the aniline spray reagent. The authentic samples of d-glucoheptulose, d-mannoheptulose, and sedoheptulose[3] were in the area of the characteristic blue spots obtained from the plant samples when the chromatograms were treated with the orcinol spray. However, the individual pentoses and ketoheptoses were not identified.

About one-half of all of the edible materials examined and all but three of the twenty-seven varieties listed under leaves in Table 1 contained ketoheptoses. The widespread occurrence of ketoheptoses has not been previously reported. In general, the seven-carbon sugars were present in relatively small amounts; however, of the edible materials, the carrot and cantaloupe appeared to contain appreciable amounts of these.

The pentoses are assumed to be of widespread occurrence in plant materials and have received considerable attention. An appreciable quan-

[2] Mention of commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

[3] Cf. L. C. Stewart, N. K. Richtmyer, and C. S. Hudson, *J. Am. Chem. Soc.*, 71, 3532 (1949), footnote 21.

TABLE 1.—*The tentative identification, by paper chromatography, of nonfermentable sugars in various plant materials*

	SUGAR[1]		
	MELIBIOSE	KETOHEPTOSES	PENTOSES
Edible portion			
Brussels sprouts	−	−	−
Cabbage	−	−	−
Grapefruit[2]	−	−	−
Kohlrabi	−	−	−
Lemon[2]	−	−	−
Lettuce	−	−	−
Peach[2]	−	−	−
Spinach[2]	−	−	−
Yam[2]	−	−	−
Cantaloupe	−	+	−
Carrot	−	+	−
Corn	−	+	−
Egg plant	−	+	−
Kadota fig	+	+	−
Onion[3]	−	−	−
Orange	−	+	−
Orange juice	−	+	−
Peas[2,3]	+	−	−
Pepper, bell	+	+	−
Potato, white	+	+	−
Strawberry	−	+	+
Stringbean	+	+	−
Tomato	−	+	−
Red beet	+	−	−
Sugar beet[2]	+	+	−
Peel			
Grapefruit	+	+	−
Lemon[2]	−	−	+
Orange	−	+	−
Leaves			
Alfalfa[4]	−	+	−
Tree and others[5]	[5]	[5]	[5]
Corn	−	−	−

[1] + indicates the presence of the sugar; − indicates the absence of the sugar.

[2] The chromatograms of these materials revealed spots of unknown identity different from those of any of the commonly known sugars. The unknown spots of one plant material were not necessarily the same as the unknown spots of another plant, as was evidenced by tangible differences in R_f values and their reaction with various spray reagents. It is possible that some of these sugars occur in measurable quantities.

[3] The onion extract contained a "fructosan" and the pea extract a "glucosan" type of material that remained at the origin of the paper chromatograms. Both of these materials were present in appreciable amounts.

[4] In a previous paper (2) no ketoheptose was reported in the alfalfa plant. However, in this earlier work the entire plant was used. In this study the leaf only was used and the greater concentration in this portion of the plant may have been responsible for this difference.

[5] The following types of leaves were included in the study: Almond, apple, apricot, camellia, *Coprosma*, castor bean, coast redwood, Bing cherry, cork oak, *Eugenia myrtifolia*, Concord grape, guava, lemon, Monterey cypress, pear, pepper tree, plum, quince, rhododendron, silver maple, *Thuja plicata*, weeping willow, and white birch. All of the leaf varieties contained melibiose (residue from raffinose). All but two (Monterey cypress and *Thuja plicata*) contained one or more ketoheptoses and about one-half of the varieties contained free pentoses.

tity of xylose was found in the strawberry, but pentoses were not detected in any of the other edible materials. About one-half of the leaf samples contained pentoses. Some analytical methods for pentoses include both free pentoses and pentosans. This would increase the incidence and amount of five-carbon sugars in plant materials but the pentosans would not be a significant factor in sugar analysis.

Melibiose (residue from raffinose) was present in about one-third of the edible materials and in all of the leaf samples. The quantities present varied from traces to amounts that would be measurable in sugar analysis.

The edible materials were examined for another naturally occurring sugar, *viz.*, galactose, but none could be found. However, the absence of sugars reported in Table 1 and in the text is not intended to certify that these sugars are not present in the plants examined. A more concentrated extract may have provided proof of their presence. However, under the experimental conditions used in this study, quantities of the order of 0.01% of each sugar would have been observed. Such quantities would not be considered significant in our sugar analyses.

SUMMARY

1. Ketoheptoses are present in a large variety of plant materials; this fact has not been heretofore known. Some plant materials contain appreciable quantities of these seven-carbon nonfermentable sugars.

2. Because of the frequent occurrence of various nonfermentable sugars in plant materials, a preliminary investigation by means of paper chromatography would aid in the proper interpretation of the sugar values obtained by methods of chemical analysis.

ACKNOWLEDGMENT

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ABSORPTION SPECTRA OF DEHYDRATED ALFALFA MEAL EXTRACTS AND STANDARD CAROTENE SOLUTIONS

By MAXWELL L. COOLEY (General Mills, Inc., Minneapolis, Minnesota)

There are several problems in the determination of the true biological vitamin A activity of alfalfa meal by chromatographic-colorimetric assay for "total carotene." The present A.O.A.C. method (1) provides a means of determining total carotene in alfalfa meal. However, the pigment, although often expressed as beta carotene, is not actually all beta carotene and therefore does not reflect the true vitamin A activity of the meal. The provitamin A plant pigments are those which contain a beta-ionone group at either one or both ends of the polyene chain. The pigment displaying the greatest vitamin A activity is beta carotene (all-trans-beta-carotene), which contains two beta-ionone rings and is therefore capable of splitting into two molecules of true vitamin A (2).

ISOMERS OF BETA CAROTENE

Although all-trans-beta-carotene predominates in fresh alfalfa as well as in dehydrated alfalfa meal, isomers of beta carotene which have much less vitamin A activity are also present, and are part of the total carotene determined by chromatographic methods. Bickoff and Thompson (3) state that the isomers of greatest significance in this total carotene are neo-beta-carotene B and neo-beta-carotene U, which may possess only about 50 per cent or less of the vitamin A activity of all-trans-beta-carotene. These authors indicate the following variation in stereoisomer composition of the total carotene of alfalfa meals and of fresh whole alfalfa using two *cold* methods of extraction:

	All-Trans-Beta-Carotene, Per cent	*Neo-Beta Carotene B, Per cent*	*Neo-Beta Carotene, Per cent*
Dehydrated Alfalfa Meal	(38.9–56.0)	(31.6–38.7)	(11.0–23.6)
Suncured Alfalfa Meal	(72.0–76.6)	(12.1–13.3)	(11.0–15.1)
Fresh Whole Alfalfa	(83.4–90.0)	(3.0– 8.1)	(5.4– 9.0)

Heating increases the isomerization of beta carotene (5). Therefore, it seems probable that the relatively high temperatures employed in producing dehydrated alfalfa meal cause the larger proportion of isomers in this material as compared with suncured alfalfa meal and fresh whole alfalfa.

CAROTENE *VS.* VITAMIN A ACTIVITY

Because most control laboratories need a method for the determination of carotene which will furnish results within two or three hours of sampling, rapid extraction seems necessary. In most cases an overnight cold extraction is too long for efficient control work. As expected, hot extraction is conducive to the formation of even greater percentages of isomers

of beta carotene than were present in the original dehydrated alfalfa meal (4, 7).

Although the determination of total carotene provides only an estimation of the vitamin A activity of dehydrated alfalfa meal, it will probably continue to be used extensively as a rapid and convenient method of ascertaining the quality of this product.

In order to convert carotene figures to units of vitamin A, it is no longer permissible to base the calculations on the arbitrary provitamin A value for beta carotene; 0.60 microgram beta carotene = 1 U.S.P. unit (prior to February, 1950) of vitamin A, because the unit of vitamin A referred to is the old unit. *The new or present* unit of vitamin A possesses approximately 1.33 times the activity of the old unit; therefore, it is reasonable that a new arbitrary provitamin A value be assigned; thus, 0.80 microgram beta carotene = 1 new U.S.P. unit of vitamin A.

