MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

By J. W. SALE (U. S. Food and Drug Administration Washington, D. C.), *Referee*

The study of analytical methods for alcoholic beverages, which has been conducted so extensively during the last four years by the associate referees, was continued during the past year. They have also devoted considerable time to the review of the methods of analysis dealing with their subjects published in the 1935 edition of *Methods of Analysis A.O.A.C.* and have recommended various changes to be incorporated in the revised edition. Some of these changes are of such a character as to require ratification by the Association.

Methods for the analysis of diastatic power of malt, and of malt adjuncts including malt sirup, brewers' sugar, cereal flakes, rice, corn grits, and refined grits; beer, volatile acids in wine, acids in distilled spirits, aldehydes in potable spirits, and benzaldehyde, volatile esters and gamma undecalactone in cordials and liqueurs, were studied during the past year and reports submitted to the Association. The other subjects listed in the program are recommended for study next year.

The specific recommendations made by the associate referees will be given in their respective reports.

REPORT ON DIASTATIC ACTIVITY OF MALT

By CHRISTIAN RASK (Albert Schwill & Company, Chicago, Ill.), *Associate Referee*

The present method for the determination of diastatic activity, *Methods of Analysis, A.O.A.C.*, 1935, 158, **46 (b)**, fails to specify the details of the standardization of the Fehling solution, and it has been shown that the use of different methods may produce appreciable differences in results. It is therefore recommended that the last paragraph be revised to read: "Standardize the Fehling soln as directed under **XXXIV, 32, 33.**"

This method is subject to criticism on several points, but the Associate Referee thinks that no changes could be made in the basic procedure that would materially improve its accuracy. Of the several variations of the method that have been developed the so-called ferricyanide method is very promising. However, these methods need further study before any superiority in speed and accuracy can be positively established.

REPORT ON PROTEOLYTIC ACTIVITY OF MALT

By STEPHEN LAUFER (Schwarz Laboratories, Inc., New York City), *Associate Referee*

The investigations conducted in recent years on proteolytic enzymes present in cereals and their proteolytic activity deserve attention. They widen the general knowledge on this subject and assist in the development of a suitable method for its measurement.

Balls and Hale,[1] Hale,[2] and Mounfield[3] established beyond doubt that the proteinase found in whole wheat, wheat bran, wheat flour, and sprouted wheat is of the papainase type. The enzyme is activated by compounds containing a sulfhydryl group, such as cysteine, and also by hydrogen sulfide and cyanide, while oxidizing substances, such as common bread improvers, persulfate, bromate, and metavanadate inhibit its activity.

A modification of Laufer's viscometric method for measuring proteolytic activity of malt was suggested by Koch, Nelson, and Ehrnst.[4] On the other hand, Landis and Frey[5] utilized the change produced in the gelation rate of gelatin by proteolytic enzymes for determining the proteolytic activity of various substances.

It appears, however, that the proteolysis occurring in a brewers' mash is a rather complex phenomenon that cannot be measured by one single method. The Associate Referee also continued his studies along the lines indicated in his last reports, *This Journal*, **20**, 307 (1937); **21**, 160 (1938), and expects to submit the results next year.

It is recommended that the investigation on proteolytic activity of malt be continued.

No report on malt extract in malt was given by the associate referee.

REPORT ON MALT ADJUNCTS

By F. P. SIEBEL, Jr. (958 Montana Street, Chicago, Ill.), *Associate Referee*

During the past year collaborative work was done with the American Society of Brewing Chemists, many of whose members are also members of this organization. Samples of such commercially sold products as corn sugars, corn sirups, brewers' rice, brewers' flakes, corn grits, and refined grits were used. The analytical procedure followed was strictly in accord with the methods of the A.S.B.C., many of which have been adopted as

[1] *This Journal*, **18**, 135, 140 (1935); **19**, 372 (1936); *Cereal Chem.*, **13**, 54, 656 (1936); **15**, 622 (1938).
[2] *Cereal Chem.*, **16**, 695 (1939).
[3] *Biochem. J.*, **32**, 1675 (1938).
[4] *Ind. Eng. Chem., Anal. Ed.*, **11**, 35 (1939).
[5] *Cereal Chem.*, **15**, 91 (1938).

TABLE 1.—*Collaborative results on sirup*

	COLLABORATORS											
	1	2	4	5	6	7	8	9	10	11	12	14
Sample 5												
Consistency, as rec'd	thick soln	f. visc. sirup	sirup	—	thin sirup	sirup	sirup	—	sirup	thin sirup	thick	—
Appearance, as rec'd	clear	light brown	light	—	brown	yellow	—	—	amber	amber	clear	amber
Taste, as rec'd	sweet	sweet	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal
Odor, as rec'd	none	none	normal	butyric	—	odorless	normal	normal	normal	normal	clean	normal
Color, ½" cell, 10% Soln	1.1	1.25	1.4	(0.75)‡	1.45	beige	1.25	1.75	1.4	1.3	—	(2.5)‡
Clarity, 10% Soln	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear
Sp. Grav., 10% Soln	1.0308	1.03088	1.03070	1.03078	1.03074	1.03080	1.03084	1.03074	1.03074	1.03080	1.03068	1.03065
Moisture	20.1	19.90	20.3	20.1	20.2	20.1	20.01	20.2	20.2	20.08	20.3	20.4
Extract, as rec'd	79.9	80.10	79.7	79.9	79.8	79.9	79.99	79.8	79.8	79.92	79.7	79.6
Maltose, anhyd, as rec'd	—	—	none	—	—	—	—	—	—	—	—	—
Dextrose, as rec'd	49.56	49.96	50.30	47.87	50.16†	48.9	—	49.54	44.3	51.0	—	50.2
Invert Sugar, as rec'd	—	—	none	—	51.28	—	50.70	—	6.1	—	52.1	—
Sucrose, as rec'd	none	—	none	1.24	1.02	—	—	—	0.4	—	—	—
Ferm. Extract, as rec'd	47.33	48.89	50.0	51.1	50.4	51.5	49.99	48.3	48.8	(61.2)‡	51.6	47.5
Ferm. Extract, dry basis		60.99	62.6	64.0	63.1	64.4	62.5	60.5	61.1	(76.6)‡	64.8	59.75
Protein, as rec'd	0.00	—	—	0.039	none	0.04	none	—	0.1	0.24	0.33	0.03
Ash, as rec'd	0.14	0.17	—	0.19	0.168	0.18	0.16	—	0.13	0.14	0.14	0.20
pH, 10% Soln	3.8	4.7	—	5.10	—	4.95	4.9	—	5.25	4.84	5.3*	5.3
Iodine reaction	Erythro D	—	—	Erythro D	Erythro D	tr. Erythro D	negative	—	negative	Erythro D	negative	negative
Sample 6												
Consistency, as rec'd	thick sirup	clear sirup	sirup	—	thick sirup	sirup	sirup	—	thick	very thick	thick	—
Appearance, as rec'd	clear	water white	light	—	clear & white	hazy white	heavy	—	gray white	gray	milky	colorless
Taste, as rec'd	sweet	none	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal
Odor, as rec'd	none	normal	normal	sl. butyric	—	odorless	normal	normal	normal	normal	normal	normal
Color, ½" cell, 10% Soln	colorless	—	l.t. 1.0	0.13	water white	water white	none	0.0	0.0	l. t. 1	—	none
Clarity, 10% Soln	clear	clear	clear	clear	clear	clear	clear	clear	sl. hazy	clear	clear	clear
Sp. Gr., 10% Soln	1.0111	1.03122	1.03108	1.03117	1.03118	1.03118	1.03113	1.03122	1.03113	1.03120	1.03106	1.03113
Moisture	19.16	19.00	19.3	19.1	19.1	19.2	19.16	19.0	19.2	19.02	19.2	19.1
Extract, as rec'd	80.84	81.00	80.7	80.9	80.9	80.8	80.84	81.0	80.8	80.98	80.8	80.9
Maltose, anhyd, as rec'd	—	—	none	—	—	—	—	—	—	—	—	—
Dextrose, as rec'd	24.75	24.48	25.2	22.65	25.12†	23.4	24.15	22.60	26.9	24.9	25.6	26.4
Invert Sugar, as rec'd	—	—	none	—	25.28	—	—	—	—	—	—	—
Sucrose, as rec'd	none	—	none	none	0.35	—	—	—	—	—	—	—
Ferm. Extract, as rec'd	23.58	23.50	23.8	23.2	24.8	27.6	24.44	27.0	23.8	28.8	26.9	23.0
Ferm. Extract, dry basis		28.99	29.4	28.7	30.6	34.1	30.23	33.3	29.4	35.6	33.3	28.4
Protein, as rec'd	0.00	—	—	0.035	none	0.07	none	—	0.1	0.28	0.14	0.03
Ash, as rec'd	0.20	0.32	—	0.17	0.152	0.17	0.14	—	0.41	0.21	0.18	0.20
pH, 10% Soln	3.9	4.9	—	5.24	—	5.00	4.9	—	6.0	4.84	5.3*	5.4
Iodine reaction	Erythro D	Erythro D	—	Erythro D & Amylo D	Erythro D	Excess Amylo D No Erythro D	Erythro D	—	v. pl. tr. Erythro D	Erythro D	Erythro D	Erythro D

* Determined colorimetrically
† Munson-Walker method
‡ Results disregarded in summaries.

tentative by the A.O.A.C. The results of this work, together with the summaries showing low, high, and median values, are presented in the tables.

The results obtained by the majority of collaborators show satisfactory agreement, except for the following determinations: *p*H of sugars and sirups; moisture, extract, time of conversion, and assortment of cereal adjuncts. Improvement of the present methods with respect to these determinations is now under consideration. It is also planned to eliminate certain superfluous determinations and on the other hand to include determinations that are not as yet part of the methods.

TABLE 2.—*Summary for sirup samples 5 and 6*

	LOW	HIGH	MEDIAN
Color, ½″ cell, 10% soln	1.1	1.75	
Extract	79.6	80.1	79.9
Maltose	—	—	—
Dextrose Invert Sugar	47.9	52.1	50.3
Sucrose	0.0	1.24	
Ferm. Extract, as rec'd	47.33	51.6	50.0
Ferm. Extract, dry basis	59.8	64.8	62.6
*p*H	3.8	5.3	
Extract	80.7	81.0	80.9
Dextrose	22.60	26.90	24.90
Ferm. Extract, as rec'd	23.0	28.8	24.4
Ferm. Extract, dry basis	28.4	35.6	30.2
*p*H	3.9	6.0	

TABLE 4.—*Summary for sugar samples 7 and 8*

	LOW	HIGH	MEDIAN
Sample 7			
Extract	97.0	97.4	97.3
Maltose	—	64.5	
Dextrose	37.4	40.3	38.6
Ferm. Extract, as is	37.2	40.9	38.2
Ferm. Extract, dry basis	38.4	42.1	39.2
*p*H	3.8	5.8	
Sample 8			
Extract	83.6	85.0	83.9
Dextrose	67.5	70.7	68.7
Ferm. Extract, as is	62.1	65.8	64.2
Ferm. Extract, dry basis	73.1	78.4	75.9
*p*H	3.3	5.5	

The mutual satisfaction and benefits arising in the past from the cooperation between the A.O.A.C. and the A.S.B.C. make a continuance of such efforts appear highly desirable and indicate good results for the future. The present methods of the A.S.B.C. offer a valuable basis for the formulation of methods for the A.O.A.C.

Further study of these methods is planned. Samples for collaborative

TABLE 3.—*Collaborative results on sugar*

	COLLABORATORS											
	1	2	4	5	6	7	8	9	10	11	12	14
Sample 7												
Consistency, as rec'd	f. powder	powder	solid	—	powder	powder	powder	—	granular	powder	f. granules	powder
Appearance, as rec'd	—	white	light	—	white	white	—	—	white	white	white	lt. amber
Taste, as rec'd	sweet	f. sweet	normal	normal	normal	malt taste	normal	normal	normal	normal	normal	normal
Odor, as rec'd	none	none	normal	normal	—	malt odor	normal	normal	normal	normal	clean	normal
Color, ½" cell, 10% Soln	0.8	nil	l.t. 1.0	0.5	water white	straw	none	0.65	0.5	l.t. 1	—	2—3
Clarity, 10% soln	clear	clear	sl. hazy	clear	clear	clear	clear	v. sl. haze	sl. haze	clear	clear	sl. hazy
Sp. Gr., 10% soln	1.0375	1.03738	1.03737	1.03747	1.03749	1.03746	1.03735	1.03744	1.03738	1.03750	1.03735	1.03741
Moisture	2.6	2.80	3.0	2.7	2.6	2.6	3.01	2.8	2.9	2.6	2.9	2.7
Extract, as rec'd	97.4	97.20	97.0	97.3	97.4	97.4	96.99	97.2	97.1	97.4	97.1	97.3
Maltose, anhyd, as rec'd	—	—	none	—	—	64.5	—	—	—	—	—	—
Dextrose, as rec'd	39.92	38.60	38.2	(36.20)‡	38.90†	—	37.35	(34.48)‡	40.3	38.1	37.8	39.4
Invert Sugar, as rec'd	—	—	none	—	39.62	—	—	—	—	—	—	—
Sucrose, as rec'd	none	—	none	none	none	—	—	—	—	—	—	—
Ferm. Extract, as rec'd	38.5	37.95	38.0	37.7	39.9	40.0	37.24	40.3	37.5	38.2	40.9	(35.6)‡
Ferm. Extract, dry basis		39.06	39.2	38.7	40.9	41.0	38.40	41.4	38.6	39.2	42.1	(36.6)‡
Protein, as rec'd	0.00	—	—	0.035	none	0.10	none	—	0.1	00.29	0.33	0.04
Ash, as rec'd	0.20	0.27	—	0.21	0.244	0.13	0.23	—	0.23	0.31	0.26	0.35
*p*H, 10% soln	3.8	4.0	—	4.90	—	4.75	4.7	—	5.8	4.56	5.0(10)*	4.8
Iodine reaction	Erythro D	Erythro D	—	Erythro D	Erythro D	Erythro D	Erythro D	—	tr. Erthro D	Erythro D	neg.	neg.
Sample 8												
Consistency, as rec'd	small	lumps	solid	—	chips	lumps	lumps	—	lumps	chips	chips	chips
Appearance, as rec'd	lumps	white	light	—	cream	cream	—	—	cream	lt. yellow	cr. white	white
Taste, as rec'd	sweet	f. sweet	normal	normal	normal	sl. malt t.	normal	normal	normal	normal	normal	normal
Odor, as rec'd	none	none	normal	normal	—	odorless	normal	normal	normal	normal	clean	normal
Color, ½" cell, 10% soln	0.2	nil	l.t. 1.0	0.25	l.t. 0.5	water white	none	0.60	0.2	l.t. 1	—	none
Clarity, 10% soln	clear	clear	clear	clear	clear	clear	clear	clear	clear	clear	sl. hazy	clear
Sp. Gr., 10% soln	1.0324	1.03232	1.03234	1.03242	1.03267	1.03234	1.03234	1.03258	1.03220	1.03230	1.03252	1.03229
Moisture	15.8	16.07	16.1	15.8	15.1	16.1	16.07	15.0	16.4	16.3	15.3	16.1
Extract, as rec'd	84.2	83.93	83.9	84.2	84.9	83.9	83.93	85.0	83.6	83.8	84.7	83.9
Maltose, anhyd, as rec'd	—	—	none	—	—	—	—	—	—	—	—	—
Dextrose, as rec'd	70.72	68.72	69.7	67.45	(72.60)‡†	68.6	67.60	68.44	68.3	69.0	69.9	68.8
Invert sugar, as rec'd	—	—	none	—	74.86	—	—	—	—	—	—	—
Sucrose, as rec'd	none	—	none	none	none	—	—	—	—	—	—	—
Ferm. Extract, as rec'd	(59.60)‡	62.65	64.2	64.1	64.52	(67.9)‡	65.76	62.1	63.1	(74.2)‡	65.6	64.9
Ferm. Extract, dry basis		74.69	75.9	76.1	76.00	(80.9)‡	78.35	73.1	75.6	(88.5)‡	77.5	77.3
Protein, as rec'd	0.00	—	—	0.044	pr. none	0.04	none	—	0.1	0.27	0.49	0.05
Ash, as rec'd	0.15	0.18	—	0.34	0.46	0.12	0.32	—	0.38	0.35	0.31	0.40
*p*H 10% soln	3.3	4.9	—	4.17	—	4.00	4.3	—	5.45	4.10	4.7*	4.2
Iodine reaction	Erythro D	—	—	neg.	neg.	neg.	neg.	—	neg.	Erythro D	neg.	neg.

* Determined colorimetrically.
† Munson-Walker method.
‡ Results disregarded in summaries.

TABLE 5.—*Collaborative results on flakes*

	COLLABORATORS													
	1	2	3	4	5	6	7	8	9	10	11	12	13§	14
Sample 10														
Color	sl. cream	—	white	cream	white	sl. cream	white	v. l. cream	white	white	white	white	—	cream
Odor	none	—	cl. & n.	normal	clean	normal	cl. & n.	clean	cl. & n.	cl. & n.	cl. & n.	cl. & n.	—	cl. & n.
Foreign matter	none	—	none	occ. y. corn	tr. y. husks	0.6% y. corn	none	none	tr. y. corn	none	none	0.2% y.c.	—	none
Mold, weevils, etc.	none	—	none	none	none	none	none	none	none	none	none	none	—	none
Condition	small flakes	—	good	small	dusty, broken fl.	somewhat small and broken	—	partly broken	cons. amt. broken fl. and dust	presence of flake dust	normal	large amt. of dust	fines present broken fl.	normal
Thickness	medium	—	thick	coarse	medium	medium	thick	thick	medium	thick	medium	medium	thin	thick
Moisture	10.9	10.8	10.1	10.1	10.8	10.3	10.7	10.6	10.3	10.7†	10.69	10.7	11.4	10.4
Oil	0.49	0.31	0.48	0.35‡	0.39	0.53	0.40	0.23	0.46	0.50	0.44	0.50*	0.50*	0.40
Extract, as is	81.35	82.7	81.4	82.4	80.7	81.2	80.2	82.4	79.0	80.5	79.9	82.0	80.0	80.8
Extract, dry basis	91.30	92.77	90.5	91.7	90.4	90.5	89.8	92.1	88.1	90.1	89.5	91.8	90.3	90.2
Time of conversion	l.t. 15 m	normal	l.t. 15 m	10 m	10–15 m	15–30 m	l.t. 15 m	l.t. 5 m	15 m	10–15 m	30–45 m	l.t. 15 m	10 m	l.t. 15 m
Speed of filtration	normal	normal	normal	rapid	—	normal	normal	fast	normal	normal	normal	normal	fast	normal
Clarity	clear	clear	clear	clear	—	clear	clear	brilliant	—	clear	clear	clear	clear	clear
SUMMARY:	LOW	HIGH	MEDIAN											
Moisture	10.1	11.4	10.7											
Oil	0.23	0.53	0.46											
Extract, as is	79.0	82.7	81.2											
Extract, dry basis	88.1	92.7	90.5											
Time of conversion	l.t. 5	30–45												

* Ether extract.
† Samples ground.
‡ Det'd with anhydrous ethyl ether on dried sample.
§ A.S.B.C. methods *not* strictly followed.

TABLE 6.—*Collaborative results on rice*

	COLLABORATORS													
	1	2	3	4	5	6	7	8	9	10	11	12	13††	14
Sample 11														
Color	sl. cream	—	white	cream	white	sl. cream	gray	white	gray-white	pearl-white	white	white	natural	buff
Odor	none	—	cl. & n.	normal	oily	sl. rancid	cl. & n.	clean	cl. & n.	cl. & n.	cl. & n.	cl. & n.	none	cl. & n.
Foreign matter	none	—	tr. foreign seeds	none	none	0.09% seeds	none	few	tr. foreign seeds	none	none	none	none	none
Mold, weevils, etc.	none	—	none	none	none	†	none	none	2 weevils	none	none	none	none	none
Assortment, on No. 10	1.55	—	1.1	1.0	0.5	0.0	—	2.1	1.2	0.0	0.0	0.95	—	—
(a) 14	56.95	—	58.6	53.2	57.7	78.6	—	61.5	60.6	29.8	80.2	29.30	—	—
18	27.90	—	29.3	33.4	31.7	14.6§	—	26.4	28.6	44.7	13.3	15.10	—	—
30	9.65	—	8.7	10.2	8.8	3.7	—	8.3	8.6	20.6	3.6	4.10§§	—	—
60	2.95	—	1.8	1.6	1.0	1.5	—	1.1	0.5	3.5	2.0	0.45	—	—
100	0.60	—	0.3	0.1	0.5	0.6	—	0.3	0.1	0.7	0.3	0.10	—	—
thru 100	0.40	—	0.2	0.5	0.3	1.0	—	0.3	0.4	0.7	0.6	0.00	—	—
Assortment, on No. 18	85.95	87.7	—	88.8	90.0	90.4	95.4‡‡	89.0	—	75.4	92.0	94.00	—	—
(b) 60	12.85	10.8	—	10.4	9.5	8.0§	4.0	10.4	—	23.3	7.3	5.30	—	—
thru 60	1.20	1.5	—	0.8	—	1.6	0.6	0.6	—	1.3	0.7	0.70	—	—
Moisture	10.1	10.4	8.6	9.6	10.5	9.8	10.4	10.5	9.6	10.3**	10.3	10.4	11.3	9.1
Oil	0.51	0.45	0.59	0.53‡	0.5	0.43	0.45	0.37	0.52	0.64	0.56	0.45*	0.51*	0.40
Extract, as is	87.28	88.2	86.6	88.0	89.1	88.5	85.8	87.8	84.3	87.2	86.2	87.5	86.7	—
Extract, dry basis	97.08	98.4	94.8	97.4	99.4	98.1	95.7	98.1	93.2	97.1	96.1	97.7	97.6	—
Time of conversion	l.t. 15 m	10–15 m	l.t. 15 m	15 m	10–15 m	15–30 m	l.t. 15 m	5–7 m	15–30 m	10–15 m	15–30 m	l.t. 15 m.	10 m	—
Speed of filtration	normal	normal	normal	rapid	normal	normal	normal	fast	normal	normal	normal	normal	fast	—
Clarity	clear	clear	clear	clear	hazy	clear	clear	brilliant	—	clear	clear	clear	clear	—
SUMMARY:	LOW	HIGH	MEDIAN											
On No. 18 sieve	75.4	95.4												
On No. 60 sieve	4.0	23.3												
Moisture	8.6	11.3	10.3											
Oil	0.37	0.64	0.51											
Extract, as is	84.3	89.1	87.3											
Extract, dry basis	93.2	99.4	97.4											
Time of conversion	5–7	15–30												

* Ether extract.
† Living larva and adult of confused corn beetle.
‡ Det'd with anhydrous ethyl ether on dried sample
§ Pfungstadt sieves and apparatus used.
** Samples ground.
†† A.S.B.C. methods *not* strictly followed.
‡‡ 20-mesh screen.
§§ Apparently figures for Ass't (a) should be doubled (K.B).

TABLE 7.—*Collaborative results on refined grits*

	COLLABORATORS													
	1	2	3	4	5	6	7	8	9	10	11	12	13§	14
Sample 12														
Color	white	—	white	cream	white	white	white	white	white	chalk white	white	white	—	white
Odor	none	—	cl. & n.	normal	clean	normal	cl. & n.	clean	cl. & n.	cl. & n.	cl. & n.	cl. & n.	—	cl. & n.
Foreign matter	none	—	none	none	none	none	none	none	none	none	none	—	—	none
Mold, weevils, etc.	none	—	none	none	none	none	none	none	none	none	none	none	—	none
Assortment, on No. 10	20.45	—	25.8	—	27.2	13.4	—	12.8	17.8	5.5	16.0	—	—	—
(a) 14	24.00	—	28.6	—	28.2	39.3	—	22.6	23.3	15.3	44.9	—	—	—
18	28.20	—	22.4	—	26.4	18.2	—	24.4	24.0	25.8	17.6	—	—	—
30	19.80	—	16.5	—	14.8	18.7‡	—	25.0	22.6	36.9	11.9	—	—	—
60	6.85	—	6.5	—	2.9	7.6	—	13.1	11.2	14.3	8.6	—	—	—
100	0.45	—	0.2	—	0.4	1.1	—	1.6	1.0	1.4	0.5	—	—	—
thru 100	0.25	—	0.0	—	0.4	1.7	—	0.5	0.2	0.8	0.5	—	—	—
Assortment, on No. 18	70.10	—	—	—	80.3	76.4	84.7††	66.1	—	46.4	76.9	—	—	—
(b) 60	29.10	—	—	—	19.3	22.2	15.1	32.2	—	51.2	21.8	—	—	—
thru 60	0.80	—	—	—	—	1.4	0.2	1.7	—	2.4	1.3	—	—	—
Moisture	6.6	6.9	6.2	7.0	7.0	6.8	7.1	7.0	6.8	6.9§	6.64	6.8	7.4	6.7
Oil	0.05	0.04	0.1	0.06†	0.02	0.02	0.04	0.01	0.04	0.04	0.13	0.00*	0.06*	0.04
Extract, as is	96.48	97.3	96.0	97.6	97.6	97.4	95.3	97.5	93.5	96.7	96.7	97.5	94.0	96.2
Extract, dry basis	103.29	—	102.5	105.0	104.9	104.5	102.6	104.7	100.3	103.7	103.5	104.6	101.5	103.1
Time of conversion	l.t. 15 m	10–15 m	l.t. 15 m	10 m	10–15 m	15–30 m	l.t. 15 m	l.t. 5 m	15–30 m	7–10 m	15–30 m	l.t. 15 m	15 m	l.t. 15 m
Speed of filtration	normal	normal	normal	rapid	normal	normal	normal	fast	normal	normal	normal	normal	fast	normal
Clarity	clear	clear	clear	clear	hazy	clear	clear	brilliant	—	clear	clear	clear	clear	clear
SUMMARY:	LOW	HIGH	MEDIAN											
On No. 18 sieve	46.4	84.7												
On No. 60	15.1	51.2												
Moisture	6.2	7.4	6.9											
Oil	0.00	0.13	0.04											
Extract, as is	93.5	97.6	96.7											
Extract, dry basis	100.3	105.0	103.5											
Time of conversion	l.t. 5	15–30												

* Ether extract.
†† 20-mesh screen
† With anhydrous ethyl ether on dried sample.
‡ Pfungstadt sieves and apparatus used.
§ A.S.B.C. methods *not* strictly followed.

TABLE 8.—*Collaborative results on grits*

	COLLABORATORS													
	1	2	3	4	5	6	7	8	9	10	11	12	13**	14
Sample 13														
Color	sl. cream	—	white	cream	cream	white	gray	lt. cream	gray-white	lt. cream	white	white	—	cream
Odor	none	—	cl. & n.	normal	clean	normal	cl. & n.	clean	cl. & n.	cl. & n.	cl. & n.	cl. & n.	—	cl. & n.
Foreign matter	none	—	tr. y. corn	tr. y. corn	none	0.53% y.c.	none	none	‡‡	none	none	0.01% y.c.	—	none
Mold, weevils, etc.	none	—	none	none	none	none	none	none	none	none	none	none	—	none
Assortment, on No. 10	0.00	—	0.1	0.0	0.0	0.0	—	0.0	0.0	0.0	0.0	0.00	—	—
(a) 14	0.40	—	1.2	0.6	0.4	24.1	—	1.2	1.2	0.1	17.7	0.80	—	—
18	64.60	—	43.6	42.6	40.3	42.1	—	35.6	39.8	12.8	55.1	23.20§§	—	—
30	32.75	—	53.8	54.2	58.0	29.7‡	—	61.6	56.2	84.5	24.0	25.60	—	—
60	1.90	—	1.1	1.0	0.7	0.9	—	1.0	1.0	1.9	1.5	0.25	—	—
100	0.20	—	0.2	0.8	0.3	0.5	—	0.4	0.6	0.5	0.2	0.15	—	—
thru 100	0.15	—	0.0	0.8	0.5	2.7	—	0.2	1.2	0.2	1.5	0.00	—	—
Assortment, on No. 18	63.70	35.6	—	43.8	40.8	63.4	74.4††	37.6	—	12.5	69.3	36.80	—	—
(b) 60	35.65	62.5	—	54.8	58.5	63.8	25.1	61.5	—	85.3	28.8	60.30	—	—
thru 60	0.65	1.9	—	1.4	—	2.8	0.5	0.9	—	2.2	1.9	2.90	—	—
Moisture	14.1	14.4	13.5	14.0	14.7	13.9	14.4	14.3	13.9	14.0§	14.64	14.5	15.0	13.7
Oil	0.44	0.36	0.27	0.36†	0.33	0.30	0.48	0.25	0.56	0.51	0.36	0.38*	0.52*	0.5
Extract, as is	79.85	78.9	77.2	80.5	80.2	80.5	77.8	80.5	76.5	79.1	78.9	79.8	81.0	78.6
Extract, dry basis	92.95	92.2	89.1	93.6	94.0	93.5	90.9	94.0	88.9	91.9	92.4	93.3	95.8	91.0
Time of conversion	l.t. 15 m	normal	l.t. 15 m	10 m	10–15 m	l.t. 15 m	l.t. 15 m	l.t. 5 m	15 m	10–15 m	30–45 m	l.t. 15 m	10 m	15–30 m
Speed of filtration	normal	normal	normal	rapid	normal	normal	normal	fast	normal	normal	normal	normal	fast	normal
Clarity	clear	clear	clear	clear	hazy	clear	clear	brilliant	—	clear	clear	clear	clear	clear
SUMMARY:	LOW	HIGH	MEDIAN											
On No. 18 sieve	12.5	74.4												
On No. 60	25.1	85.3												
Moisture	13.5	15.0	14.3											
Oil	0.25	0.56	0.38											
Extract, as is	76.5	81.0	79.8											
Extract, dry basis	88.9	95.8	93.0											
Time of conversion	l.t. 5	30–45												

* Ether extract.
† With anhydrous ethyl ether on dried sample.
‡ Pfungstadt sieves and apparatus used.
§ Samples ground.
** A.S.B.C. methods *not* strictly followed.
§§ Apparently figures for Ass't (a) should be doubled (K.B.).
†† 20-mesh screen.
‡‡ 0.44% yellow corn.

work will be distributed during November and December of this year to the members of the A.O.A.C. who have signified their readiness to cooperate as well as to the collaborators among the membership of the A.S.B.C. Efforts will also be made to benefit from the opinions and experiences of the manufacturers of the products concerned.