CAROTENE STANDARDS

The present A.O.A.C. procedure (1, 6) for the chromatographic determination of carotene bases the colorimetry on solutions of pure beta carotene. It has been assumed that the cheaper 90% beta-10% alpha carotene standard (obtainable from General Biochemicals, Inc., Chagrin Falls, Ohio), is satisfactory for most practical purposes. In Figure 1 is shown a comparison of the absorption spectra of two standard solutions as measured with the Beckman DU Spectrophotometer. The solutions consisted of pure beta carotene and of 90% beta-10% alpha carotene (both 200 micrograms per 100 ml); both made up in 10% acetone in hexane (Skellysolve B). Although in the 90% beta-10% alpha carotene curve there is a shift in the maxima to the left of about one millimicron, the two curves are very nearly the same. Hence, it seems that either carotene standard may be used for general colorimetry.

METHODS OF EXTRACTION

As was previously mentioned, the more rapid hot extraction of carotenoids from alfalfa meal samples is desirable. Quackenbush recommends (6) refluxing 1–2 g dehydrated alfalfa meal with 30 ml of 30% acetone in hexane for one hour. The resulting solution is diluted to 100 ml with hexane; this makes the concentration of acetone about 9%. This solution then is passed through a Magnesia-Hyflo Super Cel adsorption column and the carotene is eluted with 10% acetone in hexane.

The carotene method of Cooley and Koehn (4) specifies refluxing 1–2 g dehydrated alfalfa meal for one hour with 50 ml of a solvent mixture of equal parts by volume of toluene, ethyl acetate, and ethanol. The extract is filtered and the solvents removed by evaporation under reduced pressure and mild heat. The residue is taken up in 10% acetone in hexane and chromatographed as in the A.O.A.C. method. Total carotene results

obtained by this procedure are approximately 10 per cent higher than those by the A.O.A.C. procedure (4). The solvent mixture also readily extracts the carotenoid pigments from yellow corn and corn products.

In Figure 2 are shown absorption spectra obtained by hot extraction

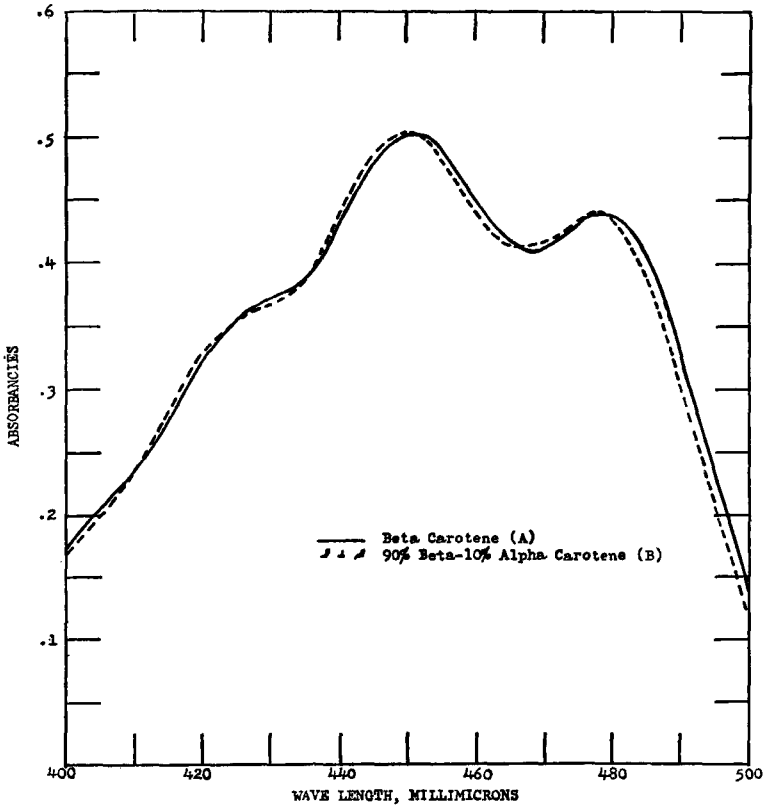

FIG. 1.—Comparison of light absorption curves. A. Pure beta carotene dissolved in 10% acetone in hexane. Solution contained 200 micrograms per 100 ml. B. 90% beta-10% alpha carotene dissolved in 10% acetone in hexane. Solution contained 200 micrograms per 100 ml.

of a sample of dehydrated alfalfa meal by the method of Cooley and Koehn and by the official method, as compared with the light absorption curve of pure beta carotene. Light absorbancies are used rather than specific absorption coefficients because of the errors introduced by attempting to calculate the coefficients for a mixture of carotenes. The standard beta carotene solution contained 200 micrograms per 100 ml, the A.O.A.C. 218 micrograms per 100 ml, and the Cooley and Koehn

solution 238 micrograms per 100 ml. Both of the latter solutions were measured at 436 micrograms and were compared with the beta carotene standard solution at that wave length. The mixture of three solvents apparently extracts the carotenoids more efficiently than does the A.O.A.C. solvent, without materially changing the light absorption characteristics.

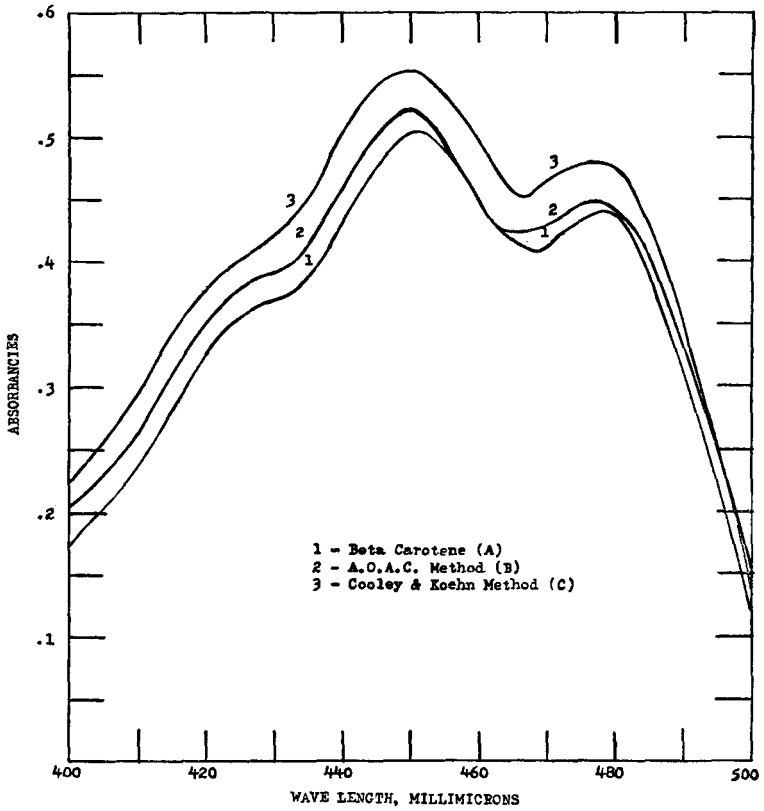

FIG. 2.—Comparison of light absorption curves. A. Pure beta carotene dissolved in 10% acetone in hexane. Solution contained 200 micrograms per 100 ml. B. Total carotene solution from a sample of dehydrated alfalfa meal extracted by A.O.A.C. hot solvents procedure. C. Total carotene solution from the same sample of dehydrated alfalfa meal used in (B) except the extraction was by Cooley & Koehn hot solvents procedure.

SUMMARY

Recent literature states that dehydrated alfalfa meal contains larger quantities of stereoisomers of beta carotene than fresh whole alfalfa or

suncured alfalfa meal because of high temperatures used in the dehydrating process. These isomers are stated to possess much less vitamin A activity than beta carotene. The ratio of the isomers to the all-trans-beta-carotene may be increased by extraction with hot solvents because of the additional heat employed. Although the "total carotene" determined by the A.O.A.C. hot solvents method contains isomers of beta carotene and therefore gives a fictitiously high figure for the true vitamin A activity of the sample, such a rapid and convenient method probably will continue to be preferred to the longer 15-hour overnight extraction in many control laboratories.

Conversion of carotene to units of vitamin A should be based on the arbitrary ratio of 0.80 microgram beta carotene equal to 1 new U.S.P. unit of vitamin A.

For most practical purposes, the cheaper 90% beta-10% alpha carotene standard may be used in place of pure beta carotene. A solvent of equal volumes of toluene, ethyl acetate, and ethanol more completely extracts the carotenoids from dehydrated alfalfa meal than hot 30% acetone in hexane. The absorption spectra of solutions by either procedure are very similar.

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STUDIES IN COAL-TAR COLORS, XI: D&C RED NO. 30

By CHARLES STEIN and KENNETH A. FREEMAN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

D&C Red No. 30 (Helindone Pink CN), 4,4'-dimethyl-6,6'-dichlorothioindigo, is certifiable for use in drugs and cosmetics under the authority of the Federal Food, Drug, and Cosmetic Act of 1938 (1). This paper describes the preparation of a pure sample of D&C Red No. 30. The purified material was used to determine the validity of the titanium trichloride titration procedure for the quantitative determination of the dye. The spectrophotometric properties of solutions of the purified dye were also determined. Pure D&C Red No. 30 has been prepared by Harley-Mason and Mann (2) and by Wyman and Brodie (3). Their data have been used to establish the authenticity of the standard sample prepared for this work.

EXPERIMENTAL

The starting material was a commercial sample of D&C Red No. 30 furnished by H. Kohnstamm & Company. The dye (25 grams) was repeatedly recrystallized from boiling nitrobenzene until samples from successive recrystallizations showed no increase in absorbancy. After each recrystallization, the dye was washed once with benzene and three times with alcohol.

ANALYTICAL DATA

The dye can be dried satisfactorily at 135°C. It is not hygroscopic.

Chlorine—The A.O.A.C. procedure (4) was used.

Found: Cl, 17.6%, 17.7%; Calc. for $C_{18}H_{10}O_2Cl_2S_2$: 18.0%.

Sulfur—Parr bomb.

Found: S, 16.3%, 16.2%; Calc.: 16.3%.

Titration with titanium trichloride.—The A.O.A.C. procedure (5) must be somewhat modified to obtain a sharp end point. The following procedure gives satisfactory results: Transfer a sample of 0.2–0.3 g to a small beaker or test tube. Add 1–2 ml of 15% fuming H_2SO_4 and heat at 100° for 30 minutes. Cool and transfer the solution to a 500 ml titration flask using about 150 ml of water. Add an equal volume of alcohol and 2–3 ml of 30% NaOH solution. Heat until a clear solution is obtained, add 20 g of sodium bitartrate and boil for a few seconds. Titrate to the appearance of a clear yellow color, then add 1 ml of a 1% solution of FD&C Green No. 2 and titrate to the disappearance of the green color. Determine and deduct the blank due to the FD&C Green No. 2.

Found (ml of 0.1 *N* $TiCl_3$ required per g of dye): 50.7, 50.9; Calc.: 50.85.

IDENTIFICATION OF THE DYE

The identity of the dye was established in two ways.

(1) *Diacetyl-dihydro derivative.*—The dye (1 g) was refluxed with 10 ml of acetic acid and 10 ml of acetic anhydride plus small portions of zinc dust until the solution was colorless. Acetic anhydride (100 ml) was added and the solution was refluxed for an additional 30 minutes, then filtered hot through a sintered glass filter. The hot filtrate was poured into a beaker containing 150 ml of water. The precipitated diacetyl-dihydro derivative was filtered off and recrystallized from acetic anhydride to constant melting point, M.P. 292–94° (Fisher block). Harley-Mason and Mann (2) have prepared this derivative of D&C Red No. 30 from a sample of dye of proved structure; M.P. 290–92°.

(2) *Spectrophotometric data.*—Wyman and Brodie (3) prepared pure D&C Red No. 30 by chromatographic methods and investigated its spectrophotometric properties. They found that solutions of the dye in chloroform consisted of mixtures of the cis- and trans-isomers, and that

the proportion of the isomers could be altered by irradiation with light of given wave lengths.

The absorption spectrum (250–600 mμ) of a chloroform solution of the standard material was determined by means of the Cary Recording spectrophotometer with quartz cells. The dye solution was then irradiated with light of wave length greater than 520 mμ in the manner described

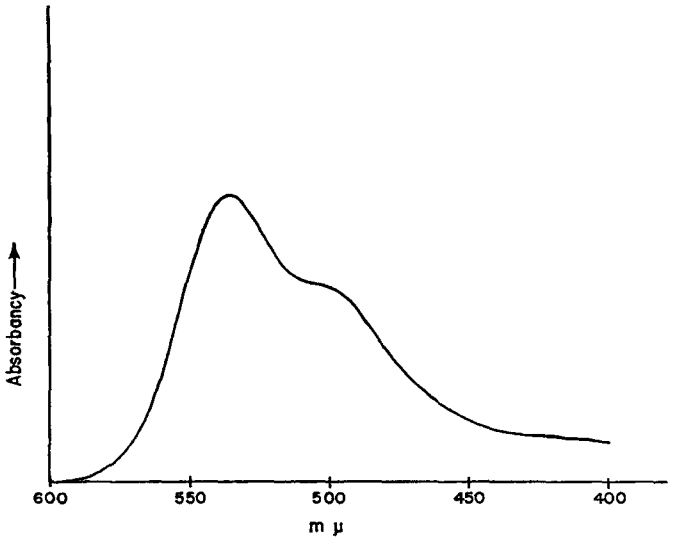

FIG. 1.—Absorption spectrum of D&C Red No. 30. Conc: 20.14 mg/liter. Solvent: $CHCl_3$. Cells: 1 cm.

by Wyman and Brodie and the absorption spectrum was again measured; the data obtained agreed with their observations.

Samples of about 20 mg of dye were dissolved in 600–700 ml of chloroform by heating near the boiling point for about two hours. After cooling to room temperature, the solutions were diluted to 1 liter. Figure 1 shows the absorption spectrum of the dye in the visible region. At the wave length of maximum absorbance, 539 ± 1 mμ, the average absorbancy was 0.0415 per milligram per liter. Absorbancies follow Beer's law. Solutions of the dye in chloroform are stable for at least a week.

DISCUSSION

The absorption spectra of the *cis*- and *trans*-forms of D&C Red No. 30 differ considerably. Since the equilibrium between the two isomers is markedly affected by light of certain wave lengths, the possibility of errors from this source in the spectrophotometric determination of the dye was investigated.

Figure 2 shows the absorption spectrum of a chloroform solution of the dye both before and after a twenty-minute exposure to light of wave length greater than 520 mμ. When the dye is so irradiated, the reestablishment of "normal" equilibrium occurs slowly; several hours are required when the solutions at room temperature are exposed to diffuse daylight. Refluxing the irradiated chloroform solution of the dye for ninety minutes failed to reestablish the equilibrium.

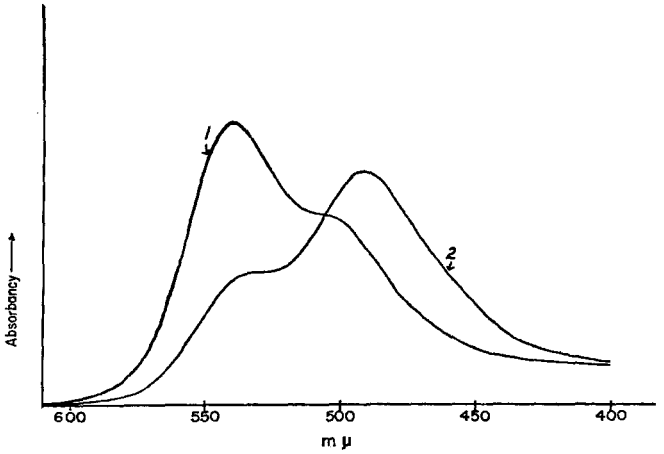

FIG. 2.—Absorption spectra of D&C Red No. 30 in $CHCl_3$.
Curve 1—Before irradiation. Curve 2—After irradiation.

Exposure of chloroform solutions of the dye to direct sunlight for more than an hour produced, at most, only very slight changes in the absorption spectrum.

The data obtained in this work indicated that determination of the dye by spectrophotometric methods is precise when the solutions are prepared under the usual conditions of laboratory lighting (diffuse "white" light) and the calculations are based on the absorbancy at 539 ± 1 mμ. When the shape of the absorption curve indicates the existence of an "abnormal" equilibrium, the alternative procedure of basing the determination on absorbancies at the isosbestic point (ca 505 mμ) gives satisfactory results. With the Cary instrument, absorbancy for the pure dye at this point was found to be 0.0282 per milligram per liter.