The collaborative results were obtained with the A.S.B.C. methods.

REPORT ON BEER

By HUGO W. ROHDE (Jos. Schlitz Brewing Company, Milwaukee, Wis.), *Associate Referee*

The 1938 report of the Associate Referee gave the results of 14 collaborators. Very satisfactory agreement was shown, particularly from those laboratories in which beer analysis is a daily routine. It did not appear to be necessary to repeat this work this year.

The following suggestions and recommendations[1] are presented for the improvement of the A.O.A.C. methods for beer. The references are to *Methods of Analysis, A.O.A.C.*, 1935, **XIV.**

(1) Par. **4,** *Apparent or Saccharometric Indication.*—The following directions should be added: "*and record to second decimal place.*"

(2) Par. **7,** *Real Extract.*—Methods **(a)** and **(c)** should be made official (final action) and Method **(b)** be dropped or retained as tentative. The latter involves the immersion refractometer reading of the beer and distillate, and the results are not always satisfactory.

(3) Par. **8,** *Extract of Original Wort.*—The following direction should be added: "Report to first decimal place."

A more convenient formula could be added:

$O = A \times 2 + E \pm$ correction, in which—

O = extract or original wort;

A = % alcohol by weight (g. per 100 g. of beer); and

E = real extract.

Correction—Added or subtracted as given in the Correction Table—Pawlowski-Doemens: "Die Brautechnischen Untersuchungs-Methoden," Fifth Edition, 1938. (This table could be reduced in size to less than one-half.)

(4) Par. **9,** *Real Degree of Fermentation.*—The direction, "Report to first decimal place," should be added.

(5) Par. **10,** *Total Acidity.*—This method should be changed to read as follows:

Run 10 or 25 ml of decarbonated beer into approximately 10 volumes of boiling distilled water, and continue boiling for 60 seconds or longer after addition of sample. Cool to room temperature and titrate immediately with 0.1 *N* alkali, using ½ ml. of 0.04% phenolphthalein; 1 ml. of 0.1 *N* alkali = 0.0090 g. of lactic acid. Report results to nearest 0.01%.

(6) Par. **12,** *Reducing Sugars.*—The Munson and Walker table, **XLII,**

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1940).

9, gives the maltose with one molecule of water. As it is customary, in beer analysis, to record the reducible sugar as *anhydrous maltose*, the table should be revised.

(7) Par. **18,** *Protein.*—The selenium method for digestion seems to be more rapid. The mixture taken consists of 250 g. of anhydrous sodium sulfate, 4 g. of copper sulfate, and 4 g. of selenium. Five grams are taken for the determination, and the titration is made with methyl red as indicator.

(8) Par. **21,** *Sulfur Dioxide.*—It is suggested that 1 cc of H_3PO_4 be changed to 4 ml. H_3PO_4 (sp. g., 1.154 containing about 25% H_3PO_4)—weigh as $BaSO_4 \times 0.2744$ equals SO_2. Report as "volatile S" in mg. per liter.

(9) Par. **22,** *Iodine Reaction for Unconverted Starch.*—The last sentence, "Abnormal beer gives red or violet coloration," which is not strictly correct, should be omitted.

(10) Par. **24,** *Metals.*—Heavy metals (Cu, Fe, Pb, Sn, Zn), even in very minute quantities, are likely to impair the stability and keeping qualities of beer. Therefore, it is recommended that an Associate Referee on Metals in Beer investigate colorimetric determinations for Fe, Cu and Sn.

(11) Par. **26,** *Pasteurization.*—It is recommended that this determination be dropped.

(12) It is recommended that the methods submitted for the determination of hydrogen-ion concentration and for end fermentation as increase in the degree of fermentation be adopted as tentative.

The Associate Referee also suggests that attention be given to the subject of hops.

No report on heavy metals in beer was given by the associate referee.

No report on carbon dioxide in beer was given by the associate referee.

No report on total sulfur in wines was given by the associate referee.

REPORT ON VOLATILE ACIDS IN WINE

By M. A. JOSLYN (University of California, Berkeley, Calif.),
Associate Referee

The work on volatile acidity of wines was limited to collaborative test of the modified Peynaud procedure outlined in the preceding report, *This Journal*, 22, 210 (1939). Three samples of wines were sent out to thirty collaborators, of whom fifteen reported. The composition of the wines sent out is given in Table 1. It is of interest that lactic acid distils over in appreciable quantities in the Fessler-type still used in this laboratory. With

wines low in volatile acidity it forms a larger proportion of the "volatile acid" content than it does with wines high in volatile acidity. The methods of analyses suggested to the collaborators were the following:

(1) Method II, *Methods of Analysis A.O.A.C.*, 1935, p. 167; (2) Same as (1) but corrected for the SO_2 in the distillate as directed in *Methods of Analysis, A.O.A.C.*, 1935, p. 167, 25; (3) modified Peynaud procedure as given in the second report of this Associate Referee, *This Journal*, **22**, 210 (1939). *Caution:* In this procedure some wines foam quite badly so that a sufficiently large distillation trap is necessary to avoid entrainment of fixed acids and frothing over.

TABLE 1.—*Description of wine samples prepared*

SAMPLE	A	B	C
Description	Dry Sauterne type	Dry red	Blended wine, to 16 liters of which was added 40 g. of 85% lactic acid, 10 g. of glacial acetic acid, and 10 g. of $K_2S_2O_5$.
Total acidity, grams tartaric/100 ml.	0.69	0.81	0.93
Lactic acid, grams/100 ml.	0.22*	0.20	0.51
Volatile acid, grams acetic/100 ml.	0.057*	0.181	0.198
Lactic acid in distillate, grams/100 ml.	0.037*	0.038	0.039–0.050

* Analyses by L. A. Hohl.

The results obtained are summarized in Table 2. It is evident that there was less divergence in the results obtained for Sample A, which was low in volatile acidity even though it contained some sulfur dioxide. The more sour wine, Sample B, gave more difficulty, although the results for collaborators in a given laboratory agree fairly well. Much more difficulty was experienced with Sample C, in which the combined high lactic acid content and the high sulfur dioxide content resulted in more erratic results between workers in a given laboratory and also between workers in different laboratories. Part of the difficulty is due largely to the effect of conditions of distillation and titration on the sulfur dioxide coming over. As shown in Table 3 there is considerable variation in the sulfur dioxide corrections, this variation being greater in the wines containing more sulfur dioxide. Thus for Sample A the spread was 0.006–0.023, for Sample B 0.001–0012, and for Sample C 0.015–0.045. Part of this difficulty is due to oxidation of sulfur dioxide, in the distillate, particularly after neutralization. Thus the iodine titration on distillates in ml. of 0.1 *N* sodium hydroxide per 10 ml. of wine was found to vary, depending on promptness of addition of the sulfuric acid (1+3) as follows: A, 0.15–0.20; B, 0.08–0.09; and C, 0.48–0.60. The higher figure is for un-neutralized distillate. However, if the distillate is quickly acidified after neutralization, the io-

TABLE 2.—*Volatile acidity (grams of acetic acid per 100 ml.) on wines obtained by several collaborators using three procedures*

COLLABORATOR	LABORATORY	METHOD I			METHOD II			METHOD III		
		A	B	C	A	B	C	A	B	C
G. F. Beyer	Bur. of Internal Revenue, Washington, D.C.	0.06	0.20	0.22	0.047	0.196	0.197	0.048	0.180	0.178
A. C. Blaisdell	Bur. of Internal Revenue, Washington, D.C.	0.053	0.197	0.203	0.045	0.185	0.167	0.047	0.185	0.177
L. Burritt	Bur. of Internal Revenue, Washington, D.C.	0.057	0.200	0.232	0.043	0.195	0.206	0.042	0.182	0.171
H. de Bussieres	Curtis & Tompkins, Ltd., San Francisco, Calif.	0.055	0.199	0.213	0.046	0.195	0.198	0.054	0.186	0.186
C. T. Carson	Frankfort Distilleries, Baltimore, Md.	0.054	0.192	0.210	0.047	0.188	0.184	0.048	0.180	0.162
H. B. Dixon	U.S.D.A. Agr. Marketing Service, Washington, D.C.	0.057	0.192	0.213	0.046	0.186	0.183	0.042	0.183	0.177
V. de F. Henriques	Shewan-Jones Winery, Lodi, Calif.	0.052	0.210	0.211	0.043	0.200	0.187	0.048	0.187	0.184
L. A. Hohl	Div. of Fruit Products, Univ. of California	0.057	0.181	0.200				0.054	0.202	0.187
M. A. Joslyn	Div. of Fruit Products, Univ. of California	0.052	0.189	0.209	0.043	0.183	0.179	0.048	0.180	0.155
S. Laufer	Schwarz Laboratories, New York City	0.047	0.193	0.185	0.041	0.188	0.175	0.043	0.195	0.166
H. B. Madsen	Washington State Liquor Control Board	0.057	0.199	0.223	0.042	0.192	0.190	0.050	0.187	0.193
J. B. Robb	A.B.C. Board, Richmond, Va.	0.050	0.199	0.199	0.038	0.193	0.165	0.049	0.198	0.199
T. M. Scott	Mt. Tivy Winery, Fresno, Calif.	0.061	0.194	0.211	0.045	0.193	0.166	0.048	0.179	0.171
S. R. Snider	U.S.D.A. Agr. Marketing Service, Washington, D.C.	0.060	0.178	0.192	0.049	0.175	0.175	0.042	0.186	0.163
T. E. Twining	The Twining Laboratories, Fresno, Calif.	0.043	0.186	0.192	0.035	0.179	0.172	0.040	0.187	0.171
P. Valaer	Bur. of Internal Revenue	0.051	0.197	0.228	0.053	0.192	0.210	0.049	0.180	0.171
J. B. Wetmore	K. Arakelian, Inc. Winery, Fresno, Calif.	0.063	0.204	0.222	0.040	0.192	0.179			
Average		0.055	0.195	0.211	0.044	0.190	0.183	0.047	0.186	0.174
Maximum		0.063	0.204	0.232	0.053	0.200	0.210	0.054	0.202	0.199
Minimum		0.043	0.178	0.185	0.038	0.175	0.165	0.040	0.179	0.155
Number of collaborators within ±0.1 ml.		17/17	13/17	7/17	15/16	12/16	5/16	14/16	12/16	8/16

dine titer agrees with that obtained on the un-neutralized to better than ±0.03 ml. In this way it is not necessary to run a duplicate distillation for the sulfur dioxide correction.

The results obtained by the modified Peynaud procedure are generally more consistent than those obtained by other procedures. The average values are somewhat lower than those obtained by Method II. This was not found to be due to a difference in the lactic acid distilled over since L. A. Hohl obtained essentially the same results for both the distillates from the barium-treated and the barium-untreated wine. The extent of

TABLE 3.—*SO_2 corrections in distillate (grams of acetic per 100 ml.)*

COLLABORATOR	WINE		
	A	B	C
G. F. Beyer	0.013	0.004	0.023
A. C. Blaisdell	0.018	0.012	0.030
L. Burritt	0.014	0.005	0.038
H. de Bussieres	0.009	0.004	0.015
C. T. Carson	0.007	0.004	0.026
H. B. Dixon	0.011	0.006	0.030
V. de F. Henriques	0.009	0.010	0.025
M. A. Joslyn	0.009	0.006	0.030
S. Laufer	0.006	0.005	0.020
H. B. Madsen	0.015	0.007	0.032
J. B. Robb	0.012	0.006	0.034
T. M. Scott	0.016	0.001	0.045
S. R. Snider	0.011	0.003	0.017
T. E. Twining	0.008	0.007	0.020
P. Valaer*	−0.002	0.005	0.018
J. B. Wetmore	0.023	0.012	0.043

* Reported earlier a correction of 1.1 ml. of 0.02 *N* iodine, or 0.013.

neutralization of the wine and the time the neutralized wine is allowed to stand have a large effect on the Peynaud procedure for wines of high sulfur dioxide content. Thus Hohl obtained a volatile acidity of 0.187 on Sample C, but on repetition after allowing the neutralized wine to stand longer she obtained 0.154, in agreement with 0.155 obtained by the Associate Referee. The latter modified the suggested procedure in two respects; first he omitted removal of carbon dioxide, which is considered to be unnecessary to this procedure, and second he increased the time of standing to 30 minutes. Some difficulty was had with neutralization of the red wine (Samples B and C) because of the masking of the end point by the anthocyanin pigments present. The Associate Referee merely added enough barium hydroxide to change the pigments from light red to a definite dark bluish purple. An external indicator changing at *p*H 8 may be used also, since the solution must be slightly alkaline rather than neutral in order to decompose bound sulfites.

Several of the collaborators made additional experiments. They comment as follows:

C. T. Carson.—The distillations were carried out in a Hortvet-type apparatus equipped with standard taper ground-glass joints. It is noted that there is better agreement in the figures for Samples A and B than for Sample C, a blended wine with added SO_2, and acetic and lactic acid.

H. B. Dixon.—Sample A required 80 ml. of distillate for Method I, while Samples B and C required 120 ml. for the same method. These quantities of distillate for the respective samples were used for Methods II and III.

The initial distillate from Sample C with Methods I and II produced a strong odor of SO_2, which indicates the need of a closed system for absorption with this type of samples. Method III should be more specific concerning the end point upon the addition of $Ba(OH)_2$ for the colored wines. No caution against frothing was necessary with the use of a large Sellier tube (100 ml. capacity) and a large modified Kjeldahl trap.

V. de F. Henriques.—We use a 10 ml. sample in the Sellier tube and do not necessarily use boiled water. The wine is not free from CO_2 either by precipitation or vacuumization or any other treatment, not even if it still is fermenting wildly. We do not ordinarily use paraffin or oil or tannin or anything else to cut down foaming. Distillation usually requires 12–18 minutes and during this time 100 ml. of distillate is collected. This is brought to a boil and boiled 30–90 seconds, and is then titrated hot with 0.1 *N* NaOH, phenolphthalein being used. The receiving flask is then covered with an inverted 50 ml. beaker and is cooled under running water in the sink so that it will give a starch end point. The distillate is then acidified with 2 or 3 drops of H_2SO_4 (1+3), starch is added, and it is then titrated with 0.02 *N* iodine. The equivalent iodine is subtracted from the alkali and volatile is calculated.

I believe that a correction must be made for SO_2. I question (without actually knowing) whether vacuumization is very effective in the removal of CO_2. I believe that CO_2 must be removed. Peynaud's method checks our method and checks itself. It also checks our standard procedure (which we have checked with you on previous collaborative analyses) more closely than the other methods, at least in the range of high volatile acids. However, there is still much work to be done.

S. Laufer.—The work was carried out by one of our chemists, Leonard Saletan. He followed the methods exactly as specified. Considerable foaming was experienced with Method III. Owing to the fact that we did not have available a larger foam trap, the results obtained on Sample C by this method are not satisfactory.

H. S. Madsen.—Although in theory the use of $Ba(OH)_2$ should remove SO_2 effectively, the distillates from each distillation were titrated with iodine solution under the conditions of the official method and it was found that SO_2, or other substances capable of reducing iodine, were present. Assuming that SO_2 was present and calculating in terms of acetic acid, the following results were obtained: Samples A and B, .005 and Sample C, .018.

If these amounts were to be subtracted from the total volatile acidity, obtained by Method II, in each case the results would be as follows: Sample A, .045; Sample B, .182; and Sample C, .175.

Little trouble was experienced in the distillation in Method II if sufficient water was provided in the outer flask. The proper quantity was found to approximate 250 ml. With the apparatus used in this laboratory, approximately 15 minutes was required to distil 100 ml.

This laboratory uses, as a sorting method in routine wine analyses, essentially the same method as that described under *Method 5* in *This Journal*, **22**, 217 (1939), except that the water in the outer flask is heated until boiling freely and the wine

in the inner tube is at its boiling point before the apparatus is connected with the trap and condenser. The results by this method, corrected for SO_2, are as follows: Sample A, .045; Sample B, .192; and Sample C, .192.

T. M. Scott.—Modified Peynaud method used. At the time of the first analysis I had no $Ba(OH)_2$ and made up some from $BaCl_2$ and NaOH. Being in doubt as to the correctness of the results I re-ran them, using C.P. $Ba(OH)_2$ in the procedure. The following are the results: Sample A, .048 grams; Sample B, .180 grams; and Sample C, .175 grams acetic acid/100 ml. Only Sample C varies any appreciable amount from the original results and it differs hardly more than the experimental error.

S. R. Snider.—A large size Sellier tube (100 ml. capacity) was used instead of the small tube generally advertized by glassware manufacturers. A modified Kjeldahl trap, similar to trap S-63545, 1937 Sargent and Co. catalogue, was used instead of the trap furnished with the Sellier assembly. It is suggested that distilling in a closed system that has been freed from CO_2 might permit more accurate estimations and better checks between replicate determinations. It seems that the design of the apparatus might be improved to facilitate handling, for instance the use of ball and socket ground-glass connections at the junction of the trap and the Sellier tube that could be easily disconnected when the distillation is completed. No particular caution against frothing was necessary for the three samples analyzed when the modified Kjeldahl trap was used.

P. Valaer.—With Method I there is a very close agreement; with Method II, which involves the use of iodine, it would seem that our interpretation of the method is different from that of the other collaborators.

Two collaborators ran the SO_2 on 50 ml. of the white wine before they realized it should first be steam distilled, then there was a question of using Na_2CO_3. Some used the distillate from 10 ml. but used all of .5 gram of Na_2CO_3 and 5 ml. of H_2SO_4 (1+3). Others believed that if 10 ml. only were steam distilled in a Sellier, then proportionate quantities of Na_2CO_3 and dilute H_2SO_4 should be used; that is, 0.1 gram of Na_2CO_3 and 1 ml. of H_2SO_4. Others were of the opinion that distillate should be neutralized and then titrated with 0.02 *N* iodine without any Na_2CO_3 and acid.

Owing to the trouble with this method and lack of explicit details, the iodine method is ranking a poor third.

With Method III (modified Peynaud procedure) we had some disagreements but not so many as with Method II. It was easy to neutralize Sample A by this third method with $(BaOH)_2$, but the neutralization end point of B and C was difficult, possibly owing to over-neutralization and because of the 20 ml. aliquot used for steam distillation there may have been enough material carried over by foaming and mechanically to give a few differences in titration.

It would seem that Method I is not satisfactory in the presence of SO_2, while Method II is difficult to check, consumes more time, and is not reliable. Method III (Peynaud) seems to be the most satisfactory one provided the results indicate the true value of the volatile acids in the samples.

RECOMMENDATIONS[1]

In view of the results obtained this year and those previously reported it is recommended:

(1) That the modified Peynaud procedure be adopted as tentative pending further investigation. The directions will be given in the 1940 edition of *Methods of Analysis, A.O.A.C.*, to issue about July 1st.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 23, 69 (1940).

(2) That the effect of lactic acid on the volatile acidity of wines be reinvestigated for the various types of apparatus now in use.

(3) That in Method II, p. 167 of *Methods of Analysis, A.O.A.C.*, 1935, the parenthetical phrase "(Preferred for wines containing an abnormal quantity of acetic acid)" be stricken out, and that the method be revised (see 1940 edition).

(4) That section 25, page 167, be revised (see 1940 edition).

REPORT ON SULFUR DIOXIDE IN BEER AND WINE

By L. V. TAYLOR, Jr. (American Can Company, Research Dept., Maywood, Ill.), *Associate Referee*

The study of sulfur dioxide methods for alcoholic and carbonated beverages during the current year was confined to ale and beer.

A previous paper by the Associate Referee, *et al.*, *This Journal*, **20**, 610 (1937), showed the A.O.A.C. tentative method, *Methods of Analysis, A.O.A.C.*, 1935, 152, for sulfur dioxide in beer and ale to be unreliable as a routine procedure, and that accurate and reproducible results could be obtained by the Monier-Williams sulfur dioxide method, *Ibid.*, 440, either by the gravimetric or volumetric technic.

An attempt made in 1938 to corroborate the above findings by collaborative studies, was unsuccessful, because of difficulties encountered in preparing and distributing collaborative samples containing a uniform sulfur dioxide concentration. No way was found to eliminate these difficulties prior to the 1939 studies. Consequently, samples having sulfur dioxide contents of the same order of magnitude were supplied to each collaborator this year, but no attempt was made to ascertain that each collaborative sample contained exactly the same quantity of sulfur dioxide. Each collaborator was instructed to make simultaneous duplicate determinations by each method on the composite samples of beer and ale received. This procedure permitted the evaluation of the merits of the A.O.A.C. and Monier-Williams methods, as shown by the results of the individual collaborators in applying the methods to the sample of beer submitted to each.

This Laboratory and three of the collaborators submitted results. They are recorded in Table 1. It will be noted that all the results except one set obtained by the Monier-Williams gravimetric and volumetric technics are in close agreement for the same determination. Occasionally difficulties have been met in the volumetric technic owing to acid carry-overs during distillation and lack of uniformity in reading end points. Consequently, the more time-consuming gravimetric method appears to be slightly preferable to the volumetric procedure for this work.

A comparison of the results obtained by the tentative A.O.A.C. method for beer with the values obtained simultaneously with the Monier-Wil-

liams method (Column 3 as opposed to Columns 1 and 2 for specific determination numbers) shows, in general, that the results by the steam

TABLE 1.—*SO_2 in beer and ale*

COLLABORATOR	DETERMINATION NO.	METHOD		
		MONIER-WILLIAMS METHOD		A.O.A.C. STEAM DISTILLATION METHOD MG./LITER
		VOL. MG./LITER	GRAV. MG./LITER	
		(1)	(2)	(3)
		Beer		
1	1	35	35	31
	2	39	40	34
	3	40	40	31
	4	41	41	25
2	1	37	37	29*
	2	37	37	35*
3	1	30	43	31
	2	31	45	36
4	1	42	43	42
	2	42	42	32
	3	43	43	39
	4	42	41	29
	5	39	39	29*
	6	38	39	31*
		Ale		
1	1	21	22	17
	2	20	22	13
2	1	19	19	20*
	2	19	19	16*
3	1	21	26	23
	2	19	26	21
4	1	25	25	18
	2	25	25	14
	3	25	24	13
	4	25	25	15
	5	19	18	17*
	6	20	20	18*

* Results obtained by using J. B. Thompson's suggestion referred to in text.

distillation method are low and quite variable. In addition, most collaborators, in both the 1938 and 1939 studies, reported difficulties with the steam distillation method due to foaming of the sample during analy-

sis. J. B. Thompson reported that this objection could be overcome to a great extent by substituting a 2 liter flask equipped with a Kjeldahl nitrogen trap for the 1 liter flask suggested in the procedure. This was corroborated by tests conducted by C. G. Ryberg, but, as indicated in the tables, it did not entirely overcome the frequent poor recoveries.

On the basis of the collaborative work described here, which confirms the work of Taylor, *et al.* (1), the Associate Referee recommends[1] that the tentative A.O.A.C. method for the determination of sulfur dioxide in malt beverages be deleted, and that the method suggested be made official (first action) and inserted in its place (see 1940 edition of *Methods of Analysis, A.O.A.C.*, to issue about July 1st).

In the 1935 edition of *Methods of Analysis*, Chapter **XV**, *Wines*, makes no provision for the determination of sulfur dioxide except by a reference to a method under the title "Total Acidity—Exclusive of SO_2", **25**, p. 167. In this instance the analyst is referred to the method bearing the title "Free Sulfurous Acid—Official" (**XXXII, 33**, p. 441), with an indication that the method is especially adapted to wine. This procedure bears no reference to indicate that the method has been studied collaboratively. In addition, this method, sometimes referred to as the Ripper method, is said by Leach[2] to be suitable for white wines and was shown by Taylor, *et al.* (*loc. cit.*) to be unsuitable when applied as a routine procedure to all types of wines.

The Associate Referee therefore suggests that a tentative procedure for the determination of sulfur dioxide in wines be inserted in the forthcoming 5th edition of *Methods of Analysis* in the chapter on wines. In addition, it is suggested that the notation, "Especially adapted to wines," be deleted from the method referred to above (**XXXII, 33**, p. 441).

On the basis of experience in the determination of sulfur dioxide in wine, and because of the lack of collaborative studies on methods for this product, the Associate Referee also suggests that the procedure submitted be included in the chapter on wines in the forthcoming edition of *Methods of Analysis* and that further collaborative studies be conducted on this determination.

Acknowledgment is made to the following collaborators:

R. A. Osborn, U. S. Food and Drug Adm., Washington, D. C.
J. B. Thompson, State Laboratories, Bismarck, N. Dak.
P. P. Gray, Wallerstein Laboratories, New York City.
C. G. Ryberg, American Can Company, Maywood, Ill.

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, 23, 69 (1940).
[2] Food Inspection and Analysis, 4th ed., p. 898.

REPORT ON VOLATILE ACIDS IN DISTILLED SPIRITS

By G. F. BEYER (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.),
Associate Referee

It has been recognized for a long time that the methods used in determining total, volatile and fixed acids in whisky and other distilled spirits are unsatisfactory. It is difficult to obtain a good end point and the distillation of the volatile acids is either time-consuming or requires extensive and special apparatus. Therefore, the following methods and two samples of whisky were sent to 19 laboratories for collaborative study and comment.

(a) *Total Acids.*—Neutralize about 250 ml. of boiled distilled water in a porcelain evaporating dish (a 7½ inch dish was found to be convenient). Add 25.0 ml. of the sample to be tested and titrate with 0.1 *N* NaOH.

(b) *Fixed Acids.*—Evaporate 25–50 ml. of the sample to dryness in a platinum dish on a steam bath and dry for 1 hour in a constant temperature oven at 100° C. Dissolve and transfer the residue with several portions of neutralized alcohol of about the same proof as the sample, using 25–50 ml. in all, to the porcelain dish containing about 250 ml. of neutralized, boiled water. Titrate with 0.1 *N* NaOH, using a 10.0 ml. buret graduated in 0.05 ml.

(c) *Volatile Acids.*—Volatile acids = total − fixed acids.

The results obtained are quite consistent—considerably more so than when the present A.O.A.C. method, *Methods of Analysis, A.O.A.C.*, 1935, 170, is used. No mention was made of the indicator, as it was taken for granted that phenolphthalein would be used, since this is the indicator specified in the A.O.A.C. method. However, where any inconsistencies in the results occurred it is quite probable that this was the cause, as varying quantities of the indicator were used. The method should have called for 1.5 ml. of a 1 per cent solution of phenolphthalein. The same quantity of indicator should be used in titrating the fixed acids as well as total acids.

Several collaborators stated that they had difficulty in obtaining a satisfactory end point, while others said that the end point could be seen much more easily than when the present A.O.A.C. method is used. Some offered no comments whatever, while quite a number stated that the proposed methods were a decided improvement.