D&C Red No. 30 is commonly submitted for certification as talc lakes. Five such samples were analyzed, both spectrophotometrically and by titration with titanium trichloride. For the spectrophotometric determination, samples of about 30 milligrams were refluxed with about 350 ml of chloroform for 2–3 hours. The solutions were cooled to room temperature and diluted to 500 ml. After the talc had settled, analytical samples

were decanted and the absorbancy curves of the solutions were determined. The results for the five lakes were 28.9, 29.2, 28.8, 29.8, and 28.4 per cent, respectively, based on the absorbancies at 539 mμ. Essentially the same results were obtained when the calculations were based on the absorbancies at 505 mμ. The corresponding results obtained by titration with titanium trichloride were 28.2, 28.5, 27.9, 29.1, and 28.5 per cent.

SUMMARY

A purified sample of D&C Red No. 30 has been prepared.

Both the spectrophotometric and titanium trichloride titration procedures give good results in the quantitative determination of the dye. Spectrophotometric data for chloroform solutions of the dye have been presented. Chloroform solutions of the dye obey Beer's law.

ACKNOWLEDGMENT

The writer wishes to thank S. S. Forrest for making the titanium trichloride determinations.

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STUDIES IN COAL-TAR COLORS, XII: D&C ORANGE NO. 3

By CHARLES STEIN and KENNETH A. FREEMAN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.)

The di-sodium salt of 1-phenylazo-2-naphthol-6,8-disulfonic acid (Orange G) is certifiable as D&C Orange No. 3 (1). This paper describes the preparation of a purified sample of D&C Orange No. 3. The purified sample was used as a standard to investigate the validity of the titanium trichloride titration procedure for the quantitative determination of the pure color and to determine the spectrophotometric properties of solutions of the dye.

The dye was prepared by coupling diazotized aniline with an alkali metal salt of G-acid (2-naphthol-6,8-disulfonic acid) following the general procedure described by Fierz-David (2). The product was precipitated and purified as the calcium salt.

The principal problem in the preparation of a purified sample of this dye is that of obtaining suitable G-acid. G-acid is prepared by the direct sulfonation of β-naphthol. More or less R-acid (2-naphthol-3,6-disulfonic acid) is formed at the same time.

It was decided to prepare the purified compound by first making and purifying some of the arylamine salts. The G-acid is readily recovered when the arylamine salts are decomposed by hydrolysis in alkaline solution.

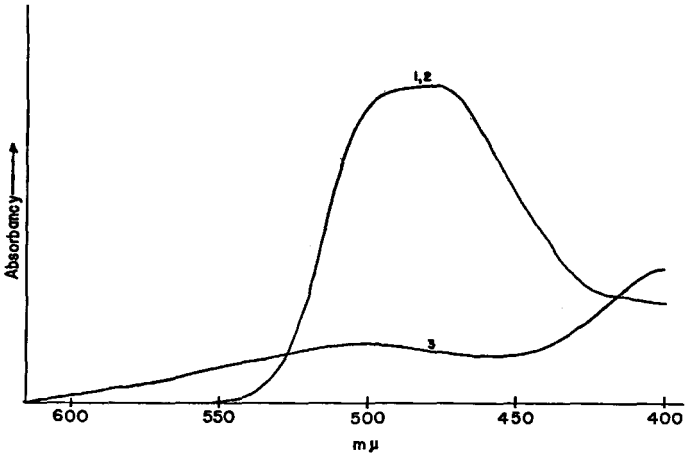

FIG. 1.—Absorbancy curves of D&C Orange No. 3. Conc: 17.85 mg/liter. Solvent: Curve 1—0.1 *N* HCl; Curve 2—0.04 *N* NH_4Ac; Curve 3—0.1 *N* NaOH. Cells: 1 cm.

EXPERIMENTAL

G-Salt.—Technical grade G-salt was recrystallized once from water after decolorizing with carbon. On the basis of its solubility and flame test, this material appeared to consist mainly or entirely of the potassium salt.

The *p*-toluidine, β-naphthylamine, and benzidine salts of G-acid and the *p*-toluidine salt of R-acid were prepared by mixing at 20°C. solutions of the hydrochlorides of the respective amines with solutions of equivalent portions of G-salt (or R-salt). After standing at about 20°C. for at least 12 hours, the amine salts were filtered off, recrystallized several times from 1% acetic acid, and dried at 60°C. Although the procedure used is that described by Forster and Keyworth (3), the derivatives appeared to decompose below the melting points given by these authors.

Approximately one millimole of each of the arylamine salts was decomposed by boiling with 1–1.5 ml of 2.5 *N* sodium hydroxide solution and 100 ml of water for 30 minutes. The *p*-toluidine liberated from the *p*-toluidide salts was removed by steam distillation. The β-naphthylamine or benzidine from the respective salts was removed by extracting the cooled alkaline solutions with six successive 100 ml portions of chloroform; the aqueous solutions of G-salt were then boiled to remove the dissolved chloroform.

The solutions of G-salt were made up to 250 ml, and solutions containing about 30 mg per liter of G-salt were prepared by diluting suitable aliquots with 0.1 *N* hydrochloric acid. The ultraviolet absorption spectra of the acid solutions were obtained with a Cary Recording Spectrophotometer.

The absorption curves of the solutions of G-acid obtained from the three arylamine salts were identical. The ratios of the absorbancies at the peaks

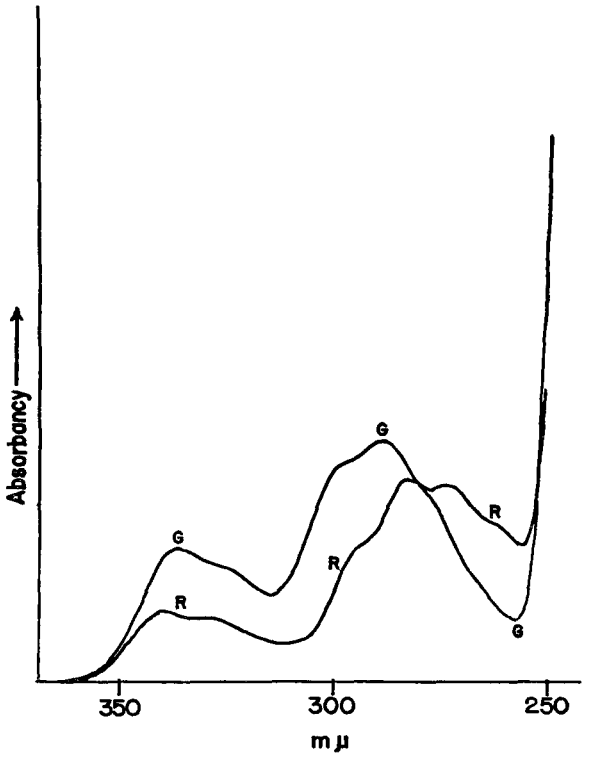

FIG. 2.—Ultraviolet absorption spectra of R- and G-acids.

at 289 and 338 mμ were 1.75 ± 0.02 in each case. The absorption spectrum of R-acid differs considerably from that of G-acid (Fig. 2). Application of the method of variable reference spectrophotometry (4), using the G-salt obtained from the *p*-toluidide as a standard, showed the technical grade of G-salt to be free of R-salt. In fact, this procedure showed the technical material to contain no substance having an ultraviolet absorption spectrum different from that of G-acid.

A single recrystallization of the technical G-salt gave material having

an ultraviolet absorption spectrum identical with that of the material obtained through the arylamine salts.

Preparation of D&C Orange No. 3.—Redistilled aniline, 9.3 g (0.1 mole), was agitated with 30 ml of hot water and then 25 ml of conc. hydrochloric acid was added. The solution was allowed to cool to 40°C. when sufficient ice was added to lower the temperature to 0° and leave a small excess of ice. A solution of 7.0 g of sodium nitrite in 35 ml of water was added rapidly with stirring. After several minutes, the excess nitrous acid was destroyed with sulfamic acid.

In the meantime, a solution containing 39.0 g of G-salt, 15 ml of 30 percent sodium hydroxide, 25 g of sodium carbonate, and sufficient water to prevent precipitation was prepared and cooled to below 5°. The solution of benzene diazonium chloride was added slowly, with continuous mechanical stirring, to the cold solution of G-salt. The total volume was about 600 ml. Cooling and stirring were continued for one hour. The solution was then allowed to warm to room temperature, made slightly acid with hydrochloric acid and boiled to expel carbon dioxide. A solution of 20 g of calcium chloride in 50 ml of water was then added. The solution was cooled and filtered. The precipitated calcium salt of the dye was dissolved in about 400 ml of boiling water containing 20 g of calcium chloride, cooled and filtered. The calcium salt was again dissolved in about 400 ml of hot water, the solution filtered hot, and the dye allowed to crystallize. The recrystallization from water was repeated twice. The final filtrate gave no test for chlorides. The product was dried at 135°C.

ANALYTICAL DATA

All samples were finally dried in vacuo (2 mm Hg) at 130° for 5 hours.

Sulfur (Parr bomb).

Found: S, 14.36%, 14.35%. Calc. for $C_{16}H_{10}O_7N_2S_2Ca$: 14.36%.

Calcium. The procedure given by Kolthoff and Sandell (5) was followed. Found: Ca, 8.90%, 8.96%. Calc.: 8.98%.

Nitrogen (semi-micro Kjeldahl). Found: 6.12%, 6.12% Calc.: 6.28%.

Titration with $TiCl_3$. The A.O.A.C. procedure (6) was followed. The end point is a pale lemon yellow. Found: 88.8, 88.7 (ml of 0.1 *N* $TiCl_3$ required per g of dye). Calc.: 89.6.

SPECTROPHOTOMETRIC DATA

Spectrophotometric measurements were made with a Cary Recording Spectrophotometer. Weighed samples of about 0.6 g were dissolved in one liter of water and appropriate dilutions were made from these stock solutions.

Figure 1 shows the effect of *p*H on the absorption curve of the dye. Solutions in 0.1 *N* hydrochloric acid and in 0.04 *N* ammonium acetate have very nearly identical absorbancies. In 0.1 *N* sodium hydroxide the dye undergoes a marked change.

In 0.04 N ammonium acetate solution, the average absorbancy of the calcium salt at the wave length of maximum absorbancy, 478 mμ, was 0.0502 per milligram per liter. This is equivalent to an absorbancy of 0.0495 per milligram per liter for the sodium salt. Absorbancies follow Beer's law. Solutions of the dye in 0.04 N ammonium acetate are stable for at least two days.

Six samples of commercial dyes were analyzed both by titration with titanium trichloride and by the spectrophotometric procedure, using the absorbancy data obtained from solutions of the purified sample. The percentages of pure dye obtained by titration were: 90.6, 86.0, 88.1, 90.0, 84.6, and 94.2. The corresponding values obtained spectrophotometrically, in solutions 0.04 N with respect to ammonium acetate, were 91.8, 86.5, 89.1, 90.8, 85.5, and 94.8.

SUMMARY

A purified sample of D&C Orange No. 3 has been prepared. Both the titanium trichloride titration procedure and the spectrophotometric method are satisfactory for the quantitative determination of the dye. Spectrophotometric data for aqueous solutions of D&C Orange No. 3 have been presented. Aqueous solutions, buffered with ammonium acetate, obey Beer's law.

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THE DETERMINATION OF IRON IN MOLASSES

By LOUIS SATTLER and F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.)

This laboratory occasionally receives requests for determinations of iron in molasses samples. In the past we have been using for this purpose the method of ashing, dissolving the ash in acid, oxidizing, and determining the iron colorimetrically with thiocyanate by visual comparison with a series of standards in Nessler tubes. Our experience with this method has not been satisfactory. The standards had to be prepared freshly each time, the results varied with the detailed conditions of the ashing procedure, and the tint of the sample solution was in many cases different from that of the standards. Similar experiences with sugar products have been reported by Boyd (1) who states that the method gave not only inac-

curate, but discordant, results even in the hands of experienced operators. In Java it has been found (2) that the thiocyanate method is unsuitable for use upon raw juice ash, although it gave good results with colorless or light colored sugar sirups. It was therefore desirable to develop a more satisfactory method. The Association has already adopted α-α'-dipyridyl (2,2'-bipyridine) as colorimetric reagent for the determination of iron in a variety of food products (3), and this chemical has been recommended by Boyd for use on sugar products.

In the case of flour and other food products the method of the Association (3) leaves a choice between dry ashing and digestion with sulfuric and nitric acids. Because of our experience with molasses ash it was decided to adapt the method to molasses treated by the wet method. Blackstrap molasses usually contains fine grain and adventitious impurities and this makes it necessary to use relatively large samples for analysis. Accordingly, samples of 5 grams of the well mixed molasses were used to insure an adequate, representative quantity. The sample was dissolved in a small quantity of water and washed into a 800 ml Kjeldahl flask. The ordinary c.p. grades of sulfuric and nitric acid usually contain relatively large quantities of iron, and because of this it was preferable to use acids specially manufactured for microanalysis. Five ml of this type of sulfuric acid was pipetted into the flask containing the sample, and 10 ml of the special nitric acid was added. The mixture was gently heated until charring began. After allowing the flask to cool, 2 ml more of nitric acid was added and the flask heated cautiously until the resulting brown fumes disappeared. This process was repeated until a total of 35 ml of nitric acid had been added. A too vigorous reaction, causing dense fumes to be ejected explosively from the flask after the addition of an increment of nitric acid, was carefully avoided. Finally, the flask was heated until white fumes of sulfur trioxide were evolved.

The final solution was usually water white. It was cooled to room temperature, and 25 ml of water was cautiously added. If the final solution was still slightly yellowish, the solution in water was gently boiled for a minute or two.

The solution was allowed to stand overnight to permit the salts to crystallize out, and filtered by means of the vacuum filtration apparatus shown in Figure 1. The contents of the Kjeldahl flask are drawn by suction through a bent glass tube of 3–4 mm inside diameter, the lower end of which reaches to the bottom of the flask, into a Pyrex Buchner funnel,[1] with a disk 40 mm wide, and a height of 50 mm above the disk. The filtrate was collected in a 100 ml volumetric flask. The inside wall of the Kjeldahl flask was repeatedly washed with distilled water. After taking out the rubber stopper on top of the Buchner funnel, the latter was also rinsed with wash water. The vacuum was broken, the receiving flask

[1] Pyrex Catalog No. 36060, 60 UF.

removed, and the volume completed to the mark. If there was any residual turbidity in the filtrate, it was removed by refiltering it through acid washed analytical grade filter paper (Baker and Adamson Grade "O," 11 cm diameter).

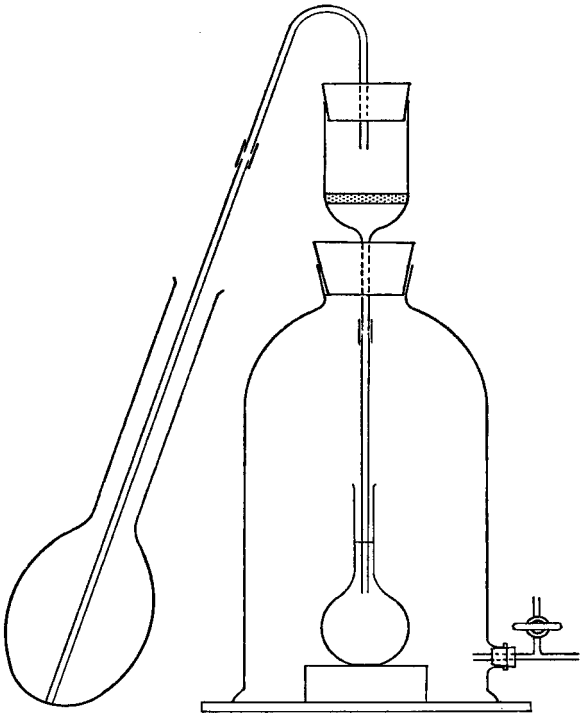

FIG. 1.—Vacuum filtration apparatus.

A 20-ml. portion of the final filtrate was pipetted into a 50 ml volumetric flask, 2 ml of 10% solution of hydroxylamine hydrochloride was added, the flask rotated and allowed to stand for a few minutes. Then 4 ml of an aqueous solution of 0.1 gram α-α'-dipyridyl in 100 ml, and 19 ml of 2 molar sodium acetate buffer solution (272 g of $CH_3COONa \cdot 3H_2O$ per liter) were added, and the volume was completed to 50 ml. If the *p*H of this solution, tested colorimetrically with bromophenol blue or a corresponding indicator paper, was substantially below 3.5–4.5, more of the acetate buffer solution was added to a new 20 ml portion of the final filtrate plus the indicated quantities of hydroxylamine and α-α'-dipyridyl solution, to bring the *p*H within that range.