Further observations relative to the work on whisky show that in a large number of analyses the use of the modified Sellier tube consistently produced slightly lower results than did the proposed method. Another series of determinations was run on a standard sample made by evaporating a definite quantity of whisky to dryness and heating the residue for an hour at 100° C. in a constant temperature oven, dissolving the residue in neutral alcohol of the same proof as the whisky, adding a definite quantity of acetic acid, and making the solution to the original volume. Another sample was made in the same manner except that a definite

quantity of lactic acid was also added. A number of determinations of these samples show definitely that all the acetic acid is recovered or re-

Results of analyses for total, volatile, and fixed acids in whisky by various collaborators

COLLABORATOR	LABORATORY	SAMPLE 1 ACIDS AS ACETIC			SAMPLE 2 ACIDS AS ACETIC		
		TOTAL	VOLATILE	FIXED	TOTAL	VOLATILE	FIXED
		g./100 liters			*g./100 liters*		
O. B. Mahaffie	Pittsburgh, Pa.	52.4	43.1	9.4	62.4	51.2	11.2
G. W. Romig	New York, N. Y.	54.5	42.5	12.0*	66.5	51.5	15.0*
L. Burritt	Washington, D.C.	50.4	40.8	9.6	61.2	49.2	12.0
		50.4	40.8	9.6	61.2	49.2	12.0
G. F. Beyer	Washington, D.C.	51.6	42.0	9.6	61.7	48.7	13.0
P. Valaer	Washington, D.C.	50.4	40.8	9.6	60.0	48.0	12.0
		50.4	40.8	9.6	60.0	48.0	12.0
		50.4	40.8	9.6	61.2	47.5	13.7
E. J. Nealon	Detroit, Mich.	51.4	41.5	9.8	61.2	49.0	12.2
J. W. Fonner	Chicago, Ill.	49.2	40.4	8.8	58.5	47.1	11.4
G. D. Williams	St. Paul, Minn.	48.4	41.1	7.3	59.5	49.3	10.2
L. E. Dale	Kansas City, Mo.	50.2	43.7	6.5†	60.2	51.3	8.9
		49.7	42.5	7.2	60.2	51.3	8.9
A. J. Mottern	Philadelphia, Pa.	50.4	40.8	9.6	60.0	48.0	12.0
		50.4	40.8	9.6	60.0	48.0	12.0
		48.0	38.4	9.6			
A. L. Morawski	Boston, Mass.	51.1	41.1	10.0	61.2	49.1	12.1
C. T. Carson	Baltimore, Md.	52.8	43.2	9.6	62.4	49.2	13.2
J. B. Robb	Richmond, Va.	51.0	43.8	7.2	60.6	51.0	9.6
F. Smith	Richmond, Va.	50.4	43.2	7.2	60.0	50.4	9.6
		50.4	43.2	7.2	60.0	50.4	9.6
M. Rosenblatt	New York, N.Y.	50.8	42.4	8.4	61.3	49.6	11.7
		50.8	43.3	7.5	60.6	49.2	11.4
R. F. Love	San Francisco, Calif.	52.2	43.0	9.0	61.8	49.8	12.0
A. Herman	Louisville, Ky.	55.4	47.3	8.1	65.9	55.4	10.5
		55.1	47.7	7.4	64.2	53.2	11.0
		49.5	42.0	7.5	61.2	55.5	5.7†
					61.2	55.5	5.7†
G. E. Mallory	Los Angeles, Calif.	50.4	39.6	10.8	61.2	48.0	13.2
S. W. Holman	Atlanta, Ga.	63.0*	53.0*	9.4	61.2	51.0	10.2
		48.4	41.1	7.3			
J. W. Quillen	Baltimore, Md.	51.8	42.8	9.0	60.6	47.4	12.0
J. B. Wilson	Washington, D.C.	52.6	40.2	12.4	64.3	55.7	8.6
M. A. Joselyn	Berkeley, Calif.	52.4	43.1	9.33	60.1	48.2	11.95
C. S. Boruff	Peoria, Ill.	50.9	42.4	8.48	63.02	52.72	10.30
H. Walker's Research Lab.		51.3	43.0	8.3	62.6	51.7	10.9
Average		51.3	42.4	8.8	61.2	50.0	11.2
Maximum		55.4	47.7	10.8	66.5	55.5	13.2
Minimum		47.5	39.6	7.2	58.5	47.1	9.6

* Omitted from maximum.
† Omitted from minimum.

moved by the use of the Sellier tube and that none of the lactic acid distils over.

In view of these experiments it is quite apparent that there is present some acid, like lactic, that is not volatile with steam, but is volatile under the conditions prescribed in the proposed method. Since the term "volatile acids" ascribed to whisky does not necessarily mean volatile by steam distillation, the proposed method is considerably more simple than any steam distillation process, and since the residue from the solids determination for fixed acids may be used (which practically combines two operations into one), it is the opinion of the Associate Referee that the proposed methods offer considerable advantage over the present methods.

In connection with the length of time that the residue should be heated, several determinations were made by heating the solids from 15 minutes to 3 hours. The results indicate that the loss from 15 minutes to one hour was negligible, but that when the residue was heated 3 hours the loss was appreciable, probably due to some decomposition of organic matter. Therefore, the residue should not be heated over an hour, and preferably only 30 minutes.

The methods presented by the associate referee were adopted as tentative and will appear in the 1940 Edition of *Methods of Analysis, A.O.A.C.*, to issue about July 1, 1940.

REPORT ON ALDEHYDES IN WHISKY AND OTHER POTABLE SPIRITS

By PETER VALAER (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.), *Associate Referee*

While the determination of aldehydes in whisky, rum, brandy, and other potable spirits is not generally considered of the utmost importance, it is often made when the quality or the identity of the spirits is in question and a complete analysis is required. Its presence in any unusual quantity is not desirable because of the unpleasant taste it imparts to a spirit. In *This Journal*, 22, 222 (1939), there were discussed the objections to the present A.O.A.C. method and the need for a new one. The results of analyses of three samples, whisky, rum, and brandy, by 14 collaborators were given. While these results were considered rather good, it was decided to give the proposed method a more thorough test in 1939. To each of 18 laboratories there were sent two 1 pint samples labeled "A.O.A.C. Samples 1 and 2." With each pair of samples was sent a mimeographed copy of the proposed method of analysis. Tabulated results of the analyses accompany this report.

Comments on the method were requested, and some interesting and valuable suggestions were obtained.

Williams points out that the lengthy calculations are unnecessary, as all that is required is the difference between the two thiosulfate titrations, i.e., for the sample and for the blank, as the values for the iodine and the thiosulfate solutions are known or may readily be determined.

M. Rosenblatt states that his laboratory "would be more than gratified to see the method for aldehyde determination change from colorimetric to titrometric. In the former method the preparation and maintenance of standard aldehyde solution are never satisfactory, and the reproducibility of results from laboratory to laboratory is not good. The new method offers an absolute determination of aldehydes and depends on but one standard solution, which can be easily checked against a reliable primary standard. One preliminary test with this method indicated very good check results."

Nealon commends the method. He suggests a more condensed method of calculating the results of the aldehyde determination. A specimen is as follows:

	ml.
0.05 thiosulfate used	8.40
0.05 thiosulfate blank	4.65
	3.75

$3.75 \times 0.0011 \times 2 = .00824$ grams per 100 ml.
8.24 grams per 100 liters.

By this scheme it is only necessary to have an accurate factor for the thiosulfate solution, which is standardized against the 0.05 *N* dichromate solution, and an approximate 0.05 *N* iodine and bisulfite solution. This eliminates standardizing the iodine solution.

Romig found the method consistent. He experienced some bumping during distillation and suggested glass beads be used. Fonner suggests that the aldehydes be distilled slowly into a glass-stoppered flask in order to avoid the possible loss of distillate in transferring from one container to another. Morawski and Forbes, of the same laboratory, prefer the titration method. Mottern feels that better checks can be obtained by this method than by the present official one.

It was pointed out by Dale that there was some possibility of loss of aldehydes during distillation. He suggests that the receiver be immersed in a bath of ice-water during distillation as experiments indicate that more aldehydes are found in the chilled distillates.

Mallory states that very good agreement was obtained between the present A.O.A.C. method and the proposed method. He prefers the titration method and thinks it should be more accurate. Beyer found by experiment that slightly more aldehydes were always obtained when the receiver contained a portion of boiled distilled water. He also recommends the use of small pieces of carborundum to avoid any bumping during distillation.

Carson and Burham comment as follows:

Our distillations were carried out in a closed system equipped with standard taper joints and mercury valves. It has been suggested that it might be advisable

Collaborative results on aldehydes in whisky with proposed method

COLLABORATORS	LABORATORY	SAMPLE 1	SAMPLE 2
		g./100 liters as acetaldehyde	
O. B. Mahaffie	Pittsburgh, Pa.	6.64	9.50
Geo. W. Romig	New York, N.Y.	7.22	9.81
L. Burritt	Washington, D.C.	6.60	9.00
		6.60	8.80
		6.60	8.58
Geo. F. Beyer	Washington, D.C.	6.80	8.20
		6.80	8.20
Peter Valaer	Washington, D.C.	6.00	8.58
		6.16	8.58
E. J. Nealon	Detroit, Mich.	5.79	8.25
John W. Fonner	Chicago, Ill.	7.03	8.46
G. D. Williams	St. Paul, Minn.	7.81	10.45
		7.81	10.56
Lloyd E. Dale	Kansas City, Mo.	7.41	10.71
		7.50	10.65
A. J. Mottern	Philadelphia, Pa.	6.25	8.65
		6.66	8.49
		6.53	8.84
A. L. Morawski	Boston, Mass.	7.92	10.12
C. T. Carson	Baltimore, Md.	5.72	9.79
J. B. Robb	Richmond, Va.	6.07	8.78
		7.05	9.65
F. Smith	Richmond, Va.	6.61	9.32
		6.40	9.11
M. Rosenblatt	New York	7.00	9.70
		7.20	9.80
R. F. Love	San Francisco, Calif.	7.48	9.92
A. Herman	Louisville, Ky.	6.10	8.80
G. E. Mallory	Los Angeles, Calif.	7.92	9.79
S. W. Holman	Atlanta, Ga.	6.82	9.36
		7.33	
G. K. Hamill	Washington, D.C.	6.70	9.06
		6.90	9.15
A. Herman	Louisville, Ky.	8.01	10.89
		7.30	10.60
J. W. Quillen	Baltimore, Md.	6.60	8.80
J. B. Wilson	Washington, D.C.	5.51	8.61
Average		6.81	9.35
Maximum		8.01	10.89
Minimum		5.72	8.20
C. S. Boruff	Peoria, Ill.	8.75	12.10
		8.50	11.20
M. A. Joslyn	Berkeley, Calif.	8.10	11.34

to have the receiver held in a low temperature bath to avoid any loss of aldehyde. However, without taking this precaution we have had no difficulty whatever in obtaining perfect checks. We find the Ripper method to be much more satisfactory from the standpoint of obtaining concordant figures, and it requires less manipulation than the present fuchsin-sulfite method, which has been a source of continual annoyance to us in endeavoring to obtain comparative results between our different laboratories on the same samples. We find that our sodium thiosulfate and iodine reagents have remained quite stable over a period of several weeks.

Boruff comments as follows:

(1) The method proposed for the determination of aldehydes is definitely more satisfactory than the method in current use.

(2) In working with the proposed method for aldehydes in this laboratory, a considerable difference was observed in blank determinations made at intervals of several hours. This was believed to be a consequence of an unstable bisulfite solution. If this observation is accurate, it may be advisable to emphasize that blank determinations accompany each series of aldehyde determinations.

(3) The example given for the calculation of the aldehyde content of whisky appears needlessly involved.

(4) Aldehyde determinations were made on a series of 10 straight whiskies by the proposed method and by a modified colorimetric method. The results indicate that the aldehyde values obtained by the proposed method are quite consistently 3.3 (range 2.8–3.9) grams per 100 liters lower than those obtained with the use of the colorimetric method.

After careful consideration of the suggestions offered by the collaborators, the Associate Referee incorporated the following improvements in the method.

(1) Provision for small pieces of carborundum to be used during the distillation in order to avoid bumping.

(2) Immersion of the delivery tube in about 100 ml. of boiled distilled water during the distillation of the aldehydes.

(3) Provision for a receiving flask made of ground-glass and stoppered so that the transfer of the distillate is unnecessary.

The collaborators that obtained low and high results were requested to give the details and conditions of their analyses. It was found that most of the "lows" were obtained during the hot weather of July and August and most of the "highs" were submitted by those working during cooler fall weather and also by those using more elaborate precautions to trap and retain all the aldehydes present in the sample during distillation.

Inasmuch as the results of this two-year collaboration are considered good and have been endorsed by so many well-known and capable chemists, it is recommended[1] that this method be adopted as official, first action, but that the present A.O.A.C. method be retained. The method will be published in *Methods of Analysis, A.O.A.C.*, 1940, to issue about July 1st.

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[1] For report of Submittee D and action by the Association, see *This Journal*, 23, 70 (1940).

No report on detection of adulteration of distilled spirits was given by the associate referee.

No report on wood alcohol in brandy was given by the associate referee.

REPORT ON CORDIALS AND LIQUEURS

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

As recommended in last year's report, further collaborative work was done on the determination of benzaldehyde, volatile esters, and gamma-undecalactone. Imitation apricot and peach cordials, which may contain all three of these constituents, were analyzed.

In each case about 650 grams of dried fruit was chopped finely and macerated with 3 liters of dilute alcohol (1+1). After several days the liquid was drained off through a large funnel containing a cotton plug. A second extract was made by macerating the extracted fruit with 2 liters of dilute alcohol (1+1) in the same manner, and this was added to the first extract.

A sugar sirup was prepared by dissolving 5.9 kilograms of sucrose in 3.9 liters of water to make 7.6 liters of sirup. The sirup had a refractive index of 1.4423, corresponding to 60.2 per cent sucrose, sp. gr. 20°/20° 1.28991, and 77.65 grams of sucrose per 100 ml. Calculated from the quantities used, 5.9 kilograms of sucrose added to 7.6 liters of sirup make 77.63 grams of sucrose per 100 ml. of sirup.

A solution of artificial flavor was then made from the following ingredients:

	Grams
Oil of cognac	3.15
Amyl butyrate	5.74
Peach aldehyde	6.26
Benzaldehyde	3.16
Alcohol	q. s.
	2 liters

To determine the esters in the flavor solution, 50 ml. was added to 100 ml. of water in a 250 ml. flask and steam distilled. The distillate was neutralized and saponified with standard alkali. The solution was found to contain in 50 ml., 154.88 mg. of esters as ethyl acetate.

The cordials were then prepared from these ingredients in the proportions given in Table 1.

Last year's procedure was repeated. About 1 liter of each cordial was submitted to seven collaborators with instructions to determine alcohol, solids, benzaldehyde, esters, and gamma-undecalactone. The results of the collaborators are given in Table 2.

TABLE 2.—*Collaborative results on imitation apricot and peach cordials*

	BATTISTA	HAMILL	CHRISTENSEN	EDWARDS	SMITH	WHITING	WILSON	CALCULATED
				Apricot Cordial				
Alcohol by vol. (%)	32.05							
	33.0	32.96	30.42	32.12	32.92	32.44	33.08	32.95
Solids per 100 ml. (g.)	—	35.08	35.64	33.41	*	32.24	34.25†	35.3
Benzaldehyde per 100 ml. (mg.)	—	11.7	5.3	8.7	11.8	10.4	11.1	11.6
Esters as E. A. per 100 ml. (mg.)	33.7	25.8	23.9	23.5	29.04	23.3	21.1	21.7
	31.1		24.7		27.87		20.5	
Gamma-undecalactone per liter (mg.)	65	13.5	31.0	48	38	130	97	220
Qualitative	+	+	+	+	+	+	+	
				Peach Cordial				
Alcohol by vol. (%)	34.98	38.36	35.82	37.04	37.60	37.30	38.36	38.50
	35.15							
Solids	—	31.52	32.46	29.88	*	29.10	29.6†	31.5
Benzaldehyde per 100 ml. (mg.)	—	17.6	8.17	11.7	16.4	13.2	15.2	15.8
Esters as E.A. per 100 ml. (mg.)	33.3	35.4	29.9	33.3	33.7	34.3	29.6	31
	29.6		29.1		32.6		30.8	
Gamma-undecalactone per liter (mg.)	75	80	26.3	40	33	69	145	313
Qualitative	+	+	+	+	+	+	+	

* Smith found 33.11 in apricot and 28.49 in peach by **XVI, 37 (a)**.

† Wilson found 33.89 and 34.01 in apricot and 30.00 and 30.01 in peach by drying for 18 hours 5 ml. and 3 ml. portions on dried asbestos in a small dish in vacuo at 70°C.

The Associate Referee was informed that decimal points were misplaced in reporting last year's results for benzaldehyde by Burritt and Valaer of the Bureau of Internal Revenue. These chemists now report 20.3 mg. and 18.1 mg. of benzaldehyde in the apricot cordial and 15.9 mg. and 13.2 mg. in the peach cordial, respectively.

TABLE 1.—*Ingredients used in making imitation apricot and peach cordials*

	APRICOT *liters*	PEACH *liters*
Fruit extract	5.0	4.9
Sugar sirup	4.0	3.5
Artificial flavor solution	0.7	1.0
Alcohol	0.3	0.6
Total volume	10.0	10.0

In last year's report mention was made of the separation of thin films of oily matter on the surfaces of the two stock bottles of cordials used for analysis. These oily films were separated by transferring the remainder of the cordials to large separatory funnels. After being allowed to settle for some time, some of the underlying liquid was used to wash out the oily matter that had clung to the large bottles. The clear liquid was drawn off until only about 50 ml. remained, which quantity was extracted with ether. The ether was allowed to evaporate, the remaining oil was dissolved in alcohol and made to 100 ml., and esters were determined by steam distillation upon a 25 ml. aliquot. The results are given in Table 3.

TABLE 3.—*Volatile esters from cordials (1938 report)*

	ESTERS AS ETHYL ACETATE	
	APRICOT *mg.*	PEACH *mg.*
From entire sample (14 liters) (Undissolved)	335	434
From 100 ml. sample (calc.) (Undissolved)	2.4	3.1
Present 1938 report	31.1	38.8
Present 1938 samples corrected	28.7	35.7

The corrected values for esters in the cordials reported in 1938 bring the majority of determinations of collaborators within the limit of experimental error. The ester values reported this year are of about the same degree of accuracy as those submitted in 1938.

Except for two collaborators, who seem to have sustained a loss of benzaldehyde in both samples, the results for benzaldehyde and the averages (11.2 and 15.6) are nearer the calculated quantities than were the results in 1938, indicating improvement with increased familiarity with the method.

Results for gamma undecalactone show definitely that the method is suitable as a qualitative test, but that it cannot be depended upon for quantitative data in its present form.

The agreement obtained in the alcohol and solids determinations indicates that these procedures are worthy of official recognition.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for benzaldehyde in cordials, *Methods of Analysis, A.O.A.C.*, 1935, p. 183, **60–61,** be adopted as official (first action).

(2) That the method for volatile esters in cordials, p. 181, **57,** be adopted as official (first action).

(3) That the method for gamma-undecalactone (qualitative), p. 181, **47,** be adopted as official (first action).

(4) That the method for total solids, **(c)** *From the refractive index of the dealcoholized sample,* p. 180, **41(c),** be adopted as official (first action).

(5) That the method for specific gravity, p. 180, **35,** be adopted as official.

(6) That the method for alcohol, p. 180, **36,** be adopted as official (first action).

(7) That the method for glycerol, p. 180, **38,** be adopted as official (first action).

REPORT ON SOILS AND LIMING MATERIALS

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

Corrections and clarifications, and deletion of obsolete procedures and unnecessary duplications for Chapter I, in the forthcoming fifth revision of *Methods of Analysis, A.O.A.C.*, are provided by the following recommendations.[1] To facilitate consideration by the Committee, reasons for the several recommendations will be stated parenthetically.

2. PREPARATION OF SAMPLE

Substitute in **(a)** either "crush" or "disintegrate" for "reduce," in line 1. ("Reduce" connotes a chemical transition.)

Substitute "0.5 mm." for "1 mm.," line 3. (The change is prompted to bring conformity with general usage.)

3. MOISTURE

Substitute "Place 2 g of the prepared soil, **2(a),** in a wide-mouthed weighing bottle and heat at 105° in an electric oven for 5 hours." (This obviates effect of variation in altitude. An electric oven of the Freas type is available in practically every laboratory.)

Delete "taken," last word of line 3.

4. LOSS ON IGNITION

Delete "or suitable substitute," line 1. (It is being prescribed that the ignited charge and platinum crucible be used for the fusion directed under Section **14.**)

Delete "If soil contains appreciable quantities of carbonates. . . . NH_4 salts,"

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 23, 70 (1940).
[1] For report of Subcommittee A and action by the Association, see *This Journal*, 23, 52 (1940).

following the word "destroyed," line 2, and make the subsequent reading, "Cool in desiccator, weigh, and report percentage loss in weight as 'loss on ignition'." (The several reasons for this change are apparent.)

7. GRAVIMETRIC METHOD

Substitute "anhydrone" for "$CaCl_2$" at end of first sentence. (This is in harmony with general practice.)

Insert "with provision for condensation," following "suspension" in the footnote. (The experienced analyst would anticipate this need, but the student analyst probably would not.)

ORGANIC MATTER

10 & 11. Wet Oxidation Method

Delete this alternative. (It is unnecessary and uncertain in spite of many attempts to perfect the procedure for attainment of absolute results.)

13. Kjeldahl Method

Delete. (This method has been practically obsolete in soil chemistry laboratories for a number of years.)

14. SODIUM CARBONATE FUSION

Method I

Insert, "Mix residue from **4**, ground to 100-mesh, with 10 g of Na_2CO_3 in 30 ml. Pt. crucible. Cover crucible and heat at low redness until fusion begins; increase heat to a clear, quiet fusion, then give full heat of Meker burner 20 minutes with flame oblique. Cool the melt, place in 250 ml. porcelain evaporating dish, add water, and digest to disintegration on water bath. Cover dish, add 50 ml. of HCl, digest 15 minutes, and wash. Evaporate to dryness and bake for 2 hours at 110° C., or substitute $HClO_4$ for HCl and thereby obviate baking." (This change is advantageous and time saving.)

15. *Method II*

Delete. (This alternative produces an unwieldly bulk of silica. The charge requisite for the alternative procedure, **27**, is unnecessarily large and its solution gives an undue concentration of NaCl. The larger charge prescribed under Section **27** is essential, however, for "sulfur," which usually occurs in relatively small quantities in soils.)

16. SILICA

Delete "at 20° C.," line 8. (Reason for this is self-evident.)

Insert "with hot H_2O containing 5 ml. of HCl per liter," following "wash," line 6. (This stipulation is merely a repetition of the previously prescribed essential, which is equally essential at this point.)

17. OXIDES OF IRON, ALUMINUM, MANGANESE, PHOSPHORUS, AND TITANIUM

Insert in lieu of first two paragraphs the following:

To 250 ml. aliquot of **16**, add 10 ml. of concentrated HCl and few drops of methyl red indicator; heat to gentle boiling, add NH_4OH (1+1) until precipitate forms and color of solution changes to a distinct yellow. Boil no longer than 2 minutes and filter rapidly. Wash the precipitate 6–8 times with hot 2% NH_4NO_3 solution. Return precipitate and filter to original beaker, add 10 ml. of concentrated HCl; macerate filter by policeman, dilute with water, and heat to dissolve the precipitate; dilute to about 200 ml. and re-precipitate as before. Wash thoroughly with hot NH_4NO_3 solution until free of chlorides. Combine the first and second filtrates and save for Ca and Mg determinations.

Place precipitate in Pt crucible; dry and ignite over low flame of Bunsen burner until carbon has been oxidized. Heat to bright red for 10 minutes. Cool in desiccator and weigh in covered crucible as F_2O_3, Al_2O_3, P_2O_5 and TiO_2.

(This section has been the subject of considerable study and the directions are brought into conformity with the usage developed at the Bureau of Standards. The modified reading takes cognizance of the fact that the use of ammonium persulfate may result in the precipitation of calcium sulfate in the extracts of soils of appreciable calcium content. It is prescribed that 250 ml. aliquots be used. This will admit of duplicate determinations, but smaller aliquots would not give precipitates sufficient for the determination of Ca and Mg in some soils.)

Delete the last two paragraphs, beginning respectively with, "or, in lieu of . . . ," and "evaporate 50–100 cc. . . ." (The reason for this is apparent.)

18. CALCIUM

Modify third sentence to read, "Dissolve any precipitate remaining on filter by washing with hot HCl (1+9) into beaker containing main portion of decanted precipitate and then re-precipitate, boiling hot, etc."

(c) *Amend*, to read, "Perforate apex of cone; wash Ca oxalate precipitate into beaker used for precipitation; and then wash filter with hot H_2SO_4 (1+4) and titrate hot (85–90°) with 0.1 *N* $KMnO_4$."

19. MAGNESIUM
Method I

Delete. (This method is ideal when the solution to be metathesized is not heavily impregnated with non-volatile salts; but metathesis of the ammonium salts is not effected so well when considerable quantities of NaCl are also present. The present method of double precipitation is the one used most extensively and is prescribed by authoritative texts in dealing with solutions carrying considerable quantities of non-volatile solutes.)

20. *Method II*

REAGENT

Delete "and" between the words "cool" and "dilute," and insert phrase "and introduce 5 ml. of $CHCl_3$," following the present final word "liter." (This stipulation is made to preclude growth of mold in the reagent.)

21. DETERMINATION

Insert "2 ml. of M/1 citric acid," following the word "add," in the first line. (This is an obvious and accepted provision to retain in solution any Fe and Al that may have escaped previous precipitation, or that may have been introduced through reagents.)

Insert concluding sentence, "Correct weight of the $Mg_2P_2O_7$ for occluded $Mn_2P_2O_7$," as directed in **XXXVII, 75.** (This assurance against the recognized possibility of contamination through occlusions of manganese by heavy precipitates of Fe and Al, and also by reagents, is in harmony with the stipulation already "Official.")

22. MANGANESE
Method I

Insert, "Treat 1 g. of 100-mesh soil with 5 ml. of HF and 5 ml. of H_2SO_4 (1+1); evaporate and heat gently to dryness. Repeat addition of HF until all silicates are decomposed, etc." (A preliminary incineration of the soil at approximately 550° C. is adequate, and there is no necessity for ignition and fusion of the residue with $KHSO_4$.)

23 & 24. *Method II*

Delete **23** and **24.** (The alternative method is more tedious and is not generally used.)

NEW METHODS

The new methods submitted by the referee were adopted as tentative. They will be published in *Methods of Analysis, A.O.A.C.*, 1940, to issue about July 1, 1940.

REPORT ON H-ION CONCENTRATION OF SOILS OF ARID AND SEMI-ARID REGIONS

By W. T. McGeorge (Agricultural Experiment Station, Tucson, Ariz.), *Associate Referee*

The work of the former Associate Referee on H-ion Concentration of Alkaline Soils, *This Journal*, **19**, 256 (1936), showed that both the quinhydrone and antimony electrodes were unreliable for soils in this group. Previous investigations by the present Associate Referee, *Ibid.*, **21**, 246 (1938), showed that in alkaline soils there is a great increase in *p*H with increase in soil-water ratio. This is due to a hydrolysis of the potentially alkaline salts and minerals in the soil. The *p*H values thus obtained do not represent the *p*H of the soil under field conditions. In fact they represent conditions rarely if ever met in the field, and *p*H determinations on soils should be made at field moisture content when possible.

Since the hydrogen electrode is not suited to *p*H determinations at low soil-moisture contents a study of the glass electrode was made. This study has shown that it is well adapted to *p*H determinations at low moisture contents. On the basis of this investigation the following method is recommended[1] for the *p*H determination of alkaline soils.

METHOD

Weigh 20–25 grams of soil into a 50 ml. beaker and add boiled distilled water until the soil is soft enough to permit the ready penetration of the electrodes. (This moisture content is slightly above the moisture equivalent and well below the water-holding capacity of the soil. The mass may be stirred with a glass rod if desired to produce uniformity.) Gently tap the beaker and contents on the table top, press the glass electrode and its companion calomel electrode into the soil, and make the *pH* reading. Make several readings on each sample by withdrawing the electrodes and pressing them again into the soil mass. The first reading is often in error because equilibrium is not always attained by the first contact between the electrodes and the soil mass.

In collaboration with W. P. Martin, Associate Referee McGeorge also presents the following report.

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, 23, 53 (1940).

pH DETERMINATION OF ALKALI SOILS

By W. T. McGeorge and W. P. Martin (Agricultural Experiment Station, Tucson, Ariz.)

With the development of knowledge of the chemical properties of alkali soils and improvement in equipment and methods for determining hydroxyl-ion concentration, a need for standardization of methods used by soil laboratories interested in these soils has arisen. Such a standardization is imperative if comparisons are to be made among the several states where alkali soils prevail. The introduction of the glass electrode has done more to make this possible than any previous development in soil technology. A report of a former associate referee, P. L. Hibbard, *This Journal*, **20**, 256 (1935), has shown that the antimony electrode is not dependable. Also general experience has proved that the quinhydrone electrode will not give accurate results on alkali soils and that the hydrogen electrode is dependable only when certain essential precautions are taken. The glass electrode is dependable under practically all alkaline conditions and therefore the studies presented in this report were made with it.

In December 1936, the following questionnaire was sent to seven of the Western states:

1. H electrode or glass electrode?_____.
2. What soil-water ratio?_____.
3. CO_2-free distilled water?_____.
4. Air-dry or fresh soil?_____.
5. How long does soil-water mixture stand before determination?_____.
6. Are you satisfied with present method?_____.
7. What interpretation do you make of *p*H value?_____.
8. Do you consider the *p*H value as determined is in any way an indication of the *p*H value of the soil in the field?_____.

Six answers to the questionnaire were received. They are summarized as follows:

1. Four laboratories were using the glass electrode and two the hydrogen electrode.
2. Five laboratories were using a 1:5 soil-water ratio and 1 laboratory a 1:10 ratio.
3. Four laboratories were using carbon dioxide-free distilled water and two laboratories regular distilled water.
4. Three laboratories were using air-dry soil, two were using fresh soil, and one used either.
5. All laboratories were allowing the soil-water mixture to stand for different periods of time; namely, 12–18 hours, 2½ hours, 30 minutes, 15 minutes, 45 minutes and 1 hour.
6. Two laboratories expressed themselves as satisfied with their present method while four were only fairly well satisfied.
7. Varied interpretations of the *p*H determination were being made: (a) It aids in diagnosing the condition of the soil; (b) can predict general nature of soil

solution; (c) in doubt about the interpretation of *p*H data; (d) depends upon the nature of the investigation; and (e) used to measure degree of alkalinity.