Twenty ml of the solution of correct *p*H in the 50 ml flask was diluted

to 100 ml in a volumetric flask. At this dilution the transmittancy could be read in the 5 cm cell of the Coleman Universal Spectrophotometer at 510 mμ. Blanks were run with the same quantities of sulfuric and nitric acid as used for the digestion, and of the other chemicals used in the analysis.

A standard iron solution was prepared by dissolving 0.1 gram of analytical grade iron wire in 20 ml of hydrochloric acid (for microanalysis) and 50 ml of water, diluting to one liter, and again diluting 100 ml of this solution to one liter. Ten solutions, containing from 2 to 45 ml of this dilute solution and 2 ml of the same hydrochloric acid, were each made up to 100 ml, and each of these solutions analyzed for iron with α-α'-dipyridyl in the same manner as described above. Blank solutions, containing only the hydrochloric acid, were run in like manner, and the iron content of the standards corrected accordingly. The corrected iron values, in mg. of iron per kilogram of molasses, were plotted on semi-logarithmic paper against the corresponding transmittancies, and the resulting straight line preserved as the reference curve. When using this curve, the time interval between the addition of the α-α'-dipyridyl and the reading of the spectrophotometer must be the same as when the standards were prepared (4). If new lots of reagents are used for the analysis of molasses samples, new blanks must be run, and the results corrected accordingly.

With uniform molasses samples, the analysis can safely be made upon a 1 gram subsample. The digestion is carried out in a 500 ml Kjeldahl flask, with 5 ml of sulfuric acid and an initial quantity of 2 ml of nitric acid. After heating to incipient charring, the flask is cooled, and 6 ml more of nitric acid is added, a few drops at a time, the flask being cautiously heated between additions as described above. After further heating, until the fumes of sulfur trioxide are evolved, the flask is again allowed to cool, and the digest is diluted with 25 ml. of water. The use of a smaller sample has the advantage that it requires less time, less trouble with fumes, and a smaller correction for iron in the blank. After standing overnight, the filtration of the digest is carried out as described above, and the filtrate collected in a 100 ml volumetric flask. Twenty ml is transferred to a 50 ml volumetric flask, the same quantities of reagents are added as specified above, and the transmittancy is read in the 5 cm cell.

Twelve raw sugar blackstraps were analyzed with the use of 5 gram samples and the results of duplicate determinations are shown in Table 1.

A 1 gram sample of molasses No. 12, run as described above for this quantity, gave, after correction for the corresponding blank, an iron content of 114 milligrams, in close agreement with the result of the 5 gram sample.

For comparison, five samples of beet molasses were analyzed for iron, with the use of 5 grams each, and the results are given in Table 2. The digestions were made with 5 ml of sulfuric acid and a total of 40 ml of

TABLE 1.—*Milligrams of iron in one kilogram of cane molasses*

SAMPLE NO.	ANALYSIS 1	ANALYSIS 2	AVERAGE
1	164	162	163
2	151	151	151
3	188	189	189
4	131	124	128
5	110	114	112
6	187	184	186
7	62	62	62
8	105	104	105
9	141	141	141
10	207	217	212
11	193	202	198
12	117	116	117

nitric acid, added intermittently. Even with a 1 gram sample the readings would be within the range of the master curve.

TABLE 2.—*Milligrams of iron in one kilogram of beet molasses*

SAMPLE NO.	ANALYSIS 1	ANALYSIS 2	AVERAGE
13	107	107	107
14	101	101	101
15	101	101	101
16	113	113	113
17	168	171	170

SUMMARY

The old thiocyanate method, with ashing, and the use of Nessler tubes, has been found unsatisfactory for the determination of iron in molasses, and the dipyridyl method of the Association has been adapted to this purpose.

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(2) HONIG, P., Private communication.
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(4) Moss and MELLON, *Ind. Eng. Chem., Anal. Ed.*, **14**, 862 (1942).

BOOK REVIEWS

The Chemistry and Action of Insecticides. By HAROLD H. SHEPARD. McGraw-Hill Book Co., 330 W. 42nd St., New York 18, N. Y. 1951. vii+504 pages. $7.00.

This book is the outgrowth of a mimeographed edition published in 1939 under the title "The Chemistry and Toxicology of Insecticides." The subject matter deals with the facts and theories relating to insecticides and includes not only chemical, physical, and toxicological aspects but also historical and commercial information. Fungicides, rodenticides, and other economic poisons are frequently mentioned and described because of their chemical and toxicological relationship to insecticides. The insecticides are presented according to their chemical classification. First the inorganic arsenicals, fluorides, sulfur and copper compounds are discussed, then miscellaneous inorganic and metal-organic substances are followed by insecticides derived from plants. Petroleum, oils, soaps, creosotes, and synthetic organic insecticides are separately treated.

Separate chapters are devoted to the more general aspects of chemical control (particle size, wetting and spreading agents, etc.), to relative toxicity and mode of action, and to attractants and repellents.

The subject matter is presented in a simple, orderly, and interesting style with many references to original source material. However, the treatment of the newer organic insecticides appears to be less complete when compared to the thorough treatment of the inorganic compounds. For example, approximately 30 pages are devoted to arsenical compounds whereas DDT, Methoxychlor, TDE, benzene hexachloride, chlordane, and toxaphene are covered in 22 pages. Chemists who have a primary interest in analytical methods will be disappointed because of the paucity of such references. Nevertheless, the book is a worthwhile contribution to the field and should be well received by the entomological profession.

BERNARD DAVIDOW

Trace Elements in Plant Physiology, a symposium, with a preface by T. WALLACE, Chronica Botanica Co., Waltham, Mass.; Stechert-Hafner, Inc., New York City. 1950. xviii+144 pages plus 10 plates. Price $4.50.

This book is composed of 14 papers presented at a symposium organized by the International Union of Biological Sciences with the support of UNESCO at the Rothamsted Experimental Station, Rothamsted, Harpenden, Herts., England, November 5–6, 1947.

Methods used for diagnosing the mineral status of plants and for growing plants in sand and solution culture are discussed in four papers, one each by T. Wallace and E. J. Hewitt of the Long Ashton Station (England) and two by D. I. Arnon of the University of California. Four papers are concerned with the functions of trace elements (or micronutrients) in plant metabolism. E. G. Mulder (Netherlands) discusses the importance of copper and molybdenum in the nutrition of higher plants and micro-organisms, while Lavollay (France) refers to the use of *Aspergillus niger* in demonstrating quantitative interactions of nutrients. Erkama (Finland) reports the effects of copper and manganese on the iron status of higher plants, and Burstrom (Sweden) discusses the action of manganese on roots.

Trace elements problems in crops of several European countries are discussed in five papers. These are: (1) deficiencies of minor elements caused by excesses of alkali, Gisiger (Switzerland); (2) studies with copper, Steenbjerg (Denmark); (3) injury through excess of manganese, Lohnis (Netherlands); (4) zinc deficiency of fruit trees in Europe, D. Mulder (Netherlands); and (5) the role of trace elements in the agriculture of Finland, by Jamalainen. A final paper by Seckles of the Nether-

lands deals with the mode of action and occurrence of trace elements in pastures and in the blood of farm animals.

The book brings together in one volume much critical discussion of the importance of micro-nutrients in both the theoretical and practical aspects of plant nutrition. The student of this subject will find the discussion stimulating and informative.

NEIL W. STUART

Organic Reactions. Volume VI. 1951. By ROGER ADAMS, Editor-in-Chief. John Wiley & Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. viii+517 pages. $8.00.

Applications of organic chemistry are rapidly becoming more frequent in food and drug work. Previously, many analyses have involved little more than the application of inorganic or the simpler organic reactions; now the regulatory chemist must occasionally apply the more specialized organic techniques. Two such reactions are discussed in detail in this volume: 1. "The Oppenauer Oxidation," by which alcohols are oxidized to ketones, and 2. "Reductions by Lithium Aluminum Hydride," by which most organic compounds containing the carbonyl group are reduced to alcohols. Both reactions have special uses in the chemistry of steroid hormones.

The excellent manner in which each chapter is developed was mentioned in the review of Volume V.* Each chapter begins with "Contents," a detailed listing of the topics covered. The "Limitations" which are given for each reaction help the chemist to avoid situations where the reaction will not work. Very little, if any, analytical information is included.

In addition to the two topics mentioned above, Volume VI includes: The Stobbe Condensation in which an aldehyde or ketone is condensed with an ester of succinic acid to form an alkylidene succinic acid, and the synthesis of the following types of compounds: Isoquinolines; 3,4-Dihydroisoquinolines; Tetrahydroisoquinolines; Thiazoles; Thiophenes and Tetrahydrothiophenes; Phosphonic and Phosphinic Acids. The Halogen-Metal Interconversion Reaction with Organolithium Compounds is also discussed.

W. I. PATTERSON

Hydrogenation of Fatty Oils. By H. I. WATERMAN, with the collaboration of C. BOELHOUWER and L. J. REVALLIER. Elsevier Publishing Company, New York, N. Y. 1951. ix+254 pp. Price $6.50.

This volume presents the results of investigations carried out by the author and his collaborators in recent years at Delft Technical University, Netherlands. Certain selected data from the voluminous literature on the hydrogenation of fatty oils are included, since one of the aims of this book is to review the development of the fat hardening industry.

Only slightly over half of this volume is devoted to fatty oil hydrogenation, with the rest of the space given over to a superficial description of oil and fat characteristics, refining methods, and a discussion of analytical methods. The Bertram oxidation method for saturated fatty acids and a few other methods are described in detail, but otherwise this volume does not provide much information for the analytical chemist.

The author hoped to have this book serve as a guide for further investigations in the field of hydrogenation and at the same time make it possible for a reader to become familiar with this complicated field in a rather short time. In his attempt to

* See *This Journal*, **33**, 144 (1950).

appeal simultaneously to the specialist and the beginner, he has included much material of little use to either reader.

The printing and presentation of data are excellent. However, some of the translation is awkward, so that meanings of sentences are sometimes obscured.

J. FITELSON

Selective Toxicity with Special Reference to Chemotherapy. By ADRIEN ALBERT. John Wiley & Sons, Inc. New York. 1951. 228 pages. Price $1.25.

The subject of this book is the scientific basis behind the killing of an undesirable group of cells, the uneconomic species, without injuring a neighboring, desirable group of cells, the economic species. This is the author's definition of what he visualizes to be a newly emerging field of science, selective toxicity. The proposed new science cuts across the older academic fields of entomology, botany, bacteriology, and pharmacology. The author is primarily concerned with its structural organization and the laying down of its basic principles. These principles and definitions are drawn broadly enough to fit weed killers and insecticides as well as drugs. However, so little is known of the mechanisms of the selective toxicity of the former substances that they are scarcely mentioned, and the bulk of the book deals with drugs. In fact, it may be said that this book is primarily a pharmacological discussion of the relation of chemical structure to biological activity. As such, it is a worthwhile contribution. Whether there is actually any particular benefit to be derived from drawing generalizations between such largely unrelated fields as insecticides and the chemotherapy of cancer, for example, seems to be open to question. The author also makes a plea for more scientific attention to basic research on the principles of selective toxicity and less to screening programs designed to produce selective toxic agents at the fastest possible rate.

JACK L. RADOMSKI

Principles of Weed Control. By G. H. AHLGREN, G. C. KLINGMAN, and D. E. WOLF. John Wiley & Sons, New York, 1950. vii+368 pages, illus. Price $5.50.

The almost fantastic developments that have occurred in the new science of weed control in the past ten years have created a critical need for an up-to-date text. This book is the first text in this rapidly expanding science and helps to fill an almost complete vacuum. Plant scientists, chemists, students, and other agricultural workers who have not followed the field closely will be somewhat amazed at the number and kind of weed control practices that have been developed in the past few years. The authors have done a good job in organizing this material into book form.

There are 17 chapters which cover a variety of subjects arranged in the following sequence: the importance of weeds, general principles of control and eradication, chemicals used for weed control, and physiological effects of herbicides on plants; seven chapters are devoted to weed control practices under different cropping conditions or in special areas, such as field crops, vegetable and fruit crops, irrigation ditches, brush and turf; five chapters are devoted to special topics including soil sterilants, defoliation, poisonous weeds, specific weed problems, and application equipment.

One of the most serious deficiencies of the book from the teacher's viewpoint is the superficial treatment of the all-important ecological aspects of weed control. Less than three pages are devoted to this subject, which is so essential for a basic understanding of weed control problems. And the critical reviewer will find some of the recommendations based on single experiments, questionable recommendations, and in some cases, errors. The authors may be partially excused because nothing short of a miracle could have prevented such mistakes. The science of weed control

is in such a state of dynamic transformation that it would be impossible to attain perfection in a text. Hence, the book will require constant revision in later editions if it is to remain useful.

Proof that weed control is in a state of rapid change is the fact that the research on the use of radioactive isotopes for studying the translocation of herbicides in plants has not been discussed or referred to.

The authors are to be commended for their choice of illustrations, especially those showing weed control equipment. This is one of the few agricultural books in which the authors use pictures of a self-propelled mechanical cotton picker in preference to the overused photo of a field of hand laborers, tractor powered four-row cultivators in preference to the mule drawn mold board plow, and modern low gallonage sprayers especially designed for weed control.

The authors have taken the first step towards reorganizing the field of weed control as a science. The neophyte in the field will find the book of greatest value, because for the first time since 1942, it is possible to find in one place a reasonably accurate summary of the scope and importance of weed control.

WARREN C. SHAW

Organic Syntheses, Volume 31. By R. S. SCHREIBER, Editor. John Wiley & Sons, Inc., 440 Fourth Avenue, New York 16, New York. 1951. 122 pages. Price, $2.75.

This is the 31st in the series of well-known books entitled "Organic Syntheses." The format is essentially the same as that of previous volumes. Detailed directions are given for the preparation of the following compounds: α-acetyl-δ-chloro-γ-valerolactone, δ-acetyl-*n*-valeric acid, *p*-aminobenzaldehyde, *p*-bromophenylurea, *p*-ethoxyphenylurea, benzofurazan oxide, N-bromoacetamide, *o*-chlorophenylcyanamide, *o*-chlorophenylthiourea, coumalic acid, cyclohexylidenecyanoacetic acid and 1-cyclohexenylacetonitrile, 4,4′-dibromobiphenyl, 1,6-diiodohexane, diisopropyl methylphosphonate, 2,3-dimethoxycinnamic acid, β-dimethylaminoethyl chloride hydrochloride, β,β-dimethylglutaric acid, 3,5-dimethylpyrazole, 2,6-dinitroaniline, N,N′-diphenylbenzamidine, α,β-diphenylcinnamonitrile, ethyl α-acetyl-β-(2,3-dimethoxyphenyl)-propionate, ethyl pyruvate, hexamethylene diisocyanate, iodocyclohexane, laurone, *o*-methylcaprolactim, methyl cyclopropyl ketone, 9-nitroanthracene, 1-(*p*-nitrophenyl)-1,3-butadiene, pentaerythrityl tetrabromide, γ-phenylallylsuccinic acid, phenyldichlorophosphine, quinacetophenone monomethyl ether, syringic aldehyde, dl-4,4′,6,6′-tetrachlorodiphenic acid, 2,2,6,6-tetramethylolcyclohexanol, tetraphenylethylene, thiolacetic acid, 2-thiophenecarboxaldehyde, triethyl phosphite.

A cumulative index of material contained in Volumes 30 and 31 is given.

KENNETH A. FREEMAN

Quantitative Organic Microanalysis. By AL STEYERMARK (Microchemist, Hoffmann-La Roche, Inc.). The Blakiston Company, Philadelphia, Pa. 387 pages. Price $7.00.

This book describes in detail the various microanalytical procedures used in the author's laboratory and contains at the end of each chapter an excellent list of references to publications related to the various phases of the author's method and to other commonly used procedures. To present in book form all the procedures used in one of the foremost microanalytical laboratories in this country is a very worthwhile contribution to the literature. This is particularly true because modern, and where possible, automatically controlled electrical apparatus is used. Description of the apparatus is ample and the directions for carrying out the various de-

terminations are concise and clear. Numerous illustrations add greatly to the value of the book. The book is well bound and the paper and print both add to its readability.