8. The answers to this question varied as follows: (a) A fair approximation adequate for the type of soil examination being conducted; (b) yes, it checks usually within .2–.3 *p*H; (c) it certainly gives some indication concerning the *p*H value of the soil in the field, but it is not regarded as being highly exact; (d) yes; and (e) yes, roughly.

It is quite evident from the answers to this questionnaire that among these few states there is considerable variation in all phases of the procedure as well as in the interpretations deemed permissible.

At the 1938 meeting of the Western Society of Soil Science, a resolution was passed suggesting a study of the *p*H determination of alkali soils. As chairman of the committee that was appointed by this group, it has been possible for the Associate Referee to obtain the cooperation of most of the Western States, and therefore to make the rather extensive cooperative study that has been conducted during the past year.

Ten soils from widely separated sections of the West were collected. Their description and location are as follows:

	Moisture equivalent	*Total soluble salts (p.p.m.)*
1. Acid soil from Humbolt Co., California	28.5	255
2. Melbourne acid soil, Washington	37.7	250
3. Alkaline-calcareous soil, Tucson, Arizona	11.0	265
4. Black alkali soil, Tucson, Arizona	15.8	3,080
5. Alkaline soil from Kearney Park, Fresno, California	10.2	14,750
6. Alkaline soil from Wapato Indian Reservation, Yakima, Washington	30.1	3,625
7. Acid soil from Estes Park, Colorado	17.3	315
8. Approximately neutral soil, Logan, Utah	24.9	985
9. Alkaline soil from Montrose, Colorado	32.3	3,020
10. Alkaline soil from Vale, Oregon	23.8	1,510

These soils were carefully mixed, and portions of each were sent to laboratories in each of the eleven Western States, except where the quantity was not sufficient for eleven portions.

Investigations at the Arizona Station have shown that when tap water is used as a medium for the *p*H determination, its buffered condition reduces the hydrolysis of the potentially alkaline compounds to such an extent that the *p*H value obtained corresponds very closely to the *p*H of the soil at field-moisture content. In view of this finding it was requested that the collaborators use both boiled distilled water and tap water for the determinations. The following is a detailed outline of the instructions sent out with the sample:

(1) *Determination at field moisture capacity.*—Weigh 20 grams of soil into a 50 ml. beaker, add sufficient moisture to bring this content to approximately the moisture equivalent, cover with a watch-glass, and let stand for 5 hours under a bell jar or other means of protection from evaporation. Determine the *p*H by gently pressing glass and calomel electrodes into the soil and noting the *p*H value.

(2) *1:2 distilled water.*—Weigh 10 grams of soil into a stoppered bottle or flask. Add 20 ml. of boiled distilled water, shake intermittently for 1 hour, and determine *p*H.

(3) *1:2 tap water.*—Same as (2) except to use tap water instead of boiled distilled water. Allow water to run for 5 minutes from tap before drawing water for the determination.

(4) *1:5 distilled water.*—Same as (2) except to use 10 grams of soil and 50 ml. of boiled distilled water.

(5) *1:5 tap water.*—Same as (3) except to use 10 grams of soil and 50 ml. of tap water.

(6) *1:10 distilled water.*—Same as (2) except to use 10 grams of soil and 100 ml. of boiled distilled water.

(7) *1:10 tap water.*—Same as (3), except to use 10 grams of soil and 100 ml. of tap water.

It was suggested that care be taken to make the reading in a well stirred suspension and that the *p*H and total soluble salt contents of the tap water be determined.

Analyses were made by the following collaborators:

A. W. T. McGeorge.
B. R. H. Kellner, Arizona Agricultural Experiment Station, Tucson, Ariz.
C. P. L. Hibbard, University of California, Berkeley, Calif.
D. R. Gardner, Colorado Experiment Station, Ft. Collins, Colo.
E. F. T. Donaldson, Montana Agricultural Experiment Station, Bozeman, Mont.
F. T. J. Dunnewald, Wyoming Agricultural Experiment Station, Laramie, Wyo.

TABLE 1.—*pH values for different soils in both distilled and tap water, at various dilutions and after 1 hour and 24 hours, respectively*

SOIL-WATER RATIO	DISTILLED WATER				TAP WATER			
	1	2	3	4	1	2	3	4
After 1 hour								
1:1	8.2	8.1	8.1	8.7	8.0	8.0	8.0	8.6
1:5	8.7	8.6	8.8	9.6	8.2	8.1	8.0	9.1
1:10	8.9	8.8	9.1	9.9	8.2	8.1	8.0	9.1
1:20	9.2	9.1	9.2	10.1	8.2	8.1	8.0	8.8
After 24 hours								
1:1	8.2	7.8	8.0	8.5	8.1	7.8	7.9	8.4
1:5	8.5	8.3	8.4	9.4	8.2	8.0	8.0	8.9
1:10	8.5	8.4	8.4	9.7	8.2	7.9	7.8	8.9
1:20	8.7	8.6	8.7	9.9	8.1	8.0	8.0	8.7

G. W. L. Powers, Oregon Agricultural Experiment Station, Corvallis, Ore.
H. C. M. Keaton, Washington Agricultural Experiment Station, Pullman, Wash.

The data submitted by the collaborators are given in Table 2 for distilled water, and in Table 3 for tap water.

TABLE 2.—*pH values for different western soils as determined by analysts from several Western States, using distilled water with the glass electrode standardized against the same phosphate buffer solution.*

SOIL-WATER RATIO	SAMPLE NO.	ANALYST								*p*H* RANGE
		A	B	C	D	E	F	G	H	
Field moisture	1	4.70	4.50	—	5.30	4.76	5.06	5.19	4.64	0.80
	2	5.55	5.55	—	5.75	5.70	5.98	6.86	5.93	1.31
	3	7.65	7.60	—	7.25	7.69	7.85	8.15	6.74	1.41
	4	9.25	9.80	—	8.60	9.72	9.55	—	8.75	1.20
	5	9.45	9.35	—	9.55	9.65	9.70	10.41	8.20	2.21
	6	9.65	9.50	—	9.57	10.03	10.13	10.30	8.97	1.33
	7	5.15	5.00	—	5.25	—	—	—	—	0.25
	8	7.55	7.60	—	—	—	—	—	7.29	0.31
	9	8.85	—	—	8.95	—	—	—	8.10	0.85
	10	9.35	9.50	—	—	—	—	—	8.59	0.91
1:2	1	4.70	4.50	4.77	4.81	4.58	5.00	5.50	4.74	1.00
	2	5.50	—	5.56	5.54	5.43	5.85	6.37	5.70	0.94
	3	8.00	7.80	8.01	7.80	7.70	8.08	8.42	7.81	0.72
	4	10.25	10.35	10.40	10.40	10.18	10.35	—	9.27	1.13
	5	9.95	10.05	10.17	10.12	10.05	10.22	10.78	9.08	1.70
	6	10.40	10.50	10.47	10.62	10.22	10.53	11.02	9.62	1.40
	7	5.25	5.10	5.31	5.38	—	—	—	—	0.28
	8	7.70	7.50	7.71	—	—	—	—	7.43	0.28
	9	9.70	—	9.77	9.65	—	—	—	8.46	1.31
	10	10.05	—	—	—	—	—	—	9.25	0.80
1:5	1	4.85	4.70	4.88	4.91	4.73	5.00	5.61	4.55	1.06
	2	5.70	5.55	5.73	5.70	5.65	6.12	6.60	5.37	1.23
	3	8.20	8.20	8.28	8.00	7.76	7.87	8.40	7.86	0.64
	4	10.25	10.00	10.40	10.60	10.48	10.32	—	9.64	0.96
	5	10.10	10.20	10.38	10.35	10.25	10.23	10.91	9.53	1.38
	6	10.30	10.10	10.38	10.78	10.40	10.55	11.22	9.94	1.28
	7	5.45	5.30	5.64	5.52	—	—	—	—	0.34
	8	7.80	7.68	7.80	—	—	—	—	7.52	0.28
	9	9.75	9.68	9.77	10.14	—	—	—	9.24	0.90
	10	10.00	9.88	—	—	—	—	—	9.46	0.54
1:10	1	5.00	4.80	5.06	5.06	4.70	4.95	5.78	4.63	1.15
	2	5.70	5.50	5.65	5.80	5.90	6.15	6.60	5.24	1.36
	3	8.40	8.55	8.46	8.20	8.29	8.25	8.40	7.89	0.66
	4	10.20	10.10	10.67	10.68	10.49	10.35	—	9.73	0.95
	5	10.10	9.92	10.40	10.50	10.35	10.33	10.90	9.61	1.29
	6	10.35	10.18	10.38	10.71	10.60	10.62	11.17	9.94	1.23
	7	5.60	5.58	5.76	5.81	—	—	—	—	0.23
	8	8.00	7.90	8.00	—	—	—	—	7.63	0.37
	9	10.00	9.85	10.29	10.40	—	—	—	9.44	0.96
	10	10.30	10.40	—	—	—	—	—	9.61	0.79

* Difference between highest and lowest *p*H values.

TABLE 3.—*pH values for different western soils as determined by analysts from several Western States using the glass electrode, with tap water as the diluting medium*

SOIL-WATER RATIO	SAMPLE NO.	ANALYSTS								*p*H* RANGE
		A	B	C	D	E	F	G	H	
1:10	1	5.45	5.40	5.05	5.15	5.43	5.75	6.75	5.46	1.70
	2	6.30	5.82	5.74	5.80	6.00	6.34	7.30	6.05	1.56
	3	7.60	7.48	8.44	8.00	7.90	8.05	8.40	7.08	1.36
	4	9.05	9.15	10.70	10.45	9.85	10.10	—	8.34	2.36
	5	9.55	9.60	10.50	10.40	10.10	10.28	10.53	8.59	1.94
	6	9.65	9.70	10.94	10.70	10.30	10.45	10.51	8.81	2.13
	7	6.40	6.28	5.76	5.90	—	—	—	—	0.62
	9	8.80	8.90	10.36	9.80	—	—	—	—	2.36
	10	9.25	9.30	—	—	—	—	—	8.61	0.69
		*p*H of tap water used by each analyst								
		7.40	7.40	8.48	7.61	8.00	7.70	8.78	7.44	
		Solids in tap water (p.p.m.)								
		285	285	40	43	149	—	270	240	

* Difference between highest and lowest *p*H values.

RESULTS WITH TAP WATER

The request that the collaborators use both tap water and boiled distilled water as a medium for the soil suspensions was based upon the fact that the tap water would be more representative of field conditions for obviously distilled water is not used for irrigation, and when distilled water is used for the *p*H determinations the soils hydrolyze more completely. Also the tap water is slightly buffered and contains some soluble salts that appear to bring the soil-water mixture to equilibrium more rapidly. This is illustrated in Table 1.

The data in Table 1 show that there is little or no change in *p*H with variation in soil-water ratio when tap water (taken from Arizona Station laboratory) is used and also that the *p*H values are apparently constant over a period of 24 hours. On the other hand, when distilled water is used, there is a marked increase in *p*H with dilution and a change in *p*H value on standing for 24 hours. In this laboratory tap water has been used in many experiments, and the results show that the values obtained at 1:10 soil-water dilution approach very closely the values obtained at the moisture equivalent.

In Table 3, figures are given to show the *p*H and total soluble salt contents of the tap waters used by the various collaborators. It may be noted that they vary through rather wide limits. The soil *p*H values for tap water are also given in this table at a soil-water ratio of 1:10. The

values obtained at 1:2 and 1:5 soil-water ratios are omitted because, like the values obtained at the 1:10 ratio, they show that the use of tap water is entirely out of the question except for specific information in individual laboratories. The variation in *p*H is too great.

RESULTS WITH DISTILLED WATER

Variability of Results.—The *p*H determinations made with boiled distilled water (Table 2) show rather wide variation among the eight analysts. Whether this results from faulty technic or from sources of error inherent in the use of the glass electrode is a matter for further study. The greatest variation occurs in the data submitted by Analysts G and H. The *p*H values submitted by Analyst G are quite uniformly too high, while those submitted by Analyst H are often too low. In order to determine whether or not the extreme variation in the results of these two analysts was from a constant source of error, the deviations from the sample means (calculated from the data submitted by Analysts A to F inclusive) were determined.

It was found that the results reported by Analyst G are, on an average, 0.66 *p*H units too high, with a standard deviation of only 0.24 (the usual variation for the data submitted by Analysts A to F is approximately equal to this, the average standard deviation being 0.20 *p*H units). The data of Analyst H average −0.48 units too low, with the large standard deviation of 0.36. The results reported by Analyst H are much more variable than are those of Analyst G.

Corrected values for Analysts G and H are given in Table 4. Also included in this table is the mean *p*H value for Analysts A to F. Whenever the corrected *p*H value is outside the range of the mean, plus or minus the average standard deviation for Analysts A to F, it is starred. It will be noted that only 4 of the corrected data for Analyst G are outside this range, whereas 15 of Analyst H's samples are outside.

It may be concluded, therefore, that the high results reported by Analyst G are caused by a more or less constant source of error, but that those reported by Analyst H cannot be explained in this manner. In all subsequent analyses, therefore, the writers feel justified in eliminating from consideration the data submitted by Analyst H. The corrected values for Analyst G, however, are used.

The average standard deviation for the different samples is 0.189 *p*H units. A *p*H range equivalent to twice the standard deviation, or approximately 0.4 *p*H units, includes most of the samples. This is the usual variation between analysts, but the writers believe that it is too great. A single analyst can narrow his range to approximately 0.2 *p*H units. These data definitely indicate that there is need for further collaborative work and standardization of the glass electrode method for alkaline soils

so that the results reported by analysts from different laboratories will not vary through a range greater than 0.2 *p*H units.

After the data had been tabulated they were sent to all collaborators for comments. Analyst H attributed his low results to the presence of a mold growth in the phosphate buffer sent with the soil samples, but this does not account for his lack of uniformity. Analyst E suggests that closer attention to time and temperature may decrease the error, but also

TABLE 4.—*Corrected pH values for Analysts G and H, together with mean values for Analysts A to F inclusive*

SOIL-WATER RATIO	SAMPLE NO.	MEAN pH VALUE ANALYSTS A TO F	ANALYST†	
			G	H
Field moisture	1	4.890	4.55*	5.12*
	2	5.706	6.20*	6.41*
	3	7.610	7.49	7.22*
	4	9.380	—	9.23
	5	9.540	9.75	8.68*
	6	9.780	9.64	9.45*
1:2	1	4.730	4.86	5.22*
	2	5.576	5.71	6.18*
	3	7.900	7.76	8.29*
	4	10.320	—	9.75*
	5	10.090	10.12	9.56*
	6	10.457	10.36	10.10*
1:5	1	4.840	4.95	5.03
	2	5.740	5.94	5.85
	3	8.050	7.74*	8.34*
	4	10.340	—	10.12*
	5	10.250	10.25	10.01*
	6	10.420	10.56	10.42
1:10	1	4.930	5.12	5.11
	2	5.780	5.94	5.72
	3	8.358	7.74*	8.37
	4	10.415	—	10.21*
	5	10.270	10.24	10.09
	6	10.470	10.51	10.42

* *p*H outside range $m \pm$ Av. St. Dev. (=0.20) for Analysts A to F.
† Recorded values obtained by subtracting 0.66 from data submitted by Analyst G and by adding 0.48 to those submitted by Analyst H.

suggests the possibility of errors in technic. Analyst D suggests time, failure to clean the electrodes, or a neglect to attain proper equilibrium after changing the glass electrode from one sample to another as possibilities. Analyst C suggests that temperature differences and variations in electrode bulbs may affect the results. Analyst A points out that failure to attain proper equilibrium at the recorded time of reading, particularly

TABLE 5.—*pH values for different alkaline soils as determined by analysts from several Western States using the glass electrode standardized against the same phosphate buffer solution*

SOIL-WATER RATIO	SAMPLE NO.	ANALYST							MEAN pH VALUE	STANDARD DEVIATION	STANDARD ERROR
		A	B	C	D	E	F	G†			
Field moisture	3	7.65	7.60	7.55*	7.25	7.69	7.85	7.49	7.583	0.185	0.070
	4	9.25	9.80	9.39*	8.60	9.72	9.55	9.40*	9.387	0.398	0.150
	5	9.45	9.35	9.59*	9.55	9.65	9.70	9.75	9.577	0.142	0.054
	6	9.65	9.50	9.77*	9.57	10.03	10.13	9.64	9.756	0.238	0.090
1:2	3	8.00	7.80	8.01	7.80	7.70	8.08	7.76	7.879	0.147	0.056
	4	10.25	10.35	10.40	10.40	10.18	10.35	10.29*	10.317	0.092	0.035
	5	9.95	10.05	10.17	10.12	10.05	10.22	10.12	10.097	0.092	0.035
	6	10.40	10.50	10.47	10.62	10.22	10.53	10.36	10.443	0.128	0.048
1:5	3	8.20	8.20	8.28	8.00	7.76	7.87	7.74	8.007	0.225	0.085
	4	10.25	10.00	10.40	10.60	10.48	10.32	10.37*	10.346	0.190	0.072
	5	10.10	10.20	10.38	10.35	10.25	10.23	10.25	10.251	0.095	0.036
	6	10.30	10.10	10.38	10.78	10.40	10.55	10.56	10.439	0.216	0.082
1:10	3	8.40	8.55	8.46	8.20	8.29	8.25	7.74	8.270	0.264	0.100
	4	10.20	10.10	10.67	10.68	10.49	10.35	10.36*	10.407	0.222	0.084
	5	10.10	9.92	10.40	10.50	10.35	10.33	10.24	10.263	0.195	0.074
	6	10.35	10.18	10.38	10.71	10.60	10.62	10.51	10.479	0.184	0.069

* Estimated values calculated by Yates' missing plot technic, *Empire J. Expl. Agr.*, **1**, 129–142 (1933).
† *p*H values in this column 0.66 *p*H units less than those reported. Correction necessitated by analysts' use of distilled water from still recently cleaned with HCl.

at the moisture equivalent, often leads to erroneous results. He found it necessary to withdraw the electrodes from soils at the moisture equivalent and to press them again into the soil several times before the readings became constant. This source of error was suggested because his data represent two sets of analyses made at two periods of time nearly 4 months apart, during which time the original Beckman *p*H meter had been returned to the factory and made over to the new Model G instrument with shielded electrodes. The two sets of data are in very close agreement.

Differences between Analysts and between Soil-Water Ratios.—It was thought desirable to submit the data to a statistical analysis to determine whether or not significant differences exist between the values obtained at the different soil-water ratios when boiled distilled water was used for the determination and whether or not the results submitted by the different analysts are significantly different from one another. Inasmuch as acid soils and alkaline soils react differently on dilution, each group was analyzed separately. Since it was not possible for every collaborator to analyze all the soil samples, it was not possible to use all the data in the statistical analyses. Therefore, the samples that had been analyzed by most of the collaborators were chosen. Missing values were estimated by the method of Yates (see Table 5).

Alkali Soils.—The data used for the alkaline soils are given in Table 5, together with the mean *p*H values, standard deviations, and standard errors. It may be noted that the standard deviations, with one or two exceptions, do not vary appreciably at the different soil-water ratios. Accuracy of measurement, therefore, seems to be independent of the ratio of soil to water used.

The data in Table 5 were submitted to an analysis of variance according to the method of Fisher, as modified by Snedecor.[1] The results of this analysis are given in Table 6.

TABLE 6.—*Analysis of variance of pH values for different alkaline soils as determined by analysts from several Western States. Various soil-water ratios used.*

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between means of samples	3	
Between means of analysts	6	0.0750*
Between means of soil-water ratios	3	3.4761†
Interactions:		
Analyst:soil-water ratios	18	0.0677
Analyst:samples	18	0.0549
Samples:soil-water ratios	9	0.1102†
Error	46	0.0320
Total	103‡	

* Significant. † Highly significant.
‡ Deduction of 8° of freedom for estimated values.

[1] R. A. Fisher, Statistical Methods for Research Workers, Chap. VII, Oliver and Boyd. London (1936)

Variation with Analysts.—The variance between means of analysts is significant. The least significant mean difference is 0.135 *p*H units. The mean *p*H values for the different analysts are as follows:

Analyst:	A	B	C	D	E	F	G
Mean *p*H:	9.531	9.512	9.669	9.608	9.616	9.683	9.536

It may be noted that the mean *p*H values for Analysts A, B, and G are significantly lower than those for Analysts C and F. The mean *p*H values for Analysts D and E are not significantly different from the others. The greatest mean difference shown is between Analysts B and F and is equal to 0.17 *p*H units. It is interesting that the differences between the results reported by the different analysts should be within an average *p*H range of only 0.17 *p*H units and yet be so consistent that a statistically significant variance was obtained. This would seem to indicate that differences in technic rather than carelessness were operative at the different laboratories, with resulting consistency, otherwise more variable results would be expected. If the method can be standardized to the point where such variations are not possible, closer agreement between the results reported from the different laboratories may be possible.

Variation with Soil-Water Ratios.—The variance between means of the *p*H values at the different soil-water ratios is highly significant. The mean *p*H differences at the various soil-water ratios are given in Table 7. The average apparent increase in *p*H of the 4 samples at a soil-water ratio of 1:2, as compared with the values obtained when the moisture content was near the moisture equivalent, is the large figure of 0.608 *p*H units. The values determined at a soil-water ratio of 1:5 are not significantly greater than those determined at 1:2, and those determined at 1:10 significantly greater than those at 1:5. The two exceptions to this are Sample 5, whose *p*H at 1:5 soil-water ratio is significantly greater than that at 1:2, and Sample 3, whose *p*H increased significantly at each of the soil-water ratios.

It is quite apparent, therefore, that if *p*H determinations made at different laboratories on alkaline soils are to be properly comparable, the soil-water ratios at which the determinations are made must be standardized. McGeorge[2] considers the true *p*H of a sample to be the one that is made at or near the moisture content found in the field (corresponding to the determinations made at field moisture content). The standard deviations of the samples (see Table 5) show that no more difficulty was experienced in obtaining accurate results at this moisture content than at the higher soil-water ratios. A method that would determine *p*H values at field moisture conditions and at the same time be subject to variations no greater than 0.2 *p*H units would be highly desirable. Therefore it is recommended that further collaborative work be done.

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[2] *J. Am. Soc. Agron.*, 29, 841–844 (1937).

TABLE 7.—*Difference between mean pH values for some alkaline soils at various soil-water ratios*

SOIL-WATER RATIO	SAMPLE NO.	MEAN pH VALUE	MEAN pH DIFFERENCES FOR FOLLOWING SOIL-WATER RATIOS			
			FIELD MOISTURE	1:2	1:5	1:10
Field moisture	3	7.583	0	—	—	—
	4	9.387	0	—	—	—
	5	9.577	0	—	—	—
	6	9.756	0	—	—	—
1:2	3	7.879	0.296†	0	—	—
	4	10.317	0.930†	0	—	—
	5	10.097	0.520*	0	—	—
	6	10.443	0.687†	0	—	—
1:5	3	8.007	0.424†	0.128*	0	—
	4	10.346	0.959†	0.029	0	—
	5	10.251	0.674†	0.154†	0	—
	6	10.439	0.683†	−0.004	0	—
1:10	3	8.270	0.687†	0.391†	0.263†	0
	4	10.407	1.020†	0.090	0.061	0
	5	10.263	0.686†	0.166†	0.012	0
	6	10.479	0.723†	0.036	0.040	0

* Significant mean difference.
† Highly significant mean difference.

TABLE 8.—*pH values for three acid soils as determined by different analysts using the glass electrode standardized against the same phosphate buffer solution*

SOIL-WATER RATIO	SAMPLE NO.	ANALYST				MEAN pH VALUE
		A	B	C	D	
Field moisture	1	4.70	4.50	4.79*	5.30	4.822
	2	5.55	5.55	5.77*	5.75	5.655
	7	5.15	5.00	5.25*	5.25	5.162
1:2	1	4.70	4.50	4.77	4.81	4.695
	2	5.50	5.53*	5.56	5.54	5.532
	7	5.25	5.10	5.31	5.38	5.260
1:5	1	4.85	4.70	4.88	4.91	4.835
	2	5.70	5.55	5.73	5.70	5.670
	7	5.45	5.30	5.64	5.52	5.478
1:10	1	5.00	4.80	5.06	5.06	4.980
	2	5.70	5.50	5.65	5.80	5.662
	7	5.60	5.58	5.76	5.81	5.688

* Estimated values calculated by Yates' missing plot technic, *Empire J. Exptl. Agr.*, 1, 129–142 (1933).

The highly significant interaction between the samples and the soil-water ratios indicates that the samples reacted differently with dilution. This interaction probably resulted from the fact that the *p*H of Sample 3 increased progressively with dilution, whereas the significant difference in the case of the other samples was between the *p*H values obtained at field moisture content and those obtained at a 1:2 soil-water ratio.

Acid Soils.—The samples used for the analysis of variance in the case of the acid soils are given in Table 8. As before, it was thought desirable to determine whether or not significant differences existed between the values reported by different analysts and at different soil-water ratios. The analysis of variance of the data is given in Table 9.

TABLE 9.—*Analysis of variance of pH values for different acid soils as determined by various analysts. Several soil-water ratios used*

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
Between means of samples	2	
Between means of analysts	3	0.1639*
Between means of soil-water ratios	3	0.1883*
Interactions:		
Analyst:soil-water ratios	9	0.0084
Analyst:samples	6	0.0104
Samples:soil-water ratios	6	0.0514*
Error	14	0.0103
Total	43†	

* Highly significant.
† Deduction of 4 degrees of freedom for estimated values.

Variation with Analyst.—Here again the difference between analysts is highly significant. The mean *p*H values for the different analysts are as follows:

Analyst:	A	B	C	D
Mean *p*H:	5.262	5.134	5.347	5.402

The least highly significant mean difference is 0.13 *p*H units. The *p*H values submitted by Analyst B are significantly lower than those submitted by the other three analysts; those submitted by Analyst A are significantly lower than those submitted by Analyst D; the other differences are not significant.

Variation with Soil-Water Ratios.—The variance between means of soil-water ratios is highly significant. The differences between the mean *p*H values at the various soil-water ratios are given in Table 10. While the *p*H differences at the various ratios are often significant, these soils, with dilution, did not act like the alkaline soils and the differences are not so great. For Sample 7, the *p*H increased progressively with dilution. The *p*H values at a 1:5 ratio are significantly higher than those deter-

mined at either the field moisture content or at a soil-water ratio of 1:2. Again, those determined at a ratio of 1:10 are significantly higher than those at 1:5.

TABLE 10.—*Difference between mean pH values for some acid soils at various soil-water ratios*

SOIL-WATER RATIO	SAMPLE NO.	MEAN pH VALUE	MEAN pH DIFFERENCES FOR FOLLOWING SOIL-WATER RATIOS			
			FIELD MOISTURE	1:2	1:5	1:10
	1	4.822	0	—	—	—
	2	5.655	0	—	—	—
	7	5.162	0	—	—	—
1:2	1	4.695	−0.127*	0	—	—
	2	5.532	−0.123*	0	—	—
	7	5.260	0.098	0	—	—
1:5	1	4.835	0.013	0.140†	0	—
	2	5.670	0.015	0.138†	0	—
	7	5.478	0.316†	0.218†	0	—
1:10	1	4.980	0.158†	0.285†	0.145†	0
	2	5.662	0.007	0.130†	−0.008	0
	7	5.688	0.526†	0.428†	0.210†	0

* Significant mean difference. † Highly significant mean difference.

Samples 1 and 2, however, did not behave like Sample 7. When *p*H was measured at a soil-water ratio of 1:2 a significant decrease over that measured at field moisture content was noted. At progressively higher dilutions, however, the differences are positive and generally are highly significant. The most consistent difference for all the samples occurs between the *p*H values obtained at a soil-water ratio of 1:2 and those determined at 1:5. In no case, however, is the difference as large as the 0.609 *p*H difference between the alkaline soils at field moisture and those at a soil-water ratio of 1:2 or higher. The average difference here is but 0.165 *p*H units.

It may be concluded, therefore, that while significant differences may exist in the *p*H values obtained on acid soils, they are not so large as those obtained with alkaline soils on dilution, and they are more variable. In either case, however, *p*H determinations made at different ratios of soil to water are not comparable and standardized methods for the determination of the soils' *p*H must specify a given soil-water ratio. The writers believe that inasmuch as equally accurate results can be obtained at low moisture contents, conditions as they exist in the field should be simulated as closely as possible so that the proper ratio of soil to water for the determinations should be at or near the field moisture content.

SUMMARY AND CONCLUSIONS

Ten soils from widely separated sections of the West were collected and sent to seven collaborators in several of the Western States. It was specified that the glass electrode be used for the *p*H determinations and that each instrument be standardized against the buffer solution supplied with the samples. One series of soils was run with boiled distilled water as the diluting liquid and another series was run with ordinary tap water. The following dilutions were specified: field moisture (approximately equal to the moisture equivalent) and soil-water ratios of 1:2, 1:5, and 1:10.

Work in the Arizona Station laboratory indicates that little change in *p*H occurs with variations in soil-water ratio when tap water is used and also that the *p*H values are constant over a period of 24 hours. When distilled water was used, however, there was a marked increase in *p*H with dilution and a change in *p*H value on standing for 24 hours. Tap waters were so variable from station to station, however, that extremely wide variations in the *p*H values submitted by various analysts were obtained. Tap water, as a consequence, is entirely out of the question except when specific information may be needed at an individual laboratory.

The average standard deviation for the different samples, about 0.2 units, is believed to be too great. A more satisfactory figure would probably be 0.1 *p*H units.

The soils were divided into two groups, alkaline soils and those on the acid side of neutrality. They were submitted to an analysis of variance to determine whether or not significant differences exist between the results submitted by different analysts at a fixed soil ratio and those at the different soil-water ratios.

In both the acid and the alkaline soils it was found that significant differences did exist between the results obtained by the different analysts. The greatest difference in the mean *p*H values is but 0.17 *p*H units. Apparently different technics were used at the different laboratories.

The variance between means of *p*H values at different soil-water ratios is also highly significant. In the case of the alkaline soils the average apparent increase in *p*H at a soil-water ratio of 1:2, when compared with the values obtained when the moisture content was near the moisture equivalent, is 0.609 *p*H units. With one or two exceptions, little change in *p*H is shown at higher dilutions.

In the case of the acid soils, the differences, though significant, are not so large as those with the alkaline soils. The most consistent and highly significant difference was between the *p*H values obtained at a soil-water ratio of 1:2 and those obtained at a dilution of 1:5. The average difference between these two dilutions is but 0.165 *p*H units.

RECOMMENDATIONS[1]

The significant variation in results, with dilution, emphasizes the need for specification of the ratio at which all *p*H determinations are made.

The first definite application of *p*H values in soil science is the practical use, namely as a measure of the actual hydrogen- or hydroxyl-ion concentration of the soil solution at field moisture content. The second is in soil research where the *p*H determinations are used to study the relationship of hydrogen- and hydroxyl-ion concentration to soil behavior under any and all conditions. For practical use of *p*H values there is urgent need for a standard, uniform method, and the study of *p*H determinations at low moisture content will be continued during the coming year.

REPORT ON HYDROGEN-ION CONCENTRATION OF SOILS OF HUMID REGIONS

By E. R. PURVIS (Virginia Truck Experiment Station, Norfolk, Va.), *Associate Referee*

The choice between the glass and quinhydrone electrodes for determining the hydrogen-ion concentration of soils of the humid regions involves a decision regarding two instruments, both of which have proved quite satisfactory under average conditions. Table 1 presents the results

TABLE 1. *Comparison of glass and quinhydrone electrodes for determining the pH value of coastal plain soils*

METHOD	*p*H RANGE			
	<5.5	5.5–6.0	6.0–6.5	>6.5
Glass electrode*	4.99	5.78	6.31	6.88
Quinhydrone electrode*	5.06	5.79	6.21	6.82
Number Variations > .2 *p*H	3	6	4	2

* Mean values for 40 soils within each *p*H range.

obtained in a comparative study of the two electrodes on 160 representative coastal plain soils of the Norfolk, Sassafras, and related series. In these determinations a 1:1 soil-water ratio with distilled water was used. It is believed that the variations in tap water due to source and treatment make it unreliable for use with poorly buffered soils. The voltage measurements were made with a Coleman electrometer in case of the glass electrode, and with a Leeds and Northrup potentiometer for the quinhydrone electrode.

Although the mean values obtained with the two electrodes for all *p*H

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 23, 53 (1940).

ranges were sufficiently concordant for all practical purposes, it will be noted that in 15 instances the variation was greater than .2 of a *p*H unit. The soils showing this significant variation were distributed throughout the four ranges studied. However, it was noted that in the lower *p*H ranges the glass electrode tended to give lower values, while the reverse was true in the higher ranges. No attempt was made to determine the cause of the variation in these samples, but it was noted that with most of them there was a tendency to drift when the quinhydrone electrode was used. For this reason it is considered that the glass electrode determinations on these samples are more accurate.

Since the glass electrode is not affected by the presence of oxidizing or reducing substances and is less subject to electrode poisoning, factors that are occasionally encountered in humid acid soils, its selection over the quinhydrone electrode would seem to be warranted. Especially is this true in those laboratories where *p*H determinations are made on large numbers of soil samples taken from environmental conditions that are unknown to the operator.

The question of the proper soil-water ratio for determining the *p*H of soils is an ever-occurring one. To investigate this point with reference to soils of humid regions, eight representative soils were chosen, and soil-water ratios of 5:1, 1:1, and 1:5 were used. In all cases 50 grams of soil was used and the suspension was permitted to stand for 30 minutes, with occasional stirring. The results obtained are presented in Table 2. To

TABLE 2.—*Effect of soil-water ratio on glass electrode values*

SOIL TYPE	LOCATION	ORGANIC CARBON	EXCHANGE CAPACITY	*p*H AT VARIOUS SOIL-WATER RATIOS		
				5:1	1:1	1:5
		per cent	*M.E.-100 gram.*			
Cecil sandy loam	S.C.	.71	2.9	5.50	5.45	5.80
Kempsville sandy loam	Va.	.77	3.9	4.90	4.85	5.10
Sassafras sandy loam	Va.	.61	4.1	5.95	5.85	6.10
Norfolk fine sandy loam	Va.	.53	6.7	5.40	5.35	5.70
Portsmouth fine sandy loam	N.C.	2.34	9.2	7.75	7.75	8.00
Crosby silt loam	Ind.	1.09	9.5	5.45	5.55	5.75
Lackawana silt loam	N.Y.	3.18	12.5	6.60	6.60	6.90
Hermon fine sandy loam	N.H.	4.38	15.2	5.00	4.95	5.20

show the variability of the chemical properties of the soils studied, the exchange capacities and organic carbon contents are included in the table.

The results of this study show that practically identical values were obtained in the 5:1 and 1:1 soil-water suspensions. With the 1:5 suspension significantly higher *p*H values were obtained for all soils. Whether this was due to the presence of soluble salts or acids or to low buffer capacity within the ranges studied was not determined. However, since

the 1:1 soil-water ratio is entirely satisfactory with the sturdier types of glass electrodes now in use, and since the results obtained with this ratio closely approximate those obtained at field moisture content, it is recommended that this ratio be adopted as standard for soils of the humid regions.

Based upon the studies reported here and upon similar studies reported in the literature on the subject, it is recommended[1] that the following procedure be used for the determination of the hydrogen-ion concentration of humid soils.[2]

To 50 grams of fresh soil add 50 ml. of distilled water and let stand for 30 minutes, with intermittent agitation. Determine the reaction value by the electrometric method, using a glass electrode of either the thin wall or penetration type. The voltage or *p*H reading should be made as soon as the potentiometer registers equilibrium.

REPORT ON LIMING MATERIALS

DETERMINATION OF EXCHANGE BASES AND EXCHANGE CAPACITY OF SOILS

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

At its 1936 meeting, the Association directed study of methods for the determination of exchangeable calcium and magnesium in soils. In 1937 the Associate Referee on Liming Materials initiated and reported a collaborative study of boiling ammonium chloride for the complete extraction of soil bases, occurrent in the absorbed state and as carbonates, *This Journal*, **21**, 252 (1938). The results showed that soils limed many years had accumulated absorbed calcium far in excess of the total exchange capacity indicated by the ammonia absorption from either neutral chloride or acetate. It was also shown that variable quantities of the ammonia radical were retained by soils after being leached with the neutral salt, depending on whether or not the alcoholic wash used was adjusted with ammonia.

Further work, *Ibid.*, **22**, 242 (1939), led to the use of a current of steam to expedite the digestion procedure for soil bases. The dissolution of calcium carbonate in soils was speeded to completion in about 10 minutes' digestion, whereas the complete solution of dolomite requires digestion for about 45 minutes. It was shown that concordant and accurate determinations of the absorbed magnesium in soils can be made by this extraction procedure when manganese is eliminated and 2 ml. molar solution of ammonium citrate is added before the precipitation of the magnesium ammonium phosphate. It was also shown that the customary use of

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **23**, 53 (1940).

[2] The quinhydrone electrode gives satisfactory results with soils having a reaction value below *p*H 6.0. The various colorimetric methods may be used for approximate quantitative determinations.

magnesium oxide in the distillation of the ammonium from clay soils gives low results in the exchange capacity determination by ammonium absorption from neutral salt treatment, whereas limited concentrations of sodium hydroxide proved expeditious and satisfactory for this purpose.

During the past year the work of the Associate Referee was devoted mainly to a number of details connected with the determination of the exchange capacity of soils by ammonia absorption. To obtain more detailed information, the Associate Referee addressed a questionnaire to a number of workers that have been identified with this type of work at the various experiment stations, and the replies have facilitated greatly the formation of this report. The experimental work is presented in relation to the topics proposed in the questionnaire.

EXCHANGEABLE CALCIUM AND MAGNESIUM IN CALCAREOUS SOILS

A normal ammonium acetate procedure was proposed by Schollenberger,[1] and, with various modifications, is used extensively. Concern for dissolved calcium carbonate or magnesium carbonate, as distinguished from absorbed bases, was shown in only half of the replies. The others expressed no interest in such differentiation, either because of interest solely in acidic soils or because the carbonates and absorbed calcium and magnesium were considered in the same category, with respect to availability. The procedure generally used for distinguishing between calcium dissolved from carbonates and that dissolved from the absorption complex, is to determine the soil's carbonate content before and after the ammonium acetate treatment. This procedure is open to objections as to principle and expediency. Calcium and magnesium replacement will be incomplete so long as these ions are being dissolved by the replacing agent and their solutes continute to bathe the soil mass. This condition will affect adversely determination of both absorbed base and exchange capacity by ammonia absorption. On the other hand, the technic of carbonate determination after ammonium acetate treatment of the soil is not well defined. If the entire extracted residue is used for the carbonate determination, it will be necessary to make another extraction for the exchange capacity determination. Any replication of soil extraction will require replication of carbonate determinations also on residues, since the quantities of carbonates dissolved by the ammonium acetate treatment will not be identical in any two such determinations. The alternative procedure of dividing the extracted residue and using one part for carbonate and the other for ammonia distillation is not conducive to accurate sampling, and is not feasible for clays or clayey soils. Mere leaching with ammonium acetate will not yield satisfactory results for either supplies of available calcium and magnesium or for exchange capacity.

[1] *Soil Sci.*, 30, 161–173 (1930).

The results of Table 1 show solvent action of ammonium acetate treatment on 10 gram soil charges, in comparison with the sum of dissolved calcium and magnesium by the boiling digestion with ammonium chloride on a number of limestone- and $MgCO_3$-treated soils. These results indicate that the ammonium acetate is capable of extracting 2–3 per cent calcium carbonate or magnesium carbonate from a 10 gram charge of soil by 150 ml. leaching on Büchner filter, subsequent to overnight shaking with 100 ml. These quantities were much less than the total available content of calcium or magnesium, as shown by amounts dissolved by the boiling ammonium chloride procedure. The acetate treatment extracted from soil No. 3 only 80 per cent of the absorbed magnesium resultant from a comparatively recent incorporation of magnesium carbonate. Increase of the ammonium acetate leachate to 500 ml. yielded additional quantities of calcium and magnesium, but failed to extract all of the calcium carbonate and magnesium carbonate, or even the absorptively held magnesium. The effect of such incomplete removal of absorbed magnesium on the exchange capacity by ammonia absorption may be seen by comparing the values obtained for Soils 4 and 5 by straight acetate treatment with those obtained by ammonium chloride or by ammonium chloride followed by acetate leaching. The incom-

TABLE 1.—*Solvent effects of NH_4Cl and $NH_4C_2H_3O_2$ upon soils containing $CaCO_3$ and $MgCO_3$, and exchange capacity values (in mg. equivalents per 100 g. of soil)*

NO.	SOIL TREATMENT	CO_3= CONTENT	Ca+Mg DISSOLVED BY			EXCHANGE CAPACITY		
			BOILING NH_4Cl	AMMON. ACETATE		AMMON. ACETATE	AMMON. CHLORIDE	AMMON. CHLORIDE ACETATE
				250 ML.	500 ML.			
1	Cumberland silt loam, limestone-treated	90.7	102.1	45.2	65.0	6.7	7.2	7.2
2	Colbert silty clay loam, untreated	18.6	67.7	58.0	—	43.3	43.3	43.3
3	Cumberland silt loam, $MgCO_3$-treated	1.5	15.1	12.1	13.3	8.6	8.7	9.0
4	Same, MgO-treated	12.0	82.5	30.8	46.0	6.7	9.2	9.6
5	Same, $MgCO_3$-treated	38.8	89.0	56.2	60.0	6.3	9.0	9.0

plete removal of the absorbed magnesium in these soils resulted in lessened subsequent ammonia absorptions, only about $\frac{2}{3}$ of those that occurred after complete extraction of the absorbed magnesium.

It appears, therefore, that the most logical and accurate procedure for soils that contain appreciable quantities of calcium carbonate, magnesium carbonate, or $Ca.Mg(CO_3)_2$ is to effect complete extraction of both absorbed and carbonate bases, and to deduct the carbonate equivalence, which need be determined only once. The boiling ammonium chloride pro-

cedure described in the 1938 report, *This Journal*, **22**, 242 (1939), seems the most satisfactory method for the complete base extraction of such soils.

Special consideration is given to the proper assignment of the determined carbonate radicle between the calcium and magnesium, where the presence of both calcium carbonate and $Ca.Mg(CO_3)_2$ is suspected. The presence of either calcium carbonate or dolomite singly can be easily detected from the dissolution rate, *Ibid.*, during the regular digestion process. In the western arid regions the soil system is more complicated. In the states of California and Arizona the exchangeable calcium and magnesium is determined indirectly and approximately from the difference of the exchange capacity by ammonium acetate and the sum of the determined sodium and potassium.

The chief objectives of any neutral salt extraction of a soil are to determine (1) the supplies of available bases (Ca, Mg, K, and Na); (2) the exchange capacity of the soil; and (3) the state of base saturation, which follows from (1) and (2). It is also of scientific interest and of practical importance to ascertain the effects on the exchange complex resultant from heavy additions in liming experiments in various parts of the humid regions in the United States. The investigation has been approached from this standpoint.

EXCHANGE CAPACITY AND ABSORBED HYDROGEN DETERMINATION OF ACID SOILS

Previous studies in connection with the ammonium chloride digestion of soils demonstrated that all soil suspensions had an acidity of about 4.4 upon completion of such digestion, and it was recommended that 15 ml. of 0.1 *N* ammonium hydroxide be added to neutralize this acidity before filtering and leaching with neutral normal ammonium chloride for determination of exchange capacity. Determinations of acidity developed by such digestions of inorganic and organic soils were made by analyses of ammonium retained by residues in comparison with ammonium held by the same residues when their acidity was neutralized by either ammonium hydroxide or by treatment with neutral ammonium acetate. The results showed that the acidity developed from such digestion of essentially mineral soils rarely exceeds 10 ml. of 0.1 *N* acid. Highly organic soils or acid peats may show acidity as high as 40 ml. of 0.1 *N* acid per 5 grams determination. Because of the potentially wide range of acidity that various soils may develop upon digestion with ammonium chloride, and because of the low buffer capacity of this solution, the arbitrary use of a constant quantity of ammonium hydroxide for neutralization of soil-NH_4Cl suspension may prove inadvisable, and, even inadequate. The boiling ammonium chloride digestion of calcareous soils converts residues of such soils to an acidic state, and their neutralization for the purpose

of base exchange determination becomes a phase of the general problem of absorbed hydrogen replacement by ammonia.

Answers received to this query indicate that the practice most prevalent among soil investigators is to leach the soil with a sufficient volume of normal ammonium acetate at *p*H 7.0 to assure replacement of the absorbed hydrogen by ammonia. Several expressed doubt as to the capacity of the ammonium ion to replace completely the absorbed hydrogen of acid soils. This was the basis of barium hydroxide treatment suggested by Kelley in 1929[2] as a prelude to leaching with ammonium acetate. In 1930, Chapman and Kelley[3] reported that neutral ammonium acetate will replace the hydrogen ions of acidic soils completely, without preliminary treatment with barium hydroxide.

Schollenberger originally suggested the use of ammonium acetate of *p*H 7.07 to assure more rapid neutralization of the absorbed hydrogen. Some investigators contend, however, that correct results for exchange capacity are obtained from the addition of the replaceable ions, including the hydrogen ion, which is determined by titration of barium-acetate leachates. Values obtained for absorbed hydrogen by direct titration of barium-acetate leachates usually run higher than those computed by difference from the ammonia absorption. Hissink[4] concluded that the absorbed hydrogen of the soil can be neutralized only at high *p*H value, 10–11, and he recommends a titration procedure with barium hydroxide. According to his method, soils naturally supplied with calcium carbonate are only about 45 per cent saturated. The Hissink procedure for absorptive capacity, therefore, gives values beyond those from natural soil conditions. In the absence of a natural standard, it seems essential to adopt some convenient arbitrary standard for the saturation capacity of soils, and the ammonium acetate procedure seems best for this purpose.

AMMONIA ABSORPTION FROM AMMONIUM ACETATE BY ACIDIC SOILS

Ultimate ammonia absorption may be affected by several factors not generally recognized, namely, leaching volume, manner of washing out the ammonium salts, care of the residue, and distillation of the ammonia.

Two types of materials, a Cumberland loam red clay subsoil practically devoid of organic matter and having a *p*H of 4.8, and a peat of very low ash content and having a *p*H of 3.3 were used to determine the speed of reaction and volume of leachate requisite for maximal ammonia absorption. A preliminary experiment was carried out with the peat by mixing 4 grams of the air-dry material with 100 ml. of neutral ammonium acetate and filtering immediately. Leaching was to a combined volume of 250 ml. in one case and to 500 ml. in the other. The m.e. of ammonia absorbed per 100 grams of air-dry peat was 95.8 in each case.

[2] *J. Am. Soc. Agron.*, 21, 1021 (1929).
[3] *Soil Sci.*, 30, 391 (1930).
[4] *Trans. 3rd Int. Cong. Soil Sci.*, II, 60.

Speed and extent of ammonia absorption were measured further by a single extraction of these acidic materials in varying volumes of both ammonium acetate and ammonium chloride solutions. The results of the ammonia absorptions and resultant *p*H values are given in Table 2.

TABLE 2.—*Effect of varying volume of single extractions with NH_4Cl and $NH_4C_2H_3O_2$ on NH_3 absorptions*

MATERIAL	VOLUME OF REPLACING SOLUTION	*p*H OF EXTRACTS		NH_4 ABSORBED PER 100 G. FROM	
		CHLORIDE	ACETATE	CHLORIDE	ACETATE
	ml.			*m.e.*	*m.e.*
Cumberland loam	50	5.0	6.4	6.5	8.4
Red clay subsoil, practically devoid of organic matter, *p*H 4.8, 20 g. air-dry	100	5.3	6.6	6.6	8.5
	200	6.0	6.7	6.9	8.4
	100+150 leach.	—	—	9.0	9.2
Peat, finely ground, 95 per cent volatile, *p*H 3.3, 5 g. air-dry	50	2.8	5.6	23.2	92.8
	100	2.9	5.8	27.0	95.6
	200	3.0	6.2	32.2	96.2
	100+150 leach.	—	—	97.3	99.4

Capacity of ammonium acetate to replace absorbed hydrogen in both inorganic and organic colloids is most illuminating, in contrast with the low replacement by the chloride solution. For example, the 100 ml. extraction of the clay shows 6.6 m.e. ammonium absorbed from the chloride and 8.5 m.e. from the acetate. In each case about 3.5 ml. was due to exchange for calcium and magnesium, giving corrected hydrogen replacements of 3.1 against 5.0 m.e., respectively, for the chloride and the acetate. Taking 5.7 m.e. absorbed hydrogen by the acetate leachate as the total, hydrogen replacement by one 100 ml. extraction was 54 per cent for the chloride and 88 per cent for the acetate. Allowing 20.5 m.e. of the ammonia absorption for replacement of calcium and magnesium in a similar comparison for the peat, 6.5 m.e. hydrogen replacement by the chloride was 6.5 m.e. and 75.1 m.e. by the acetate. In terms of maximal hydrogen determined, displacement per 100 ml. was 8.2 per cent for ammonium chloride and 95.2 per cent for the acetate. Decrease or increase in volume of replacing solution in a single extraction exerted an appreciable effect on the low hydrogen replacements by the ammonium chloride, but only a slight effect on hydrogen replacement by the ammonium acetate.

The comparative *p*H values of the extracts are indicative of the buffering capacity of ammonium acetate. Moreover, the poor buffer capacity

of the ammonium chloride is shown by the low *p*H values of the peat extracts, which were diminished from *p*H 7.0 by comparatively small hydrogen-ion replacements.

EFFECT OF *p*H ON THE AMMONIUM ABSORPTION OF SINGLE 100 ML. EXTRACTION

For each extraction of 50 ml. of 2 *N* solution of ammonium acetate taken, increasing quantities of 0.5 *N* ammonium hydroxide were added and made to 100 ml. with water. Five grams of peat was shaken with the several solutions in stoppered Erlenmeyer flasks and allowed to stand overnight. The suspensions were filtered on 70 mm. Büchner filters. The filtrates were saved for *p*H determinations, and the residues washed with 95 per cent ethanol to about 250 ml. The absorbed ammonia was steam-distilled after the addition of 12.5 ml. of normal sodium hydroxide. The results are given in Table 3.

TABLE 3.—*Effect of increasing pH value of* $NH_4C_2H_5O_2$ *on the* NH_3 *absorption by a peat*

NO.	NH_4OH ADDED		*p*H OF EXTRACT	NH_4 ABSORPTION	
	APPROX. 0.5 *N*	M.E. VALUE		PER 5 G.	PER 100 G.
1	0	0	5.88	4.96	99.2
2	5	2.27	6.08	6.01	100.2
3	10	4.54	6.44	5.14	102.8
4	15	6.81	6.95	5.27	105.4
5	20	9.08	7.40	5.45	109.0
6	25	11.35	7.70	5.53	110.6

The data demonstrate the increasing absorption of ammonium with increase of equilibria of *p*H values. The exact *p*H of the starting acetate solutions was not determined, hence diminutions in *p*H value were not computable. In system No. 4, however, where the equilibrium solution was nearly neutral and the ammonium absorption was 105.m.e., the initial absorption was at higher *p*H value, and it was decreased by the subsequent hydrogen-ammonia exchange. When the same peat samples were shaken with a solution of initial *p*H of 7.0, the resultant equilibrium *p*H was much lower, actually 5.98, and it was necessary to build up the additional absorption for the *p*H 7.0 equilibrium by further renewed contacts with the equilibrium solution. It is possible, therefore, that the ammonium absorption from a single contact at a certain final *p*H may be higher than the absorption from renewed contact by leaching with the same solution of identical *p*H. The maximal ammonia absorption at *p*H 7.7 was only about 5 per cent above that at *p*H 6.95.

EFFECT OF VARYING *p*H OF AMMONIUM ACETATE LEACHINGS ON AMMONIA ABSORPTION BY ORGANIC AND INORGANIC SOILS

An effort was made to determine the effect of *p*H of the ammonium acetate on the ammonium absorption under usual conditions. The soils were selected to represent a wide range with respect to organic and inorganic colloids. A peat was used as representative of an organic colloidal material, two subsoils as representative of inorganic colloids, and a black clay loam as representative of an intermediate. Ammonium acetate solutions of various *p*H values were prepared by the addition of either acetic acid or ammonium hydroxide to the neutral solution. The soils were shaken with 100 ml. of the respective solutions in stoppered flasks and allowed to stand overnight. The suspensions were then filtered on Büchners and leached with 150 ml. of the same solution, and washed with 250 ml. of 95 per cent ethanol. The ammonium absorptions were determined by dilute sodium hydroxide distillation, as described.

The results of Table 4 show that, except for the peat sample, ammonia absorption did not increase with increases in *p*H values above 7.0 after

TABLE 4.—*Effect of varying pH of $NH_4C_2H_3O_2$ leachings on the NH_3 absorptions of various soils*

NO.	$NH_4C_2H_3O_2$ MAKE-UP		*p*H	NH_4 ABSORPTION PER 100 G. AIR-DRY			
	ADDITION PER LITER			RED CLAY	YELLOW CLAY	BLACK CLAY LOAM	PEAT
	1 *N* ACETIC ACID	1 *N* NH_4OH					
				m.e.	*m.e.*	*m.e.*	*m.e.*
1	15	—	6.45	—	35.6	—	100.0
2	10	—	6.60	—	35.5	—	100.8
3	5	—	6.80	—	35.6	—	101.6
4	0	0	6.95	9.2	35.6	38.8	104.6
5	—	5	7.18	9.4	35.4	38.6	105.2
6	—	10	7.40	9.4	35.5	38.8	106.2
7	—	15	7.58	9.6	35.6	38.6	108.2
8	—	20	7.72	9.4	35.6	38.7	108.6
9	—	25	7.81	9.6	35.6	38.8	108.2

the ammonium acetate. Increase induced by raising the *p*H value from 7.0 to 7.8 was about 5 per cent for the peat. If allowance is made for variability in the last three determinations, it is found that there is about one per cent increase in absorption for each 5 ml. excess of normal ammonium hydroxide per liter of ammonium acetate of *p*H above 7.0. In the light of these results, the absence of any increased absorption with increase in *p*H by the black clay loam, which contains 8.7 per cent organic matter, is surprising. Lack of evident increase in the ammonia absorption through increased *p*H of replacing agent in any of the several soils does

not necessarily prove that the *p*H factor is without effect upon such absorption by ordinary soils. It is possible that such effect is small and that it was nullified by subsequent washings with the 95 per cent alcohol.

Experiments were carried out to determine the *p*H effect on ammonium absorption by analyzing the change in ammonium concentration of the liquid phase. The results indicate much greater effects of *p*H on the ammonium absorptions than those shown above, but because of the high ammonium concentration and the relatively large factor on experimental error, it will be necessary to check this technic.

EFFECT OF ALCOHOLIC WASH ON THE AMMONIA ABSORPTION VALUES OF SOILS

In saturating the soils with ammonium from either ammonium chloride or ammonium acetate, it is necessary to free the soil mass of the solute before the absorbed ammonium can be determined. Both ethyl and methyl alcohol have been used for this purpose, but ethyl alcohol has been used in a wider range of concentration, from 50–95 per cent. Some investigators neutralize the alcohol with ammonium hydroxide, neutrality being registered by bromothymol blue or by the glass electrode. Such neutralization has been discussed in previous reports, *This Journal*, **21**, 252 (1938) and **22**, 247 (1939). Previous and present studies have shown differences of 2–4 m.e. per 100 grams of soil in results by use of ammonia-treated alcohol and non-treated alcohol. The U.S.P. 95 per cent used was acidic to bromothymol blue, and required approximately 2.5 ml. of normal ammonium hydroxide to neutralize one liter. On exposure to the atmosphere this adjusted alcohol became acidic to the above indicator. Others have shown that this indicator cannot be depended upon to give a sustained *p*H to the alcohol. Measured by the Beckman glass electrode, the alcohol showed a *p*H slightly above 7.0. The question arises, Is the difference in the ammonium absorption by the treated soils due to the ammoniated alcohol or to the solvent action of the ammonia-free neutral alcohol wash? To answer this question, experiments were run to determine the solvent effect of the 95 per cent ethanol.

Several soils were treated with ammonium acetate, filtered, and leached in the usual manner. One series was washed with 500 ml. of 95 per cent ethanol and the other with the same volume of ethanol adjusted with 2.5 ml. of normal ammonium hydroxide per liter. The washed residues of each series were again leached with 500 ml. of 95 per cent ethanol and their washings evaporated to about 10 ml. with the addition of a few drops of sulfuric acid. The residues were diluted and transferred to distillation flasks, and the absorbed ammonium was distilled after addition of sodium hydroxide. The amounts of ammonium found in the second 500 ml. alcoholic wash, together with that found in the soil residues after the two 500 ml. washings, are given in Table 5. The quantities of ammo-

nium dissolved from the NH_4-soil residue by 500 ml. of alcohol wash are shown in the second and fourth columns. In each case these residues had been washed with more than the usual volume of alcohol, previous to the determined solvent effects. When the ammonium absorptions by these soils were determined after one 500 ml. alcohol washing under the two conditions, two sets of ammonium values for the exchange capacity were obtained, as shown in Columns 5 and 6. Differences in results by these two procedures are shown in the last column. Data in Column 2 show the minimal solvent effect of 500 ml. of 95 per cent ethanol on the NH_4-soil complex. The fact that much larger quantities of ammonium were removed by the same wash from the ammonium soils, previously washed, however, with 500 ml. of ammoniated (0.0025 *N*) alcohol, indicates that this ammonium increment is not held so tenaciously as the remaining ammonium in the soil. This fact also suggests that the practice of ammoniating the alcohol wash may cause enhanced and variable ammonium absorption values. The solvent effect of neutral 95 per cent ethanol on the NH_4-soil complex should be taken into consideration in regulating the volume of alcohol wash. Although no experimental work has been done on this phase of the problem, it is apparent that greater solvent effects may be expected when the NH_4-soil complex is washed with alcohol of concentration below 95 per cent.