A discussion by the author of the choice and use of standard samples for proving the different apparatus and methods would have added to the value of the book. Only one omission of consequence was noted. That was the author's failure to include a blank correction in his calculations for per cent nitrogen by the Dumas method although he did mention in his discussion how large this blank normally would be. A few of the more traditional techniques described by the author, particularly those for the carbon and hydrogen determination, are being abandoned by some microanalysts. Nevertheless, many of the author's ideas and techniques are excellent and make the book a valuable laboratory manual and reference source to microanalysts and students of microchemistry.

C. L. Ogg

A Dictionary of Antibiosis. By Leonard Karel and Elizabeth Spencer Roach. Columbia University Press, 2960 Broadway, N. Y. 27, N. Y. 1951, 373 pages, Price, $8.50.

This book should have been properly titled "An Encyclopedia of Antibiosis" as it is more than a dictionary. It is a very comprehensive compilation of various facts and information on all antibiotics, including those obtained from plants as well as microorganisms.

The data included are arranged alphabetically and consist of (1) compilation of substances tested for antibiotic activity; (2) available information on source, extraction, chemical and physical properties, and spectrum in vitro and in vivo; toxicological and pharmacological, clinical, and experimental clinical results, and (3) enumeration of organisms (designated as test organisms) against which substances have been tested for antibiotic activity. Each entry has a reference to the bibliography at the end of the book, which is arranged alphabetically according to authors with appropriate cross-references and which cites the complete title of each paper recorded. No one engaged in work in the field of antibiotics can afford to be without this excellent reference book.

D. C. Grove

Table Wines, the Technology of Their Production in California. By M. A. Amerine and M. A. Joslyn, University of California Press, Berkeley and Los Angeles. 1951. 397 pages. $4.50.

This comprehensive book on the manufacture of California table wines is an outgrowth and extension of Bulletin 639 of the California Agricultural Experiment Station, by the same authors, which has so well served the technicians of the wine industry in California and elsewhere. A comparison of the two publications impresses one with the widespread advances in the technology of wine making that have taken place in the last decade. As an illustration one need compare only Chapters 19 and 20 of "Table Wines," dealing with alcoholic fermentation and consisting of 41 pages, with the corresponding subject matter in the former publication contained on 24 pages. The chapter on "Wine Disorders" has been expanded to 18 pages. These chapters alone should be well worth the price of the book to the personnel in charge of quality control in wineries.

The twenty chapters are grouped under the broad captions of Wines and Grapes Trends in the Industry, The Winery and Its Equipment, Recommended Procedures for Making Wines, Preparing Wines for Market, and Technological Principles. The appendices consist of 16 tables of analyses of production and factory data and some 500 or more titles of publications. The book is embellished with 33 illustrations,

which together with the liberal use of conspicuous captions throughout the reading matter add much to its attractiveness and readability.

The authors' attitude towards the use of questionable and unnecessary chemicals such as fluorine compounds, monochloroacetic acid, etc., in the production of wine, and towards sanitation in the winery is commendable. From the standpoint of technology, the book is beyond criticism so far as the writer is concerned.

In the next edition a slight revision of "Federal Regulations" (pages 18 and 19) might be made. The so-called regulations of the Food and Drug Administration, referred to therein, have no legal status under the current Federal Food, Drug, and Cosmetic Act, and the partial quotation of Section 21, Class 1, Grape Wine of Regulations No. 4 under the F.A.A. Act (p. 17) may lead the novice to conclude that wine may not be ameliorated and need not contain over 7 per cent of alcohol. In Chapter 14 on "Testing and Analyzing," the Etienne tube and the rapid method for the determination of alcohol appear to merit more detailed description than is accorded them.

It is the writer's opinion that these authorities in the field of wine production have in this book fully accomplished their objective which in their own words in the Preface was: . . . "This book is intended to help producers, technicians, and chemists in the wine industry find ways to lower costs and improve quality of both fine and ordinary table wines." The wine industry throughout the world will undoubtedly welcome this new addition to its standard texts.

It is hoped that the authors have in mind the preparation of another book of the type of "Table Wines," based on their Bulletin 651, Commercial Production of Dessert Wines.

J. W. SALE

Agricultural Chemistry. A Reference Text. Vol. II. Editor-in-Chief, DONALD E. H. FREAR. D. Van Nostrand Co., Inc., New York. 588 pages. Price $9.50.

This volume follows the style, form, and editorial policy of volume I, reviewed in *This Journal*, **33**, 936 (1951). The twenty-four chapters are contributed by twenty-three authors, of whom eleven are university or college specialists, four are industrial authorities, and six are with federal or state research organizations. The remaining two are from the legal profession and are authors of the chapter on Inventions and Patents Relating to Agricultural Chemistry. The work comprises five parts.

Part I deals with the chemistry of the major products of agriculture, namely: seed and cereal crops, fruits and vegetables, forage crops, meats, dairy products, and egg and poultry products. In these six chapters the properties and composition of foods are presented.

Part II is devoted to products of importance to agriculture, the fertilizers and soil amendments. The nitrogen, potassium, phosphate, and mixed fertilizers are the subjects of four of the chapters in this part. Another chapter is devoted to liming materials and the sixth to minor element supplements. In this last chapter the term "minor" is applied in a quantitative sense to those elements which are as essential as the four which for many years have been regarded as the major nutrients to be returned to the soil by means of fertilizers. The "minor" elements dealt with are boron, copper, manganese, molybdenum, and zinc.

The nutrition of farm animals constitutes the subject matter of part III. Seven chapters deal with dairy cattle, beef cattle, horses and mules, sheep and goats, poultry, swine, and domestic and fur-bearing animals. As is to be expected in the light of the strides made in animal nutrition, discussion of vitamins and the role of trace elements receive major treatment in these chapters.

In these days of modern agriculture, high production cannot be maintained without recourse to pest control practices; therefore no treatise on Agricultural Chemistry can be complete without a discussion of the important available aids. Part IV on Pesticides presents three chapters respectively on insecticides, fungicides, and herbicides.

As the designation in the title "A Reference Text" indicates, the discussion throughout parts I to IV depends almost exclusively on statements and data from the literature. Because the book is the work of so many specialists a detailed evaluation of the adequacy of each chapter by one reviewer is hardly to be expected. It seems to this reviewer, however, that in general the parts of the book dealing with products of importance to agriculture are more comprehensive than those dealing with the products of agriculture. For example, no chapter on eggs can be considered complete which (as is the case here) overlooks the work of Mitchell on the composition of eggs (*This Journal*, **15**, 310 (1932)) and on changes in composition during storage (*This Journal*, **17**, 506 (1934)).

Part V, entitled "Commercial Agricultural Chemistry," closes the book with a chapter on inventions and patents and one on chemurgic application of agricultural products. In the former chapter the principles of patent procedure are discussed, with illustrations from the agricultural field. The discussion in the later chapter is most general and appears to treat of time honored industrial uses of farm products more than those developed since the term "chemurgic" was popularized.

HENRY A. LEPPER

Statistical Methods for Chemists. By W. J. YOUDEN (Statistician, National Bureau of Standards). John Wiley and Sons, Inc., New York 16, N. Y., 1951, 123 pages. Price $3.00.

The author has presented a discussion of statistics for chemists. Because of his long association with chemistry, he views statistics realistically as a tool to help chemists, and shows that it can be usefully applied to the data chemists usually acquire. He makes use of small series of duplicate analyses, differences in these duplicates and single values and does not take the arbitrary stand that with limited populations to work with little if any information can be gained from a statistical analysis. His whole approach to the application of statistics is one of practicability rather than one based strictly on mathematics.

Of particular interest is the treatment of analysis of variance and the emphasis on the importance of design of an experiment so that this useful tool can be best utilized. The lucid explanation and examples of such common statistical tools as the *t* and F tests make the book a very useful one, particularly for those chemists who have not had formal statistical training but who realize the importance of statistics in comparing and evaluating methods in research. The book should prove to be particularly valuable to referees in their comparison and interpretation of data obtained by collaborative study of analytical methods. The author has included tables of critical *t* and F values and a table of squares which is of particular aid in making analyses of variance. The table of F values would have been more useful had it been extended to include a greater number of degrees of freedom for the numerator.

This book which is defined as "a primer of statistics for chemists" is an important contribution for it will encourage more chemists to make use of scientifically designed and statistically evaluated experiments.

C. O. WILLITS
C. L. OGG