TABLE 5.—*Effect of NH_4OH in alcohol wash upon absorbed NH_3 from NH_4Cl treatment of soils*

SOILS*	TWO 500 ML. PORTIONS OF ETHANOL		500 ML. AMMONIATED WASH FOLLOWED BY 500 NON-AMMONIATED ETHANOL		ABSORBED NH_4 IN SOILS AFTER ONE 500 ML. WASH		INCREASE IN ABSORBED NH_4 DUE TO AMMONIATE OF WASH
	NH_4 IN SOIL	NH_4 IN 2d WASH	NH_4 IN SOIL	NH_4 IN 2d WASH	AMMONIATED	NON-AMMONIATED	
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Clarksville silt loam	3.6	.8	4.6	1.3	5.9	4.4	1.5
Fullerton silt loam	4.1	.6	5.0	1.3	6.3	4.7	1.6
Dewey silt loam	6.8	.8	8.0	1.7	8.7	7.4	1.3
Dickson silt loam	3.9	.6	4.5	1.7	6.2	4.5	1.7
Hartsells sandy loam	4.2	.6	4.9	1.7	6.6	4.8	1.8
Hagerstown silt loam	6.0	.6	6.6	2.0	8.6	6.6	2.0
Colbert silt loam	13.7	.8	15.0	2.3	17.3	14.1	3.2
Colbert clay loam	41.0	1.3	42.5	3.0	45.5	42.3	3.2
Chickamauga surface	31.7	1.5	34.1	3.0	37.1	33.2	3.9
Chickamauga subsoil	33.8	1.0	35.1	2.1	37.1	34.8	2.3
Cherokee clay subsoil	8.0	.8	9.5	2.4	11.9	8.8	3.1
Bentonite	—	—	35.2	1.4	36.6	34.8	1.8
Becket sandy loam	41.4	2.9	45.5	3.5	49.0	44.3	4.7
Peat	34.4	2.1	37.8	4.6	42.4	36.5	5.9

* All were 10 g. charges except the peat, which was 4 g.

EFFECT OF DRYING ON THE NH_4-SOIL COMPLEX

The usual practice is to drain off the free liquid from the alcohol-washed ammonium soil and to transfer it immediately into the distillation apparatus, but some analysts practise drying the soil mass before distillation. Some comparisons indicate that the preliminary step of drying causes appreciable loss of ammonium. This loss is more serious with soils of high organic matter content.

AMMONIA DISTILLATION TECHNIC

The prevalent practice in the determination of the absorbed ammonium is to suspend the soil mass in about 400 ml. of water, and to add magnesium oxide and distil 200 ml. into standard acid. To obviate foaming and bumping, and possible chemical action on the soil organic matter, some investigators have resorted to aeration or to acidic leaching of the ammonium. The steam distillation eliminates all mechanical difficulties through the vigorous churning of the soil mass by the steam jet. Foaming, encountered in distillation of soils of high organic content and heavy clays, is prevented by the addition of a few drops of a mixture of mineral oil and caprylic alcohol.

The relative efficiencies of magnesium oxide and certain concentrations of sodium hydroxide in the displacement of ammonium soil by distillations was studied previously, and has been reported, *This Journal*, **22**, 246 (1939). It was shown that the ammonium displacement by the magnesium oxide is much slower than with sodium hydroxide, and that this disparity may be the cause of serious error in distillations of clay soils. It was shown also that in the concentration used sodium hydroxide exerted negligible effect on the soil organic matter.

Experience with concentrated ammonia distillates, i.e. those carrying 30 ml. of 0.1 *N* ammonium hydroxide and over, led to introduction of certain precautionary measures to assure greater accuracy in ammonium recovery. Distinct ammoniacal odor indicated the passage of ammonia through the standardized acid in the receiving vessel during displacement of air by the steam in the initial stage of distillation. This was not prevented by increasing the cooling surface of the condenser, or by collection of distillate in chilled acid. From 1 to 2 ml. of 0.1 *N* ammonium hydroxide may be lost in this manner. To remedy this defect, air is dispelled from the distillation apparatus by boiling the suspension in either neutral or slightly acid condition, releasing the steam pressure, and then introducing the alkali through a separatory funnel.

Another error may be injected at the beginning of the distillation, since the amount of ammonium hydroxide then carried over may form an alkaline surface layer and thus be partly lost by volatilization, despite the excess of acid in the receiver. It is essential, therefore, to stir the absorbing acid frequently during the first 2–3 minutes of the distillation.

By means of steam distillation and use of dilute sodium hydroxide, the complete removal of ammonium and a 200 ml. distillate can be attained in less than 15 minutes.

No report on less common metals in soils was given by the associate referee.

No report on selenium in soils was given by the associate referee.

REPORT ON FERTILIZERS

By G. S. FRAPS (A. & M. College of Texas, Texas Agricultural Experiment Station, College Station, Texas), *Referee*

The recommendations of the associate referees will be given in their reports, as usual. The revision of *Methods of Analysis* calls for some corrections; most of these are editorial, but a few on the margin between the editorial and actual changes in methods will be brought to the attention of the Association.

The direction for preparing methyl red for the Kjeldahl method should be corrected since the indicator is partly precipitated when the water is added to alcohol. Kjeldahl flasks of 800 ml. capacity should be included in the method. The term "Thomas or basic slag" should be changed to "basic slag." Authority to standardize the acid and alkali used in the Kjedahl method by the general methods now adopted should be given. Recommendations regarding these points are made later.

Suggestions have been made to change the size of the screen used in preparation of fertilizers, and to standardize the potassium hydroxide used in the volumetric estimation of phosphoric acid by means of calcium phosphate, but these suggestions involve more than editorial changes and would require collaborative work, although the method can be clarified in this respect.

The recommendation of the Referee on Fertilizers that an associate referee be appointed to work in cooperation with the Bureau of Standards on the testing of volumetric apparatus and weights and to recommend methods for discouraging the use of apparatus and weights that are too inaccurate was last year deferred for further study. It is, of course, not necessary to test flasks, burets, pipets, and weights that have been tested by the Bureau of Standards, but large numbers of other instruments are in use, and it is recognized that some of these do not have the desired accuracy. This Association should recommend the testing of all flasks, pipets, burets, and weights not already tested by the Bureau of Standards and should provide methods for making these tests. Weights should prob-

ably be tested annually. A condensed statement of methods for testing used in the Referee's laboratory follows:

TESTING GRADUATED FLASKS

Use Morse calibrating burets (Eimer and Amend No. 19218) of 500 cc., 200 cc., 50 cc., and 1 cc. capacity which have been calibrated by the Bureau of Standards. Set up the apparatus with one side of the 2-way stop-cock connected with an aspirator containing distilled water. Fill the buret and run water into the clean and dry flasks to be tested in such a way that the water strikes the neck of the flask just below the graduation mark, at the rate used in the original calibration. Fill the flask until the bottom of the meniscus is exactly on the graduation mark, and read the volume from the graduated stem of the Morse buret. If the volume comes within the limits of tolerance, the flask is marked "O.K." If the volume is beyond the limits of tolerance, the flask is either destroyed or sent back to the company from which it was purchased.

TESTING PIPETS

Fill the clean pipet with distilled water of known temperature and empty into a weighed flask or weighing bottle. Compare the weight of the water with the calculated weight at the temperature of measurement.

TESTING BURETS

Test the buret either by means of the calibrated Morse buret, or by weighing distilled water delivered as directed for the pipets.

TESTING WEIGHTS

Test the weights against weights tested by the Bureau of Standards. Clean weights and adjust brass weights by removing or adding aluminum or lead or fine platinum wire, which can easily be cut to the desired weight.

RECOMMENDATIONS[1]

The following recommendations in regard to revision of methods are presented.

(1) Page 23, **19.**—Insert "The acid may be standardized by any of the official methods given in Appendix 1 (editorial, final action).

(2) Page 23, **19 (c).**—Add "proceed as directed in any official method given in Appendix 1," in order that standard alkali solutions may be standardized by other official methods (editorial, final action).

(3) Page 23, **17.**—Change heading to read: "Citrate-soluble and available phosphoric acid, official." Add the sentence, "Subtract citrate-insoluble P_2O_5 from total to obtain available P_2O_5." This is in accordance with the definition of available phosphoric acid adopted in 1931 (editorial, final action).

(4) Page 24, **20 (a).**—Change to read "total capacity ca 550 cc. or 800 cc," and **20 (b)** to read "550 or 800 cc." This provides for the use of 800 cc. flasks (editorial, final action).

(5) Page 23, **19 (e).**—Change to "HgO of reagent grade, free from N" (editorial, final action).

(6) Page 24, **19 (i).**—Change directions for methyl red to read, "Dis-

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 23, 75 (1940).

solve 1 g. of methyl red (dimethylaminoazobenzeneorthocarboxylic acid) in 200 cc. of alcohol" (correction, final action).

(7) Page 36, 62.—Change "500 cc. Wagner flask" to "500 cc. cylindrical shaking flask (Wagner)" (editorial, final action).

(8) Page 19, 7 (a).—Insert the sentence "Cool" after the first sentence, in order to avoid precipitation of molybdic acid, which occurs when hot solutions are used (correction, final action).

(9) Page 23, 19 (g).—Change the last sentence to read as follows: "A soln having a sp. gr. of 1.36 or higher may be used" (final action).

(10) Change the official methods for the determination of P_2O_5 as given in paragraphs 4 and 5, *This Journal*, **22**, 70 (1939) (official, final action).

(11) Refer the distillation method for water in fertilizers to the Associate Referee on Nitrogen for further consideration in regard to its applicability.

It is also recommended—

(12) That the Associate Referee on Phosphoric Acid study the fineness of grinding needed for high analysis fertilizers.

(13) That the Association approve the testing of flasks, burets, pipets, and weights used for analyses of fertilizers that have not been tested by the Bureau of Standards, and consider the adoption of methods for such testing or appoint an associate referee to recommend methods for testing unstandardized flasks, burets, pipets, and weights, and for checking the accuracy of weights in use.

(14) That since the work of the Associate Referee on Phosphoric Acid shows that the McIntire-Shaw-Hardin method for available phosphoric acid gives such high results with rock phosphate that its use would permit the substitution of part of the superphosphate by rock phosphate in mixed fertilizers, the study of this method as a substitute for the present method for determining available phosphoric acid be discontinued.

REPORT ON PHOSPHORIC ACID

A COMPARISON OF THE OFFICIAL AND MacINTIRE-SHAW-HARDIN METHODS FOR DETERMINING AVAILABLE PHOSPHORIC ACID

By WILLIAM H. ROSS, *Associate Referee*, and L. F. RADER, JR. (Bureau of Agricultural Chemistry and Engineering, Washington, D. C.)

At the last meeting of this Association a recommendation was adopted, *This Journal*, **22**, 254 (1939), that the Associate Referee on Phosphoric Acid give further consideration to the method proposed by MacIntire, Shaw and Hardin[1] for the determination of available phosphoric acid. A collaborative study of the relative merits of the proposed method and

[1] *Ind. Eng. Chem., Anal. Ed.*, **10**, 143 (1938).

of the present official method was accordingly undertaken. The samples submitted to the collaborators for use in this study were as follows:

Standard Samples

1. Ammoniated superphosphate containing 8.66% of NH_3.
2. Tricalcium phosphate, precipitated, $Ca_3(PO_4)_2$.
3. Calcium hydroxyphosphate $[3Ca_3(PO_4)_2] \cdot Ca(OH)_2$.
4. Calcined phosphate, Ross and Jacob, *This Journal*, **20**, 231 (1937).
5. Tennessee brown-rock phosphate.

Sample 1 was prepared on a laboratory scale by making a slurry of a superphosphate, adding an excess of ammonia, and evaporating to dryness on a steam bath. This sample represents the maximum degree to which a superphosphate can be ammoniated.

Sample 2 was prepared by slowly adding ammonia to a slurry of monocalcium phosphate and calcium sulfate in the proper amount to satisfy the equation:

$$Ca(H_2PO_4)_2 + 2CaSO_4 + 4NH_3 = Ca_3(PO_4)_2 + 2(NH_4)_2SO_4.$$

The precipitated tricalcium phosphate was filtered off by suction, washed with cold water until essentially free from sulfates, and finally dried at 45° C. The dried material had a P_2O_5/CaO ratio of 0.819 as compared with the theoretical value of 0.845.

Sample 3 was prepared by digesting tricalcium phosphate (Sample 2) in boiling water for 42 days. The water was changed each day to facilitate hydrolysis of the tricalcium phosphate—

$$10Ca_3(PO_4)_2 + 6H_2O = 3[3Ca_3(PO_4)_2] \cdot Ca(OH)_2 + 2H_3PO_4.$$

The calcium hydroxyphosphate prepared in this way was dried over concentrated H_2SO_4 and P_2O_5. It had a P_2O_5/CaO ratio of 0.774 as compared with 0.760 for the theoretical value.

Sample 4 was prepared experimentally by calcining Tennessee brown-rock phosphate in a rotary kiln at 1400° C. in the presence of water vapor. The product was ground to pass a Tyler 80-mesh screen.

Sample 5 was a commercial grade of ground Tennessee brown-rock phosphate. About 98% of the material passed a 200-mesh screen and 86% a 400-mesh screen.

Directions for Analysis

A-1. Determine total and citrate-insoluble P_2O_5 in each of the standard samples as directed in *Methods of Analysis, A.O.A.C.*, 1935.

A-2. Determine citrate-insoluble P_2O_5 in each of the standard samples as directed in *Methods of Analysis, A.O.A.C.*, 1935, except to take a 0.5 gram sample in place of a 1 gram sample.

B-1. Determine available P_2O_5 in each of the standard samples as directed in the MacIntire-Shaw-Hardin method[1] for the analysis of mixed fertilizers.

B-2. Determine available P_2O_5 in each of the standard samples as directed in the MacIntire-Shaw-Hardin method[1] for the analysis of mixed fertilizers except to take a 0.5 gram sample in place of a 1 gram sample.

C-1. As it is desirable to test the MacIntire-Shaw-Hardin method on a wide range of fertilizers, determine available P_2O_5 by both the official and MacIntire-Shaw-Hardin methods in such other materials and mixtures as is convenient. The MacIntire-Shaw-Hardin method directs that a 1 gram sample be taken for the analysis of mixed fertilizers and phosphate rock and that a 0.5 gram sample be taken for the analysis of such concentrated and highly available materials as triple superphosphate and calcined phosphate.

Notes

(a) It is suggested that the citrate-insoluble residues obtained in the analysis

of the samples by the official method be washed with a 5% NH_4NO_3 solution at 65° C. if a cloudy filtrate is obtained on washing with water.

(b) In determining citrate-insoluble P_2O_5 in Sample 1 by the official method, the sample should be washed with water prior to the citrate digestion. This step may be omitted in the analysis of the other samples by this method.

The following analysts collaborated in this work:

COLLABORATORS

1. Allen, H. R. and Gault, Lelah, Univ. of Kentucky, Lexington, Ky.
2. Batton, H. C., Swift and Co. Fertilizer Works, Baltimore, Md.
3. Byers, C. R., Armour Fertilizer Works, Carteret, N. J.
4. Caldwell, R. D., Armour Fertilizer Works, Atlanta, Ga.
5. Carpenter, F. B. and Lazarus, Sam, Virginia-Carolina Chemical Corp., Ric h mond, Va.
6. Charlton, R. C. and Levi, R. C., The Am. Agr. Chem. Co., Carteret, N. J.
7. Fraps, G. S., Ogier, T. L., Asbury, S. E., and Walker, Waldo, Agr. Expt. Station, College Station, Texas.
8. Grattan, Geo. E., Dept. of Agriculture, Ottawa, Canada.
9. Howes, C. C., The Davison Chemical Corp., Baltimore, Md.
10. Jones, W. Catesby, Dept. of Agriculture and Immigration, Richmond, Va.
11. Koch, R. C., Swift and Co. Fertilizer Works, Hammond, Ind.
12. Morgan, W. A., E. I. du Pont de Nemours & Co., Wilmington, Del.
13. Rader, Jr., L. F., Bureau of Agri. Chemistry and Engineering, Washington, D. C.
14. Shuey, P. McG., Shuey and Company, Savannah, Ga.

RESULTS OF ANALYSIS

Table 1 summarizes the results reported by the collaborators for total, available, and citrate-insoluble P_2O_5 in the standard samples when (1) 1 gram, and (2) 0.5 gram samples were taken for analysis, while Table 2 gives corresponding results for a number of miscellaneous materials and mixtures on the basis of a 1 gram sample. Data obtained for the available phosphoric acid in miscellaneous phosphatic materials as determined by various modifications of the official and the MacIntire-Shaw-Hardin methods are given in Table 3.

COMMENTS BY COLLABORATORS

H. C. Batton.—From the point of view of time required and of attention to minute detail, I prefer the official method. However, I am convinced that the official method does not give a true determination of the P_2O_5 present in a reverted material that is actually available for plant nutrition. I believe that the MacIntire-Shaw-Hardin method comes much nearer to doing that than does the official method.

F. C. Carpenter.—We do not like the MacIntire-Shaw-Hardin method. Some of our results lead us to believe that equally satisfactory agreement with the values obtained in field tests can be obtained with the official method by changing the *p*H of the citrate solution.

G. S. Fraps.—When 0.25 gram of Sample 5 was used, the available P_2O_5 obtained by the MacIntire-Shaw-Hardin method was 16.76% of the sample, or 50% of the total quantity present. This means that the use of this method would encourage to an appreciable extent the substitution of superphosphate by rock phosphate in

mixed fertilizers. This opinion is confirmed by the following results obtained by T. L. Ogier in the analysis of six fertilizers made by mixing phosphate rock with $(NH_4)_2SO_4$, K_2SO_4, and gypsum. The available P_2O_5 was run by the MacIntire-Shaw-Hardin method. It will be noted that a large percentage of the total P_2O_5 is available by this method and that the method is therefore unsuited for use on mixed fertilizers:

	Fertilizer Mixture*					
	A	*B*	*C*	*D*	*E*	*F*
	per cent					
Total P_2O_5	9.03	8.80	9.01	8.65	9.07	4.77
Available P_2O_5	3.70	2.60	3.59	3.57	3.77	2.19
Available P_2O_5 (per cent of total)	40.97	29.54	39.84	41.27	41.57	45.91

* All mixtures contained 20 grams of $(NH_4)_2SO_4$ and 8 grams of K_2SO_4. The rock in mixtures A to F amounted to 17, 13, 13, 20, 20, and 25 grams, respectively, and the $CaSO_4$ to 5, 5, 9, 2, 2, and 12 grams.

P. McG. Shuey.—Duplicate results do not agree so well by the MacIntire-Shaw-Hardin method as by the official method, and results on some samples, especially Sample 3, seem to be entirely too high. Further, the method is more difficult to use, especially when it is necessary to make a large number of determinations. If the present method gives results that are too low, the *p*H of the citrate solution can be changed accordingly.

DISCUSSION OF RESULTS

All the collaborators used the volumetric method in the determination of available P_2O_5 and also in the determination of total P_2O_5 with the exception of L. F. Rader, Jr., who used the gravimetric method. All the results show that the MacIntire-Shaw-Hardin method gives higher values than does the official method for available P_2O_5 in all the standard samples with the exception of the calcined phosphate sample. Six of the thirteen collaborators found that the MacIntire-Shaw-Hardin method gives a lower value for available P_2O_5 in this sample than does the official method when a 1 gram sample is taken for analysis, but with two exceptions all found that the reverse was true when a 0.5 gram sample was used.

The results show good agreement in the values for total P_2O_5. The replicated results for available P_2O_5 that some of the collaborators submitted with their reports also agree very well, but there was a greater variation in the mean values reported for available P_2O_5 as determined by both the official and MacIntire-Shaw-Hardin methods. The standard deviation from the mean (Table 1) is much greater for the available P_2O_5 than for the total P_2O_5 values. This is particularly noticeable for the tricalcium phosphate and calcium hydroxyphosphate samples.

Most of the collaborators expressed a preference for the official method, but some of them suggested that the availability of a phosphatic material determined by the MacIntire-Shaw-Hardin method might be in better agreement with its relative efficiency in promoting crop growth as determined by vegetative tests.

TABLE 1.—*Results obtained on standard phosphate samples by the official method and the MacIntire-Shaw-Hardin method*

COLLABORATOR	TOTAL P_2O_5	AVAILABLE PHOSPHORIC ACID (P_2O_5)				INSOLUBLE PHOSPHORIC ACID (P_2O_5)			
		OFFICIAL METHOD		MACINTIRE-SHAW-HARDIN METHOD		OFFICIAL METHOD		MACINTIRE-SHAW-HARDIN METHOD	
		1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM
	per cent	*per cent*		*per cent*		*per cent*		*per cent*	
				Sample 1					
1	18.68	12.49	16.93	12.98	17.25	6.19	1.75	5.70	1.43
2	18.63	12.25	15.73	13.71	17.33	6.38	2.90	4.92	1.30
3	18.53	11.63	16.93	12.20	17.40	6.90	1.60	6.33	1.13
4	19.00	11.20	14.38	11.43	16.66	7.80	4.62	7.57	2.24
5	18.58	11.11	15.75	14.65	16.70	7.47	2.83	3.93	1.88
6	18.81	12.07	16.18	16.22	17.65	6.74	2.63	2.59	1.16
7	19.29	11.40	15.87	15.25	18.23	7.89	3.42	4.04	1.96
8	18.50	11.50	—	11.53	16.65	7.00	—	6.97	1.85
9	18.64	12.02	16.20	14.45	17.65	6.62	2.44	4.19	0.99
10	19.00	12.50	16.58	12.80	17.45	6.50	2.42	6.20	1.55
11	18.63	12.48	16.23	13.47	17.17	6.15	2.40	5.16	1.46
12	18.69	11.46	15.69	13.45	17.47	7.23	3.00	5.24	1.22
13	18.70	12.00	16.63	13.36	17.84	6.70	2.07	5.34	0.86
14	18.56	12.46	16.50	12.25	17.18	6.10	2.06	6.31	1.38
Mean	18.72	11.89	16.12	13.41	17.33	6.83	2.62	5.31	1.46
Standard deviation from mean	0.22	0.50	0.64	1.34	0.43	0.56	0.75	1.39	0.40
				Sample 2					
1	41.03	26.55	39.68	35.65	40.73	14.48	1.35	5.38	0.30
2	41.00	28.40	39.15	38.37	40.99	12.60	1.85	2.63	0.01
3	40.65	24.70	40.25	37.60	40.40	15.95	0.45	3.05	0.25
4	40.75	25.52	38.35	28.53	38.84	15.23	2.40	12.32	1.91
5	40.39	22.02	38.16	38.25	39.05	18.37	2.23	2.14	1.34
6	41.01	27.27	40.25	39.23	40.90	13.74	0.76	1.78	0.11
7	40.57	21.37	35.04	36.70	40.26	19.20	5.53	3.87	0.31
8	40.70	25.70	—	33.93	39.10	15.00	—	6.77	1.60
9	41.05	29.33	39.22	35.20	39.40	11.72	1.83	5.85	1.65
10	41.18	27.70	38.85	35.05	41.05	13.48	2.33	6.13	0.13
11	41.00	26.62	38.32	38.35	40.45	14.38	2.68	2.65	0.55
12	40.86	21.78	36.79	35.55	40.60	19.08	4.07	5.31	0.26
13	41.23	24.20	39.62	33.86	41.12	17.03	1.61	7.37	0.11
14	40.84	25.10	38.02	32.69	40.61	15.74	2.82	8.15	0.23
Mean	40.87	25.45	38.59	35.64	40.24	15.42	2.30	5.24	0.66
Standard deviation from mean	0.23	2.37	1.40	2.77	0.80	0.71	1.28	2.78	0.64
				Sample 3					
1	42.10	11.40	19.02	20.78	30.43	30.70	23.08	21.32	11.67
2	42.08	17.80	24.75	21.88	30.23	24.28	18.33	20.20	11.85
3	41.90	13.05	27.50	16.55	29.60	28.85	14.40	15.35	12.30
4	42.23	11.13	14.18	17.38	24.16	31.10	28.05	24.85	18.07
5	41.53	10.66	16.27	20.71	26.88	30.87	25.26	20.82	14.65
6	42.00	11.79	23.26	24.20	31.00	30.21	18.74	17.80	11.00
7	42.00	9.42	15.50	21.59	31.46	32.58	26.50	20.41	10.54
8	41.60	15.20	—	18.97	27.70	26.40	—	22.63	13.90
9	42.25	14.73	23.93	18.90	26.40	27.52	18.32	23.35	15.85
10	42.50	10.65	18.75	16.90	30.28	31.85	23.75	25.60	12.22
11	42.25	11.20	17.50	21.60	30.13	31.05	24.75	20.65	12.12
12	42.07	10.04	16.27	20.16	27.51	32.03	25.80	21.92	14.56
13	42.31	10.33	17.14	19.73	29.77	31.98	25.17	22.58	12.54
14	41.73	10.90	17.02	19.27	28.54	30.83	24.71	22.46	13.19
Mean	42.03	12.03	19.26	19.90	28.86	30.02	22.83	21.42	13.10
Standard deviation from mean	0.28	2.36	3.94	2.04	2.00	2.33	3.64	2.54	2.04

TABLE 1.—*Continued*

COLLABORATOR	TOTAL P_2O_5	AVAILABLE PHOSPHORIC ACID (P_2O_5)				INSOLUBLE PHOSPHORIC ACID (P_2O_5)			
		OFFICIAL METHOD		MACINTIRE-SHAW-HARDIN METHOD		OFFICIAL METHOD		MACINTIRE-SHAW-HARDIN METHOD	
		1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM	1 GRAM	0.5 GRAM
	per cent	*per cent*		*per cent*		*per cent*		*per cent*	
				Sample 4					
1	37.10	34.26	34.55	31.45	34.80	2.84	2.55	5.65	2.30
2	37.38	33.45	33.98	34.03	35.78	3.93	3.40	3.35	1.60
3	37.80	34.05	35.00	31.43	34.80	3.75	2.80	6.37	3.00
4	37.15	31.58	34.01	31.23	34.80	5.57	3.14	5.92	2.35
5	36.83	32.93	33.97	34.10	34.92	3.90	2.86	2.73	1.91
6	37.53	34.53	34.80	34.60	36.30	3.00	2.73	2.93	1.23
7	37.00	32.54	33.80	33.14	36.30	4.46	3.20	3.86	0.70
8	36.80	—	—	32.70	34.85	—	—	4.10	1.95
9	37.45	33.89	34.73	34.33	35.80	3.56	2.72	3.12	1.65
10	37.35	33.90	33.85	35.00	35.75	3.45	3.50	2.35	1.60
11	37.35	33.95	34.23	33.65	35.67	3.40	3.12	3.70	1.68
12	37.11	33.24	34.25	32.82	35.14	3.87	2.86	4.29	1.97
13	37.03	33.95	34.26	34.06	35.57	3.08	2.77	2.97	1.46
14	37.32	34.23	34.53	33.29	34.73	3.09	2.79	4.03	2.95
Mean	37.22	33.57	34.31	33.27	35.34	3.68	2.95	3.96	1.89
Standard deviation from mean	0.28	0.80	0.36	1.12	0.59	0.71	0.27	1.20	0.61
				Sample 5					
1	33.58	2.90	4.75	4.05	6.43	30.68	28.83	29.53	27.15
2	33.58	3.98	4.33	4.57	7.37	29.60	29.25	29.01	26.21
3	33.80	2.80	3.80	3.50	5.60	31.00	30.00	30.30	28.20
4	33.68	1.51	2.32	3.35	4.60	32.17	31.36	30.33	29.08
5	33.23	2.29	3.25	3.55	7.93	30.94	29.98	29.68	25.30
6	33.62	3.19	4.74	4.98	7.70	30.43	28.88	28.64	25.92
7	33.51	2.31	3.40	5.04	7.76	31.20	30.11	28.47	25.75
8	33.10	6.50	—	2.73	5.55	26.60	—	30.37	27.55
9	33.75	3.11	4.63	4.40	7.60	30.64	29.12	29.35	26.15
10	34.02	2.22	3.12	3.75	7.25	31.80	30.90	36.27	27.77
11	33.50	2.45	3.72	4.53	6.23	31.05	29.78	28.97	27.27
12	33.63	2.20	3.31	3.41	5.38	31.43	30.32	30.22	28.25
13	33.65	2.49	4.01	4.59	7.52	31.16	29.64	29.06	26.13
14	33.57	3.11	3.39	3.96	5.15	30.46	30.18	29.69	28.42
Mean	33.59	2.93	3.75	4.03	6.58	30.64	29.86	29.98	27.08
Standard deviation from mean	0.22	1.14	0.68	0.65	1.13	1.28	0.73	1.85	1.14

The mixed fertilizers analyzed by Batton (Table 2) had undergone reversion in storage, while the materials and mixtures analyzed by the other collaborators, whose results are given in the same table, remained substantially unchanged. The results reported indicate that the MacIntire-Shaw-Hardin method gives lower results than does the official method for insoluble P_2O_5 in mixed fertilizers that have undergone reversion in storage, but that both methods agree quite well in the analysis of superphosphate and mixed fertilizers that have not reverted in storage. MacIntire, Shaw, and Hardin recommend a 0.5 gram sample in the analysis of double superphosphate by their method, inasmuch as a 1 gram sample gives high results, as shown in Table 2.

TABLE 2.—*Results obtained on miscellaneous materials and mixtures by the official method and the MacIntire-Shaw-Hardin method*
(1 gram sample taken for analysis.)

FERTILIZER	TOTAL P_2O_5	AVAILABLE PHOSPHORIC ACID (P_2O_5)		INSOLUBLE PHOSPHORIC ACID (P_2O_5)	
		OFFICIAL METHOD	MACINTIRE-SHAW-HARDIN METHOD	OFFICIAL METHOD	MACINTIRE-SHAW-HARDIN METHOD
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
	Collaborator—H. C. Batton				
2–12–4	12.63	11.50	12.20	1.13	0.43
2–12–4	12.88	11.60	12.33	1.28	0.55
2–12–4	13.28	11.97	12.50	1.31	0.78
2–12–4	13.61	11.81	12.55	1.80	1.06
2–8–3	8.93	7.43	8.55	1.50	0.38
2–12–4	13.43	12.04	12.56	1.39	0.87
2–12–4	13.69	12.40	12.77	1.29	0.92
2–12–6	13.20	11.91	12.81	1.29	0.39
	Collaborator—R. D. Caldwell				
Superphosphate	19.75	18.44	18.32	1.31	1.43
Florida Double Superphosphate	48.83	48.43	47.63	0.40	1.20
Tennessee Double Superphosphate	47.96	47.76	46.47	0.20	1.49
	Collaborators—R. C. Charlton and R. C. Levi				
Superphosphate	21.30	20.09	20.25	1.21	1.05
Superphosphate	19.85	18.96	19.00	0.89	0.85
Superphosphate	19.65	18.85	18.85	0.80	0.70
Superphosphate	21.25	20.32	20.35	0.93	0.90
Ammoniated Superphosphate	18.25	17.20	17.35	1.05	0.90
2–12–4	13.20	12.30	12.33	0.90	0.87
2–12–6	13.20	12.17	12.50	1.03	0.70
4–10–4	11.20	10.47	10.70	0.73	0.50
4–12–4	13.30	12.34	12.60	0.96	0.70
5–10–5	10.95	10.22	10.35	0.73	0.60

Table 3 gives results obtained in the determination of available P_2O_5 in the standard phosphate samples (Samples 1–5); in a superphosphate high in iron and aluminum ($Fe_2O_3+Al_2O_3=8.15\%$, Sample A-4); and in ammoniated superphosphates that had been reverted by storage for 6 months at 75° C. (Samples 71, 141, 151, 161, and 171). Higher availability values were obtained with the MacIntire-Shaw-Hardin method than with the official method for all the samples with one exception (Sample A-4). The procedure of simply digesting the sample at 30° C. with continuous stirring in 200 ml. of MacIntire-Shaw-Hardin's citrated ammonium nitrate solution (Method E, Table 3) gave promising results when applied to the standard samples, but the values obtained with this procedure were lower than those obtained with the official method for samples containing

TABLE 3.—*Available phosphoric acid in miscellaneous phosphatic materials as determined by various modifications of the official and MacIntire-Shaw-Hardin methods.*

(*1 gram taken for analysis.*)
(Analyses by L. F. Rader, Jr.)

SAMPLE NO.	PHOSPHATIC MATERIAL	AVAILABLE P_2O_5 (PER CENT OF THE TOTAL)					
		A	B	C	D	E	F
1	Ammoniated superphosphate	64.2	74.6	71.4	41.4	68.3	37.5
2	Tricalcium phosphate	58.7	96.7	82.1	34.8	81.6	36.7
3	Calcium hydroxyphosphate	24.4	60.0	46.6	19.7	45.1	22.5
4	Calcined phosphate	91.7	92.3	92.0	42.7	91.1	66.4
5	Tennessee rock phosphate	7.4	14.1	13.6	4.9	9.2	6.9
A-4	Tennessee superphosphate high in iron and aluminum	98.0	96.3	98.0	—	55.0	—
71	Ammoniated Nauru Island superphosphate	78.8	89.0	82.2	—	75.3	—
141	Ammoniated Florida pebble superphosphate	75.5	83.1	79.4	—	66.0	—
161	Ammoniated Florida pebble superphosphate	75.5	87.3	79.1	—	65.5	—
151	Ammoniated Tennessee superphosphate	71.7	86.7	81.4	—	65.1	—
171	Ammoniated Tennessee superphosphate	75.4	89.6	80.3	—	57.9	—

A—Official method.

B—Modified official method as adapted by Smith to the determination of available MgO in dolomite. The neutral NH_4 citrate solution was replaced with a 6% citric acid solution adjusted to a *p*H of 4.0 by the addition of NH_3.

C—MacIntire-Shaw-Hardin method.

D—Modified MacIntire-Shaw-Hardin method in which the sample is digested for 30 minutes at room temperature with continuous stirring in 100 ml. of citrated NH_4NO_3 solution without subsequent steam digestion.

E—Modified MacIntire-Shaw-Hardin method in which the sample is digested for 30 minutes at room temperature with continuous stirring in 200 ml. of citrated NH_4NO_3 solution without subsequent steam digestion.

F—Modified MacIntire-Shaw-Hardin method in which the sample is steam digested for 30 minutes in 100 ml. of citrated NH_4NO_3 solution without prior washing.

iron and aluminum. The modification of the official method adapted by Smith for determining available magnesium in dolomite gave results for available P_2O_5 that were considerably higher than those obtained with the official method.

RECOMMENDATIONS

It is recommended—

(1) That the words, "Place 1 g of the sample on a 9 cm filter and wash with successive small portions of H_2O, allowing each portion to pass thru before adding more, until the filtrate measures about 250 cc," *Methods of Analysis, A.O.A.C.*, 1935, p. 21, **13**, lines 1–3, be changed to read, "Place 1 g of the sample on a 9 cm filter and wash with successive small portions

of H_2O until the filtrate measures about 250 cc. Allow each portion of the wash water to pass thru the filter before adding more, and wash with suction if the washing would not otherwise be complete within 1 hour" (final action).

(2) That the words, "Heat 100 cc of the NH_4 citrate soln to 65° in a 250 cc flask . . . Shake the flask every 5 min.," *Methods of Analysis, A.O.A.C.*, 1935, sec. **16 (a)**, p. 22, lines 1–8, be changed to read, "After washing out the water-soluble P_2O_5, **13**, transfer the filter and residue, within a period not to exceed an hour, to a 250 cc flask containing 100 cc of the NH_4 citrate soln previously heated to 65° in a water bath. Close the flask tightly with a smooth rubber stopper and shake vigorously until the filter paper is reduced to a pulp, relieving the pressure by momentarily removing the stopper. Loosely stopper the flask to prevent evaporation and return it to the bath. Maintain the contents of the flask at exactly 65°, keeping the level of the H_2O in the bath above that of the citrate soln in the flask. Shake the flask every 5 min." (Final action.)

(3) That a collaborative study be made of the fineness to which fertilizers should be ground in the preparation of samples for analysis.

REPORT ON NITROGEN*

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

It was pointed out in last year's report, *This Journal*, **22**, 263 (1939), that the manipulative features of the present official method for determining water-insoluble nitrogen in organic materials appeared to be the chief source of disagreement in results among analysts. Preliminary work was done last year on the actual method of washing organic materials by five different procedures. Two of these procedures gave results that were more consistent and reproducible than those obtained by the official procedure. Hence the collaborative study carried on this year was confined to a comparison of these two procedures with the present official method.

The detailed instructions sent out to the collaborators are as follows:

INSTRUCTIONS TO COLLABORATORS

Three samples of organic fertilizer will be submitted; namely, foreign process tankage, ground fish, and cottonseed meal. Run each sample in triplicate for water-insoluble nitrogen by the procedures listed below.

EXPERIMENTS

Series I, Regular Official Method. Place 1 gram of the sample on an 11 cm. Whatman No. 2 filter paper, moisten with ethyl alcohol (about 5 ml.) and wash with

* Journal Series of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

distilled water (20°–25° C.) to 250 ml. The washing must be rapid, and new additions of water should be added as soon as the filter goes dry. Transfer the paper and residue to a Kjeldahl flask and determine the N as directed under **21** or **23**, page 24, *Methods of Analysis, A.O.A.C.*, 1935.

Series II, Regular Method (Modified). Proceed as directed in Series I, except to do the washing slowly by allowing the funnel to drain 5 minutes between washings.

Series III, Beaker Method. Place 1 gram of the sample in a 100 ml. beaker, moisten with ethyl alcohol (about 5 ml.), add 20 ml. of distilled water, and allow to stand 15 minutes, with occasional stirring. Transfer the supernatant liquid to an 11 cm. Whatman No. 2 filter paper and wash 4 or 5 times by decantation with distilled water (20°–25° C.). Finally transfer all the residue to the filter paper and complete the washing to 250 ml. by adding fresh additions of distilled water as soon as the filter goes dry. Transfer the paper and residue to a Kjeldahl flask and determine the N as directed under **21** or **23**, page 24, *Methods of Analysis, A.O.A.C.*, 1935.

Report the results in percentage of water-insoluble nitrogen, on the accompanying sheet.

The collaborators were the following:

(1) A. L. Prince.
(2) E. K. Rist, Bur. Chemistry and Soils, Washington, D. C.
(3) R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
(4) H. C. Batton, Swift and Company, Baltimore, Md.
(5) R. L. Johnson, Consolidated Rendering Company, Boston, Mass.
(6) W. L. Adams, Agr. Expt. Station, Kingston, R. I.
(7) C. W. Hughes, Agr. Expt. Station, Lafayette, Ind.
(8) P. McG. Shuey, Shuey and Company, Savannah, Ga.
(9) A. H. Allen, Virginia-Carolina Chemical Corp., Richmond, Va.
(10) E. F. Boyce, Agr. Expt. Station, Burlington, Vt.
(11) R. C. Koch, Swift and Company, Hammond, Ind.
(12) L. J. Hardin, Agr. Expt. Station, Knoxville, Tenn.

The data from the collaborative study for process tankage, ground fish, and cottonseed meal are compiled in Table 1. Under Series I are given the data for the present official method; under Series II, the official method is modified to allow for slow washing; and under Series III, the data are given for the beaker procedure. For the data presented in this table the wash water was maintained at a temperature of 20°–25° C. (normal room temperature).

Table 1 shows that there is comparatively little difference in the results by all three of these methods. The extreme values obtained by the collaborators on any one of the three series are quite close to one another for each material. The average results by all the collaborators, given at the bottom of the table, show that the variations in the figures are not significant for the ground fish and cottonseed meal. For the process tankage the average figures do indicate a more complete washing out of the soluble nitrogen by the slower washing method and beaker method (Series II and III). Thus the average result for water-insoluble nitrogen on the process tankage by the official method was 6.23 per cent; by the modified official method to allow for slower washing, 6.12 per cent; and by the

beaker method, 6.07 per cent. Of course, it was on porous, open-structured materials, where channels might easily be formed, that the greatest variation in results occurred when the present official method was used. The beaker procedure (Series III) largely overcomes this variability. It is particularly noticeable in the individual determinations made by the various collaborators. The collaborators were able to obtain closer results in their individual determinations by the beaker method (Series III) than by the procedures prescribed in Series I and II.

The data for process tankage, under Series II, where the official method is modified to allow for slower washing, also show improvement over those obtained by the official method, since there is a more complete washing out of the soluble nitrogen. This procedure, however, does not show as good results as the beaker method and is not so practical as far as the time element is concerned.

Material such as ground fish and cottonseed meal apparently yields equally as good results with any of the three methods, the average for the three series being 8.56, 8.52, and 8.56 for the fish, and 6.71, 6.69, and 6.72 for the cottonseed meal.

Although the results in general do not indicate that the present official method gives values that are seriously in error or of great variability, it does appear that the beaker procedure is a more logical and rapid method for extracting the soluble nitrogen and would also cover more satisfactorily those types of organic fertilizers that are open-structured and subject to channel formations in the process of washing.

TEMPERATURE OF WASH WATER

The question of the effect of the temperature of the wash water has also been raised, *This Journal*, **22**, 268 (1939). Some data were acquired on this point by three of the collaborators using wash water at 30° C. The results are reported in Table 1(b). It will be noted that here again it is only the results from the process tankage that have been affected at all by the increased temperature. Even with this material, the variation in results from those where wash water at 20°–25° C. was used, was only about 0.1 of 1 per cent. It is quite possible, however, that certain types of organic fertilizers would be more severely affected by extreme variability in the temperature of the wash water, and the Associate Referee believes it would be desirable to specify a definite temperature for the wash water in the methods; namely, 20°–25° C.

In last year's report on nitrogen, attention was called to the fact that an error had appeared in the preparation of the sodium hydroxide solution, par. **(g)**, p. 23. A recommendation correcting this error was accepted and should receive final action this year. Also, due to an oversight last year, a recommendation approved by first action in 1937 concerning an addition to the Devarda method, par. **19**, pp. 26–27, was not presented for final

adoption. It will be recommended this year. It reads as follows: "In the analysis of nitrate salts, proceed as above, but use 25 ml. of the nitrate solution equivalent to 0.5 gram of the sample."

TABLE 1.—*Per cent water-insoluble nitrogen in process tankage, ground fish, and cottonseed meal*
(Each result represents an average of three determinations.)

(a) Temperature of Wash Water 20°–25° C.

COLLABORATOR	PROCESS TANKAGE			GROUND FISH			COTTONSEED MEAL		
	SERIES 1	SERIES 2	SERIES 3	SERIES 1	SERIES 2	SERIES 3	SERIES 1	SERIES 2	SERIES 3
1	6.29	6.18	6.16	8.57	8.48	8.53	6.67	6.69	6.76
2	6.19	6.07	5.99	8.57	8.49	8.52	6.60	6.59	6.73
3	6.13	5.95	6.06	8.69	8.63	8.60	6.78	6.81	6.78
4	6.24	6.27	6.16	8.58	8.64	8.49	6.68	6.79	6.71
5	6.24	6.06	6.06	8.61	8.47	8.44	6.68	6.71	6.69
6	6.33	6.26	6.21	8.72	8.61	8.72	6.86	6.81	6.81
7	6.15	6.12	5.98	8.58	8.65	8.75	6.87	6.78	6.83
8	6.40	6.29	6.40	8.62	8.46	8.66	6.67	6.65	6.75
9	6.37	6.19	6.00	8.62	8.60	8.49	6.75	6.71	6.79
10	6.16	6.10	6.04	8.29	8.46	8.54	6.73	6.70	6.76
11	6.20	5.95	5.82	8.45	8.35	8.42	6.68	6.55	6.55
12	6.08	5.96	5.93	8.48	8.44	8.49	6.55	6.51	6.48
Average	6.23	6.12	6.07	8.56	8.52	8.56	6.71	6.69	6.72

(b) Temperature of Wash Water 30° C.

1	6.19	6.02	6.07	8.45	8.55	8.50	6.68	6.72	6.73
3	6.02			8.71			6.78		
9	6.13	6.10	5.92	8.58	8.54	8.57	6.67	6.68	6.70
Average	6.11	6.06	6.00	8.58	8.55	8.54	6.71	6.70	6.72

The question of the method for standardizing acid and alkali for use in the Kjeldahl method on fertilizers should be considered this year, since the section on standard solutions of the A.O.A.C. has already adopted official methods for the preparation and standardization of these solutions. For many years fertilizer chemists have standardized hydrochloric acid for nitrogen work by means of the silver nitrate method with very satisfactory results. In fact, in many fertilizer laboratories where ammonium hydroxide is used as the base in the nitrogen work, it is necessary to standardize the acid prior to the alkali by means of a direct gravimetric procedure. However, when sodium hydroxide is used as the base, it is equally as satisfactory and possibly preferable for the fertilizer chemist to standardize the alkali, first by means of potassium acid phthalate, and, subsequently the acid by titration. Consequently, the Associate Referee

recommends that an additional sentence be incorporated in *Methods of Analysis, A.O.A.C.*, 1935, under par. **19 (a)**, to provide for the alternative method of standardizing the hydrochloric acid according to the procedure given under **5** and **6**, Appendix 1, p. 682. A similar addition should be made to par. **19 (b)** on the standardization of sulfuric acid, to allow for the alternative methods of standardizing acid solution with borax or sodium carbonate as described in the Appendix.

Another reagent used in the nitrogen determinations that has caused some difficulty in preparation is methyl red. According to the directions given under par. **19 (i)**, 1 gram of methyl red is dissolved in 50 ml. of 95 per cent alcohol, diluted to 100 ml. with H_2O, and filtered. It has been found that when the water is added, a considerable amount of the methyl red salt is thrown out of solution. Most reference books specify a much weaker solution of this reagent for general use, such as a 0.2 per cent solution. This is also the strength solution specified under par. **55 (a)** in the determination of the "Acid-forming and non-acid-forming quality" of fertilizers. However, 8–10 drops of such a solution would be required to give the same intensity of color as 1 or 2 drops of a 1 per cent solution. This stronger solution is more desirable for nitrogen work. Hence, the Associate Referee believes that a 1 per cent solution of methyl red, made up in 95 per cent alcohol without further dilution, should be used.

During the past year a new catalyst has been suggested for use in the determination of total nitrogen by the Kjeldahl method, especially on cereal products. It is a copper powder manufactured by Charles Hardy, Inc., of New York. It is used with red mercuric oxide and potassium sulfate, and is said to reduce the period of digestion considerably. The Associate Referee has made a few preliminary determinations with this catalyst on a variety of organic fertilizer materials, and the results indicate that it warrants further study.

RECOMMENDATIONS[1]

It is recommended—

(1) That the beaker method for the determination of water-insoluble nitrogen, *This Journal*, **22**, 268 (1939), be adopted as official (first action).

(2) That in the Kjeldahl method for the determination of organic and ammoniacal nitrogen (p. 23, par. **19**), the last sentence in par. **19 (g)** be changed to read "A solution having a sp. gr. of 1.36 or higher may be used" (final action).

(3) That the following paragraph be added to the Devarda method (pp. 26–27, par. **33**): "In the analysis of nitrate salts, proceed as directed above, but use 25 cc. of the nitrate solution equivalent to 0.50 gram of the sample" (final action).

(4) That in the Kjeldahl method for the determination of organic and

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **23**, 50 (1940).

ammoniacal nitrogen (p. 23, par. **19 (a)**) after the heading, "*Standard hydrochloric acid*", the following portion of a sentence be added: "Proceed as directed under **5** and **6**, Appendix 1, or" (first action).

(5) That in the Kjeldahl method for the determination of organic and ammoniacal nitrogen (p. 23, par. **19 (b)**), after the heading, "*Standard sulfuric acid,*" the following portion of a sentence be added: "Proceed as directed under Appendix 1, or" (first action).

(6) That in the Kjeldahl method for the determination of organic and ammoniacal nitrogen (p. 24, par. **19 (i)**, line 2) the phrase "dilute to 100 cc. with H_2O" be deleted (first action).

(7) That the copper catalyst, suggested in this report for use in the determination of total nitrogen by the Kjeldahl method, be studied by the Associate Referee.

REPORT ON MAGNESIUM AND MANGANESE*†

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

This fifth report in a series on this topic is a continuation of the work published last year, *This Journal*, **22**, 270 (1939). Progress has been slow because it has been necessary to establish an agronomic background for evaluation of magnesium compounds, as well as to develop analytical methods. Work on manganese was begun in 1937, and has progressed more rapidly.

MAGNESIA

Emphasis has been placed on the selection of a rapid method for acid-soluble magnesia, and the development of the acid ammonium citrate solvent for active or available magnesia. A method for the water-soluble fraction has been studied.

COLLABORATIVE ANALYSES

Three samples containing magnesium and manganese were sent to collaborators, and the results for both elements are included in this section. The samples are described in Table 1. The mixtures were formulated with the object of giving the methods a rather severe test. Sample 1 is high in phosphates, ammonium salts, and potassium, but it was taken from a commercial formula. Sample 2 contains more organic matter than is customary for a 4–12–4 fertilizer. Sample 3 is purposely abnormal, but makes possible a study of the interference of zinc, copper, manganese dioxide, and the chloride ion. Zinc and copper are frequently added to special pur-

* A review of methods for magnesia, read before the Fertilizer Division of the American Chemical Society, Boston, Mass., September 11–14, 1939, has been published in *American Fertilizer*. Tables 3, 5, and 6 of this report are taken from that review by permission.

† Contribution No. 560 of this Station.

pose fertilizers in Florida, and may find a wider use. Manganese dioxide is less likely to be used, but it has been suggested for study with other manganese compounds. Chlorides interfere in the oxidation of manganese to permanganate unless removed.

TABLE 1.—*Samples for collaborative analysis*

SAMPLE	INGREDIENTS	POUNDS	MgO				Mn
			KIESERITE	DOLOMITE	NON-CARRIER	TOTAL	TOTAL
			per cent	*per cent*	*per cent*	*per cent*	*per cent*
(1).							
12-16-12	Nitrate of potash	500					
	Sulfate of ammonia	464					
	Diammonium phosphate	380					
	Triple superphosphate	284					
	Dolomitic limestone, 21.2% MgO	200					
	Kieserite, 24.67% MgO	100					
	Tecmangam, 24.84% Mn	72	1.23	2.12	0.28	3.63	0.89
(2).							
4-12-4	Superphosphate	1090					
	Muriate of potash	156					
	Sulfate of ammonia	180					
	Cottonseed meal	350					
	Urea	50					
	Dolomitic limestone	130					
	Tecmangam	44		1.38	0.41	1.79	0.54
(3).							
0-12-0	Superphosphate	1134					
	Zinc sulfate, C.P.	88					
	Copper sulfate, C.P.	40					
	Sodium chloride, C.P.	188					
	Dolomitic limestone	300					
	Kieserite	200					
	Pyrolusite, 54.1% Mn	50					
	Tecmangam	100	2.35	3.03	0.20	5.58	2.45
(4). Kieserite, contains more than normal moisture							
(5). Sulfate of potash magnesia							

Kieserite and sulfate of potash-magnesia were analyzed for water-soluble magnesium to complete the three years of collaborative work required for an official method. The kieserite, taken from a stored sample, contained more water than do fresh commercial stocks. This accounts for the low analysis.

The methods[1] are as follows:

(1) *Acid-soluble Magnesia by the Bartlett-Tobey Method.*—The procedure published in *This Journal*, **22**, 270 (1939) is shortened by elimination of a filtration previous to the precipitation of Ca oxalate. Reduce the aliquot to contain not more than 20 mg. of MgO. Partially neutralize this solution with NH_4OH before adding methyl red and change the quantity of NH_4 oxalate to 10 cc. for 50 cc. of solution. After evaporating the filtrate to a volume of about 100 cc., use 5 cc. of 10% citric acid in place of Na citrate, and eliminate the addition of 2 cc. of HCl.

In addition to the usual gravimetric method, the volumetric modification described under Method 2 was tried.

(2) *Acid-soluble Magnesia by the Shuey Method.*—Weigh 4 g. of sample into a 200 cc. volumetric flask, add 10 cc. of HNO_3 and 10 cc. of H_2SO_4, and boil until white fumes appear. Allow mixture to cool somewhat, add 50 cc. of water, boil for a few seconds, and allow to cool. Add 100 cc. of 95% alcohol, mix by giving the flask a rotary motion, and allow to stand 2 hours or longer, swirling several times during the first half hour. Make up to the mark with 95% alcohol, mix, and pass through a dry filter, making sure that the removal of calcium sulfate is completed. Pipet 25 cc. of the alcoholic solution into a 250 cc. beaker, place on water bath, and evaporate the alcohol slowly. Dilute with 75 cc. of water, washing down the sides of the beaker. Add 1 g. of citric acid, 10 cc. of a 10% solution of $(NH_4)_2HPO_4$, then add NH_4OH until alkaline to litmus. Cool by placing in cold water. Add NH_4OH, a few drops at a time, while stirring, until the precipitate begins to form. Stand 15 minutes and add 10 cc. of NH_4OH. Stir rapidly for 30 minutes or allow to stand at least 2 hours.

Gravimetric modification.—Treat the precipitate as described under the Bartlett-Tobey Method.

Volumetric modification.—Filter through an asbestos pad on Gooch crucible. Remove excess NH_3 by washing with a solution of equal volumes of alcohol and water (6–10 washings). Transfer pad and precipitate quantitatively to beaker with water. To a volume of approximately 50 cc. add sufficient 0.1 *N* H_2SO_4 from a buret to dissolve the precipitate, and use a small excess of the acid. Titrate the excess acid with 0.1 *N* NaOH, using methyl orange as an indicator. 1 cc. of 0.1 *N* acid = 0.00202 g. of MgO.

If Mn is present, add 1 cc. of H_2SO_4 to solution from above titration, and transfer to a 200 cc. volumetric flask. Make to volume, mix, and pipet 50 cc. of the clear solution into a beaker. Add 5 cc. of 85% H_3PO_4 and 0.3 g. of KIO_4 and heat for 30 minutes or until color development is complete. Dilute to a measured volume containing not more than 20 p.p.m. of Mn and compare with a $KMnO_4$ standard in a colorimeter.

Correct the previous titration, or calculated weight of MgO, for the Mn present, taking account of the dilutions. 0.00275 g. of Mn = 1 cc. of 0.1 *N* H_2SO_4 or 0.00202 g. of MgO.

(3) *Magnesia Insoluble in Acid Ammonium Citrate.*—

Acid-ammonium-citrate solution.—Dissolve 60 g. of crystallized citric acid in 900 cc. of water. Adjust to *p*H 4.0 with NH_4OH, using a standard potentiometric method; 20–30 cc. of NH_4OH will be required. Make to a volume of 1 liter with water. The density should be 1.025 at 20° C.

Determination.—Extract a 1 g. sample with water as directed under **II, 13**, *Methods of Analysis, A.O.A.C.*, 1935, p. 21. Proceed as directed under **II, 16**, substituting the acid NH_4 citrate solution for the neutral NH_4 citrate. Omit the di-

[1] Detailed changes advocated on the basis of this work appear under "Recommendations" later in the report.

rection for washing with 5% NH_4NO_3. Dry the filter and ash in a crucible. Place the crucible in a covered beaker and boil with 15 cc. of HCl (1+2). Remove the crucible, washing it with water. Unless more than 20 mg. of MgO is present, dilute to 50 cc. and determine MgO as directed by the Bartlett-Tobey method, beginning, "Partially neutralize with NH_4OH, and add a few drops of methyl red." If the MgO exceeds 20 mg., dilute to a convenient volume, take an aliquot, and proceed as directed above.

(4) *Water-soluble Magnesia in Magnesium Sulfate and Sulfate of Potash Magnesia.*—The portion of this method published in *This Journal*, **21**, 77 (1938), is changed to bring the determination of magnesia after solution in line with the Bartlett-Tobey method. This is a minor change to conserve space in *Methods of Analysis*.

(5) *Volumetric Periodate Method for Acid-soluble Manganese.—This Journal*, **21**, 277 (1938).

(6) *Colorimetric Modification of Method 5.*—Discussed in the report of last year for acid-soluble manganese. The method follows:

(A colorimetric method applicable to low percentages.)

Place 1 g. of sample in a 200 cc. wide-necked volumetric flask or a 250 cc. beaker. Add 10 cc. of H_2SO_4 and 30 cc. of HNO_3. Heat gently until brown fumes diminish, then boil 30 minutes. If organic matter is not destroyed, cool, add 5 cc. of HNO_3, and boil. Repeat this process until no organic matter remains, and boil until white fumes appear. Cool slightly and add 50 cc. of H_3PO_4 solution (90 cc. of H_2O, 10 cc. of 85% H_3PO_4). Boil for a few minutes. Cool, make to 200 cc. in a volumetric flask, mix, and let stand to allow precipitation of $CaSO_4$. Pipet 50 cc. of clear solution into a beaker. Continue as directed in the method for Mn in grain and stock feeds as modified in 1939, *This Journal*, **21**, 291 (1938); **22**, 279 (1939), beginning, "Add 0.3 g. of KIO_4 for each 15 mg. of Mn, etc." (at final dilution solution should contain not more than 20 p.p.m. of Mn). Calculate to percentage.

Collaborators submitting results are: W. A. Bridgers, Farmers Cotton Oil Co.; R. G. Kreiling and C. R. Byers, Armour Fertilizer Works; C. A. Butt and A. O. Hallman, Int. Agr. Corp.; E. J. Deszyck; W. Y. Gary, Florida Agricultural Department; John F. Hooper and C. Harry White, Maine Agricultural Experiment Station; L. F. Rader, Jr., U. S. Department of Agriculture; P. McG. Shuey, Shuey and Company; and Oscar I. Struve, Eastern States Coop. Milling Corp. To these collaborators, several of whom have assisted in this work since its beginning, and to others who have helped and advised concerning analytical and other phases of this problem, the writers gratefully ascribe the progress that has been made.

The results submitted are shown in Table 2, and are discussed with other pertinent data under the appropriate headings.

ACID-SOLUBLE MAGNESIA

A major objective this year was to choose a method that is shorter than the official method, and sufficiently accurate. The official method has been tested thoroughly, and was not again submitted to collaborative study, but determinations made in this laboratory are recorded in Table 2.

TABLE 2.—*Collaborators' results for MgO and Mn (per cent)*

COLLABORATORS	SAMPLES					
	1	2	3	1	2	3
			Official Method, MgO			
Deszyck	3.55	1.75	5.75	—	—	—
			Bartlett-Tobey Method, MgO			
	Gravimetric			*Volumetric*		
Bridgers	3.52	1.67	5.58	3.66	1.58	5.58
Butt-Hallman	3.68	1.97	5.94	3.67	1.90	5.86
Byers	3.98	1.99	6.05	3.92	1.92	5.97
Deszyck	3.47	1.85	5.67	3.51	1.66	5.55
Gary	3.55	1.97	5.36	3.43	1.87	5.78
Hooper	3.52	1.94	6.08	—	—	—
Rader	3.53	1.76	5.60	3.69	1.81	5.78
Shuey	3.80*	1.93*	5.72*	3.80*	1.90*	5.89*
Struve	3.58	1.81	5.78	—	—	—
White	3.68	1.95	5.81	—	—	—
Average	3.63	1.88	5.76	3.67	1.81	5.77
Recovery MgO(%)	100	105	103	101	101	103
			Shuey Method, MgO			
	Gravimetric			*Volumetric*		
Bridgers	3.59†	1.57	5.47	3.57	1.60	5.49
Butt-Hallman	3.54	1.80	5.85	3.47	1.76	5.86
Byers	4.02	1.97	6.03	—	—	—
Deszyck	3.48	1.75	5.74	3.54‡	1.81	5.89
Gary	3.51	1.79	5.53	3.55	1.77	5.70
Hooper	3.59	1.72	5.52	—	—	—
Rader	3.08	1.72	5.58	3.20	1.76	5.70
Shuey	3.67*	1.90*	5.70*	3.67*	1.81*	5.89*
Struve	3.56	1.78	5.80	—	—	—
White	3.59	1.72	5.52	—	—	—
Average	3.56	1.77	5.67	3.50	1.75	5.76
Recovery MgO(%)	98	99	102	96	98	103
			Acid-Soluble Mn			
	Volumetric			*Colorimetric*		
Bridgers	0.86	0.45	2.13	—	—	—
Byers	0.85	0.41	2.11	0.82	0.40	2.08
Deszyck	0.89	0.54	2.45	0.89	0.56	2.31
Gary	0.85	0.59	2.56	0.90	0.53	2.42
Rader	0.87	0.55	2.48	0.88	0.56	2.46
Shuey	0.89	0.52	2.51	—	—	—
Average	0.87	0.51	2.37	0.87	0.51	2.32
Recovery Mn(%)	98	94	97	98	94	95

* Mn correction from averages by other collaborators.
† Aliquot after brief standing in alcohol. After 12 hours, 1.31%.
‡ Aliquot after 2 hours in alcohol. After 16 hours, 2.62%. Two gram sample, 3 hrs. in alcohol, 3.68%.

TABLE 2.—*Continued*

COLLABORATORS	SAMPLES				
	1	2	3	1	2
	Citrate-Insoluble MgO			*Water-Soluble MgO*	
				Kieserite	*Sulfate of Potash-Magnesia*
Bridgers	—	—	—	23.79	10.32
Byers	0.81	—	—	24.40	10.34
Deszyck	0.83	0.75	1.66	24.29	10.31
Gary	0.82	0.71	1.53	24.14	10.15
Rader	0.85	0.87	1.67	24.46	10.24
Shuey	0.78	0.78	1.53	23.87	10.26
Struve	0.77	0.81	1.50	24.20	10.21
Average	0.81	0.78	1.58	24.16	10.26

Bartlett-Tobey Method.—This method, developed at the Maine Agricultural Experiment Station, has been mentioned frequently in previous reports, and has been accepted as a tentative method. It has been modified in detail, but not in essentials. The special steps for dehydration of silica found in the more conventional methods are omitted. Apparently the silica that is soluble in the acid digestion mixture is largely dehydrated at the boiling temperature of the solution. Originally the insoluble matter was removed before precipitation of calcium oxalate, but these steps were combined this year. Too great excess of ammonium oxalate for the single precipitation of calcium brings down magnesium, and this detail has been guarded. Partial neutralization of the acid solution before the indicator is added prevents oxidation of the methyl red.

Iron and aluminum are not removed, but are held in solution by the citrate ion. Magnesium ammonium phosphate is filtered after a single precipitation, and minimum periods for standing during the formation of precipitates are somewhat less than those usually prescribed. The precipitates can be ignited to the pyrophosphate by the customary procedure, or washed with 50 per cent alcohol and titrated acidimetrically to ammonium dihydrogen-phosphate, as suggested by Stolba,[2] Handy,[3] and more recently by Shuey.[4]

When the results in Table 1 are considered, it must be remembered that these samples were intentionally formulated to test the methods severely. All contain manganese, and the results have been corrected for this element in the final precipitate by the individual collaborators.

Because of interference by manganese and zinc, and other difficulties, the results are somewhat more variable than those of previous years. The averages are very consistent, but the average deviations from the mean

[2] *Chem. Centr.*, 728 (1866).
[3] *J. Am. Chem. Soc.*, **22**, 31 (1900).
[4] Private communication.

for Samples 1, 2, and 3 are 0.12, 0.09, and 0.17 per cent for the gravimetric modification; and 0.12, 0.10, and 0.12 for the volumetric method. Eleven of 51 determinations differ from the mean by 0.2 per cent or more, but nearly one-half of these are for Sample 3, a very abnormal mixture. By segregating results from laboratories most familiar with this method, only 2 of 27 results differ by as much as 0.2 per cent from a new mean calculated on this basis; both of these results are for Sample 3. It cannot be assumed from past performance that results by the more elaborate official method would show better agreement. The correction for manganese introduces a second error and to this is ascribed much of the difficulty. As with all methods, experience adds accuracy, and it is believed that this method will prove satisfactory.

The volumetric method, although by no means new, is unfamiliar to most analysts. Supplementing the collaborative results, the junior author and an assistant compared this procedure with the official and the gravimetric modification on 69 samples obtained from commercial brands sold in North Carolina and Maine. The results are summarized in Table 3. Averages for the three methods agree, and the average deviations from the official method are not large; but while less than one-fifth of the results by the gravimetric method differ from the official method by as much as 0.1 per cent, one-third fell in this class by the volumetric method. In general, the titration gives results more quickly than does the ignition method, but it has a tendency to give slightly lower and probably less uniform results. However, the collaborative results for two years have shown no very serious defects in the method, and they seem to justify its adoption as a tentative method. Further study of the solubility of ammonium magnesium phosphate in the alcohol wash is desirable.

TABLE 3.—*Comparison of the gravimetric and volumetric modifications of the Bartlett-Tobey method with the official gravimetric method. Analyses of 69 samples (1–8% MgO.)*

	OFFICIAL GRAVIMETRIC METHOD	BARTLETT-TOBEY METHOD	
		GRAVIMETRIC	VOLUMETRIC
Average MgO (%)	2.58	2.63	2.58
Average deviation from official method (%)		0.074	0.091
Number greater than official method		58	27
Number less than official method		10	40
Within ±0.10 of official method (%)		81	69
Within ±0.11 to ±0.20 of official method (%)		19	24
Within ±0.20 to ±0.35 of official method (%)			7

The Shuey Method.—This is an adaptation of the official method, and is published in a previous section in response to frequent requests that it be made available. The results in Table 2 and those published in the

previous report show that it is comparable in accuracy with the Bartlett-Tobey method, and it requires about the same working time. Like the Bartlett-Tobey method, either a gravimetric or volumetric modification may be used. Its principal defect is shown by the results and footnotes for Sample 1 in Table 2. From samples containing relatively large quantities of ammonium salts, and possibly potassium salts, magnesium is precipitated with calcium sulfate on standing in the alcohol solution. This precipitation may be minimized by reducing the sample weight and the time of standing, but an occasional unusual sample may show low results despite these precautions.

Previous remarks concerning the volumetric modification of the Bartlett-Tobey method apply equally to a similar modification of the Shuey method.

Collaborators were asked to express preference for either the Bartlett-Tobey or the Shuey method. Struve and Gary prefer the Bartlett-Tobey gravimetric method, while Bridgers likes the Shuey volumetric procedure, and has used it in routine analysis for a year. Rader found high results for a standard sample from the U. S. Bureau of Standards by both methods, and by the official method as well. The percentage error averaged 4 per cent. Deszyck has also found this average true for products with high percentages of calcium and magnesium. Rader considers 10 washings of calcium oxalate too many. White, Bridgers, and Deszyck noted precipitations of magnesium compounds with calcium sulfate from Sample 1 by the Shuey method from long standing in alcohol, and from the large sample prescribed. Bridgers and Hooper comment that the Shuey method does not destroy the organic matter from Sample 2 completely, and Bridgers supplemented the acid with sodium nitrate. White believes that silica may interfere in some instances with the Bartlett-Tobey method unless dehydrated more thoroughly. W. Catesby Jones[5] has reported comparisons of seven methods for acid-soluble magnesium tried at the laboratory of the Virginia Department of Agriculture and Immigration. From the standpoint of adaptation to routine conditions he concludes: "The Shuey and Bartlett-Tobey methods ran a pretty close race for preference. . . . The two methods receiving most favorable comment by the chemist who did the work were the Bartlett-Tobey and the Shuey methods. After considerable discussion we reached the following conclusion, namely, that the Bartlett-Tobey method, using the acidimetric titration suggested by Shuey, will be an ideal method for determining magnesia in fertilizer, particularly when phosphoric acid and possibly calcium are being determined in the same sample. The three determinations can be determined from the same solution, necessitating only one weighing."

H. D. Haskins and J. W. Kuzmeski state in a private communication,

[5] *Am. Fertilizer*, 91, No. 7, 5 (1939).

"The combination of the Bartlett-Tobey method with the volumetric adaptation suggested by Shuey seems to be the best method, and the one preferred in this laboratory."

From the evidence and opinions of collaborators, it appears that the Bartlett-Tobey and the Shuey methods are about equal in accuracy and time requirements. The Shuey method as now written meets the difficulty with samples high in ammonium salts, and other salts may contribute to the error, but this can be obviated by reducing the sample weight and the time of standing in the alcohol solution. The cost of alcohol may be important, especially in commercial laboratories. The deciding factor is the coordination of the methods for magnesium and calcium, a very desirable objective to keep in mind. For this purpose the Bartlett-Tobey method has the advantage, for calcium is precipitated as the oxalate rather than as the sulfate. It is possible that the first calcium oxalate precipitate might be titrated directly with permanganate. To avoid contamination with iron and aluminum, Hoffman and Lundell[6] have suggested that calcium oxalate be precipitated at *p*H 3 to 4 rather than at *p*H 5. W. Y. Gary approves of this suggestion. If manganese interferes it may require oxidation and removal. This step would also remove iron and aluminum and avoid the present manganese correction in the final magnesium precipitate. In the Shuey method the impure calcium sulfate precipitate requires solution and reprecipitation as the oxalate, an additional step. Since the Association prefers to authorize only the minimum number of methods to satisfy actual needs, it seems best to adopt only the Bartlett-Tobey method at present.

Manganese Correction.—The fractions of the manganese present in the sample that are precipitated with the magnesium ammonium phosphate are not constant. Averages of the corrections reported by the collaborators show a general agreement for the Bartlett-Tobey and the Shuey methods, equivalent to 73, 61, and 66 per cent of the manganese present in Samples 1, 2, and 3, respectively. Each precipitate suspected to contain manganese must be analyzed. After a little experience the analyst can tell from the amount of color developed whether the correction is significant. Rader and Deszyck reported incomplete oxidation of manganese in the precipitate from Sample 3 by the volumetric modifications of both methods until more acid is added than is prescribed, but others reported satisfactory results without comment. The ratio for acidity and manganese concentration during the oxidation with periodate needs further study.

Zinc Interference.—Gary believes that zinc compounds contaminate the magnesium ammonium phosphate precipitate unless it is eliminated by reprecipitation and presents the data in Table 4 to support his belief. The differences are not large, perhaps not greater than might be developed by double precipitation for other samples free from zinc, but

[6] *J. Research, National Bureau of Standards*, 20, Research Paper RP1095 (1938).

TABLE 4.—*Effect of double precipitation of $MgNH_4$ phosphate from samples containing zinc.*
(Data furnished by W. Y. Gary)

SAMPLES	ADDED ZnO	MgO SINGLE PRECIPITATION	DOUBLE PRECIPITATION
	per cent	*per cent*	*per cent*
1	0	4.10	4.10
2	0	2.94	2.94
3	0.1	4.26	4.22
4	1.1	5.80	5.69
5	0.8	1.02	0.95
6	1.0	2.73	2.67

they indicate a possible source of contamination. Collaborative Sample 3 was formulated to test this difficulty. The averages of the results submitted show consistently high recoveries of magnesia. The differences are not significant, however, when compared with the general error of the methods. Deszyck analyzed this sample by the Bartlett-Tobey method, leaving out zinc sulfate as an ingredient, but including all other components from the same stocks that were used originally. After calculating the results on the basis of the entire formula, he found no error caused by zinc. The results with and without the zinc carrier were identical. Lundell and Hoffman[7] include zinc among the elements held in solution by the citrate ion. It seems unlikely that amounts of zinc equivalent to one per cent of zinc oxide or less in a fertilizer will cause serious difficulty with either of the methods discussed.

ACTIVE OR AVAILABLE MAGNESIA

This is the most difficult fraction to define and determine. The writers[8] have attempted a summary of the status of this topic recently, and a paper, on the same subject is to be read by E. R. Collins at this meeting (see p. 373). Therefore, only a brief outline will be included in this report.

Probably all will agree that water-soluble compounds are completely available at the time of application. Magnesium phosphates and magnesium ammonium phosphate in quantities greater than appear in fertilizers are soluble in neutral ammonium citrate and classify the phosphate as available. If the phosphate portion of the molecule is available, the magnesium must be, also.

Hydrated dolomites, selectively calcined dolomites, and magnesium oxide are more reactive than dolomitic limestone, and are assumed to be relatively available substances. The portion changed to phosphates by fertilizer reactions would be fully available.

The greatest uncertainty is caused by the cheapest source of mag-

[7] Outlines of Methods of Chemical Analyses, page 70. John Wiley and Sons, Inc. (1398).
[8] *Loc. cit.*

nesium, dolomitic limestone. This is accepted universally as a liming material, but many question its adequacy as a single source of magnesium in a fertilizer mixture, especially for seedling stages of growth before the limestone has had sufficient time to decompose, for extreme deficiency, for sandy soils, for soils of low acidity, and for surface application. It has been proved that dolomites in contact with soil are less reactive than calcic limestones, but that the rate of decomposition is increased by fine grinding, soil acidity, and acid-forming fertilizers. Other factors are the physical and chemical constitution of the limestone, buffer capacity of the soil; and, probably, soil temperatures, moisture, biological state, and the action of plant roots.

Obviously, no single laboratory method can reflect all these variables. If a method is desired, it must be based on average conditions, and interpreted on that basis.

In the paper mentioned previously, the writers attempted to make a summary of the published data on this topic. Only the portion bearing directly on the rate of decomposition during a single growing season will be repeated here. Several methods have been used to determine this. Probably the determination of undecomposed carbonates at intervals is considered the most reliable method. Others are the relative removal of magnesium by plants, comparison of the unknown compounds with sources known to be available, relative effect on soil *p*H, and the relative increase of water-soluble magnesium in soil, as compared with soluble magnesium compounds. Most of the work has been done with pot cultures.

The data available are grouped arbitrarily in Table 5, with citations of the sources. All methods were given equal weight in calculating the averages, but the data based on carbonate residues were more numerous, and have the greatest influence on the averages. Many of the results are based on three dolomites furnished by W. H. Ross. One of these is a composite from three quarries, and the results have been considered as the averages of three dolomites. The qualitative effects of soil acidity, particle size, individual dolomite, and time are evident. The quantitative averages in many instances are calculated from widely varying data, few soils and dolomites, insufficient numbers of trials, and varying methods of application and measurement of decomposition. Further work will provide more reliable data, but these indicate the general trends to be expected.

Attention is directed particularly to the averages for soils from *p*H 5 to 5.6, in contact with dolomites for 50–85 days. These are considered important for several reasons. Magnesium deficiency is not restricted to soils in this category, but much of it occurs here. Less acid soils are less likely to be deficient, unless the calcium is so high as to decrease the availability of the magnesium present. The time period is sufficient to include the most critical portion of the growth of most crops. The soils used were

TABLE 5.—*Rate of decomposition of dolomite in soil**

Decomposition of separates (per cent)

SOIL pH	DAYS	20–40 MESH			40–60 MESH			60–80 MESH			80–100 MESH			100–200 MESH			FINER THAN 100			FINER THAN 200			MIXTURE OF SEPARATES			MILL-RUN		
		DOLOMITES	SOILS	PER CENT	DOLOMITES	SOILS	PER CENT	DOLOMITES	SOILS	PER CENT	DOLOMITES	SOILS	PER CENT	DOLOMITES	SOILS	PER CENT	DOLOMITES	SOILS	PER CENT	DOLOMITES	SOILS	PER CENT	DOLOMITES	SOILS	PER CENT	DOLOMITES	SOILS	PER CENT
4.5–4.9	53	—	—	—	2	2	53	—	—	—	2	2	65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4.5–4.9	79	3	2	26	3	2	48	3	2	74	3	2	81	3	2	93	—	—	—	3	2	99	5	2	70	—	—	—
5–5.6	24– 35	4	1	2	—	—	—	4	1	20	3	1	52	—	—	—	4	1	70	—	—	—	4	1	40	—	—	—
5–5.6	50– 65	10	5	19	4	3	23	9	4	39	5	3	52	5	3	58	5	2	66	3	2	77	9	3	44	1	1	26
5–5.6	72– 85	7	6	19	5	5	40	7	6	52	5	5	57	5	5	70	4	1	99	5	5	82	9	6	56	—	—	—
5–5.6	171–176	5	2	37	1	1	34	1	1	64	—	—	—	—	—	—	5	2	93	—	—	—	4	1	76	1	1	43
6.1	63	1	1	15	1	1	10	—	—	—	—	—	—	—	—	—	1	1	22	—	—	—	—	—	—	1	1	18
6.1	114	1	1	12	1	1	21	—	—	—	—	—	—	—	—	—	1	1	47	—	—	—	—	—	—	1	1	21
6.1	176	1	1	12	1	1	22	—	—	—	—	—	—	—	—	—	1	1	57	—	—	—	—	—	—	1	1	20

* Summary of published data from the following sources: Morgan and Salter, *Soil Sci.*, **15**, 293–305 (1923); Taylor and Pierre, *J. Am. Soc. Agron.*, **27**, 764–773 (1935); Proc. 1st Ann. Meet. Comm. on Fertilizers, *Am. Soc. Agron.*, 1935; Dawson et al., Collins and Spear, *This Journal*, **22**, 137–147 (1939), and unpublished data obtained by the writers. The various technical methods have been given equal value in calculating averages and a single widely used sample made by compositing three dolomites was considered to be three separate dolomites.

of medium texture, sandy loams to very fine sandy loams. Unfortunately, data were not found for soils of *p*H 5.6 to 6, for this is an equally important group. Decomposition would be slightly slower for such soils; also for more sandy soils. Heavy, more highly buffered soils should prove more active in dolomite decomposition.

When all these factors are taken into account, it seems conservative to estimate that ordinary dolomites in the average soil at *p*H 5–5.5 for two or three months will decompose at about the following rate: 20–40 mesh, 15 per cent; 40–60 mesh, 25 per cent; 60–100 mesh, 50 per cent; 100–200 mesh, 60 per cent; finer than 200 mesh, 75 per cent. Criticism and revision of this tentative estimate are invited.

Further confirmation of these estimates is furnished by MacIntire and Shaw.[9] This work was not included in the grouping of results, because the *p*H of the soil used was not known. This has since been supplied by the authors. Dolomite equivalent to 3570 pounds of calcium carbonate was placed in the upper half of fallow pots containing fairly well buffered clay loam, although a lighter phase than many clay and silt loams of the region. The *p*H was 5.6. The pots were sunk in soil April 1, and watered only by natural rain fall. Determinations of residual carbonates showed the following rates of decomposition: 20–40 mesh, May 1, 4 per cent; June 1, 17 per cent; July 1, 30 per cent. At the same dates the decomposition of 60–100 mesh was 30, 43, and 51 per cent, respectively.

To make these estimates useful some laboratory method is necessary for fertilizer mixtures. It must dissolve water-soluble compounds, phosphates, and portions of dolomite to correspond with the fractions suggested above, or others that may be selected. Several solvents have been tried. Shaw, *This Journal*, **22**, 237 (1939), finds that steam distillation of dolomite with ammonium chloride and titration of the distilled ammonia give results proportionate to particle surface. He suggests this as a possible measure of limestone availability.

Whittaker, Rader, and Zahn, *Ibid.*, 180, compared the solubility of a 100-mesh dolomite alone, and mixed with monocalcium phosphate, superphosphate, and double superphosphate. Their solvents were 2 per cent citric acid, the official neutral ammonium citrate, a citrate and ammonium nitrate solution suggested by MacIntire, Shaw, and Hardin[10] for phosphates, and ammonium citrate at *p*H 4 as suggested by the same authors in previous reports. For use in the determination of available magnesium, the 2 per cent citric acid dissolved too much of the dolomite, and the neutral ammonium citrate too little. Of the other two solutions, digestion with the citrate and ammonium nitrate solution dissolved the more magnesium. Which is more nearly a measure of the reactivity of this particular dolomite in soil cannot be stated. From the data in Table 5 either might apply.

[9] *J. Am. Soc. Agron.*, **22**, 272 (1929).
[10] *Ind. Eng. Chem., Anal. Ed.*, **10**, 143 (1938).

Four per cent citric acid brought to *p*H 4 with ammonium hydroxide, also studied by Kuzmeski, *This Journal*, **22**, 147, 270 (1939), showed promise, but did not give satisfactory duplication in other laboratories, and manipulation near the boiling point of the solvent proved undesirable. Since all fertilizer laboratories maintain a constant temperature bath at 65° C. for the determination of available phosphoric acid, several concentrations of ammonium citrate were tried for one hour at this temperature, and the acidity was varied. The dolomites and fertilizer mixtures were those furnished by W. H. Ross and described by Dawson, Snyder, Leighty, and Reid, *Ibid.*, 137, also by Collins and Speer, *Ibid.*, 142. They form the basis of many of the results in Table 5. The greatest success was had by treating leached samples with 6 per cent citric acid brought to *p*H 4 with ammonium hydroxide. The results, which appear in Table 6, show a considerable degree of correlation with the tentative estimate of the decomposition of the various separates of average dolomites as deduced from the data in Table 5. As noted in previous work and by Whittaker et al. in the paper cited previously, the dolomite is more reactive alone than in the fertilizer mixture. This fact makes it impossible to estimate the solubility of dolomite in a mixed fertilizer from a preliminary analysis of the limestone, and reduces the usefulness of the method materially. Greater accumulation of calcium citrate in the solution from fertilizers than from the 0.2 gram samples of dolomite is a possible cause. If true, it may be possible to overcome the difficulty by adding calcium phosphate to the solution when working with dolomite alone, and thus create conditions more similar to the leached residues that react when fertilizers are used as samples. This detail is now being studied. In its present stage the method is published as Method 3 of this report. Collaborators' results (Table 2) show excellent agreement. However, if the citrate-insoluble magnesia from ingredients other than dolomite are subtracted from the averages of these results, the same dolomite in the three samples shows different degrees of reactivity. Of the magnesia added in dolomite, 66, 55, and 50 per cent were decomposed for Samples 1, 2, and 3, respectively. Sample 1 contains triple superphosphate and is composed largely of water-soluble ingredients. Samples 2 and 3 have superphosphate, and Sample 2 has cottonseed meal.

Apparently the decomposition of dolomite in these mixtures was affected by the different water-insoluble ingredients. This condition must be studied further and overcome if possible.

Manganese, even manganese dioxide, did not interfere significantly. Rader found that bromophenol blue and bromocresol green show salt effect with the ammonium citrate solutions, causing results 0.2 *p*H higher than those by the glass electrode. Deszyck has been unable to confirm this, but such errors are to be expected. Until this can be investigated

TABLE 6.—*Correlation of the rate of decomposition of dolomite in soil, and solubility in an acid ammonium citrate solution by Method 3 of this report*

DOLOMITE*	MESH	DECOMPOSITION IN SOIL†	SOLUBILITY	
			WITH 6-8-6 FERTILIZER	ALONE‡
		per cent	*per cent*	*per cent*
A	20– 40	15	21	41
A	40– 60	25	33	51
A	60– 80	50	41	71
A	80–100		50	70
A	100–200	60	52	76
A	Finer than 200	75	78	93
A	Mixture of separates	50	46	68
B	Mixture of separates		64	74
C	Mixture of separates		26	52

* Dolomites and 6-8-6 fertilizer base supplied by W. H. Ross, U. S. Dept. of Agriculture for many of the soil studies summarized in Table 5. A is a composite of 3 widely used dolomites from different quarries; B, a very soluble dolomite; and C, a less soluble type. Fertilizer base contains ammoniated superphosphate, C.P. ammonium sulfate, and potassium chloride.

† From Table 5. Data not restricted to the dolomites listed in this table.

‡ 0.2 gram charge.

more thoroughly, only standard potentiometric methods for adjusting the solution to *p*H 4 are advised.

The changes made this year seem to be improvements, and some analysts have urged recommendation of the technic as a tentative method. Others, however, believe that the variability of soil conditions affect the decomposition of dolomite to such a degree that interpretation of the results by this method would be impossible. Another criticism is that the "availability" is not effective at once, but only over the entire season. If dolomite is the only source of magnesium, seedlings might not receive a sufficient supply. The Associate Referee recognizes these limitations and others. He has stated many times that if a method is desired, it must be accepted by a sufficient number of agronomists and soil and fertilizer chemists to give it weight. It is evident that there is not sufficient support at present to justify more than continuation of this work.

WATER-SOLUBLE MAGNESIA

The method for magnesia in water-soluble carriers before admixture with other ingredients has been changed in a minor detail to allow determination of the dissolved magnesia by the Bartlett-Tobey method, now tentative, but expected to become official. This is to simplify publication and for laboratory convenience. The results reported this year are in excellent agreement for sulfate of potash magnesia. In general the results for kieserite are in agreement, although two analysts find less than the others. The sample was taken from a stock that had adsorbed considerable moisture. It was dried for several days at 100° C., reground, and

mixed thoroughly, but still contained more than the usual water of crystallization, and may have been less uniform than is desired. In general, the method as now written seems satisfactory.

No work was done this year on water-soluble magnesia in mixtures. Previous work has shown that boiling a sample with water will recover added water-soluble compounds, and include water-soluble magnesium phosphates that result from reactions with various magnesium carriers, and appreciable quantities of compounds usually considered insoluble, such as dimagnesium phosphate and magnesium ammonium phosphate. Increasing the volume of water will increase the quantities of these compounds of low solubility that may be included in this fraction.

Two products that are new as fertilizer ingredients introduce uncertainty with respect to this method. These are the selectively calcined dolomite described by MacIntire, Hardin, and Oldham,[11] and magnesium oxide obtained from sea water after removal of the sodium chloride.[12] Whittaker, Rader, and Zahn[13] found this material very reactive in fertilizer mixtures, and discuss its possibilities and limitations. Both these products are very reactive in fertilizers, and doubtless in soils as well. However, boiling a sample containing these ingredients with water will carry the reactions farther than could happen in dry mixtures, and this may be true to a lesser extent with wet mixtures. Thus, water-soluble products might be created by the technic. If these compounds form with the same rapidity in the soil, or if magnesium oxide is agronomically equivalent to water-soluble carriers, this method of measurement would be feasible. At present this appears to require proof.

MANGANESE

The question of the relative usefulness of manganese compounds has not been raised, which makes this problem more simple than the work with magnesium. The work has been a continuation of that reported last year. Methods have been rewritten in minor detail for clarity and accuracy. The most troublesome factor in the periodate oxidation is the relation between acidity and manganese concentration, and it is hoped that this is controlled by the methods as now written. Gary reports that 0.3 gram of potassium periodate for 15 mg. of manganese was insufficient, especially for Sample 3, but others obtained very satisfactory results and reported no difficulty.

The results in Table 2, both by the volumetric and the colorimetric modifications, show satisfactory agreement. Apparently copper sulfate equivalent to 0.5 per cent cupric oxide caused no trouble in the colorimetric method, although this element in sufficient concentration must interfere because of its color. Slightly less than the calculated percentage

[11] *Ind. Eng. Chem.*, 30, 651 (1938).
[12] *Am. Fertilizer*, 91 (No. 8), 14 (1939).
[13] *Ibid.*, No. 6, p. 9.

of manganese was recovered, but the differences are within allowable limits for such methods. Pyrolusite is not expected to become a fertilizer ingredient, but would be included by these methods if present.

The volumetric method is necessary for the analysis of manganese carriers, and mixtures high in manganese, and is applicable to fertilizers in general. It is preferred by Gary to the colorimetric method. Rader emphasizes the fact that the ferrous sulfate solution is unstable, even in dilute sulfuric acid solution, and requires frequent restandardization.

The colorimetric modification is simple, and much more rapid than the volumetric method. It appears to work well with manganese up to 2 per cent. To save space in *Methods of Analysis, A.O.A.C.*, 1940, it has been written in conjunction with the method for manganese in stock feeds, *This Journal*, **22**, 78 (1939), adopted last year as tentative and rewritten to change minor details this year.

Both modifications discussed in this section appear to satisfy a need, and are recommended.

RECOMMENDATIONS[14]

It is recommended—

(1) That the method, entitled "Magnesia in water-soluble compounds," *This Journal*, **21**, 77 (1938), adopted as official (first action) last year be brought in line with the Bartlett-Tobey method as recommended this year, by deletion of the remainder of the method after the words, "Transfer an aliquot," in line 2, and substitution of the words, "continue as in the Bartlett-Tobey method, beginning "Transfer an aliquot containing not more than 20 mg of MgO." That, with this change, the method be adopted as official (first action).

(2) That the Bartlett-Tobey method for acid-soluble magnesia, *This Journal*, **22**, 270 (1939), adopted as tentative last year, be changed as follows: Lines 2, 3, delete "filter thru a dry filter paper, and transfer a 100 cc aliquot to a 400 cc beaker." Substitute, "and transfer an aliquot of the clear soln containing not more than 20 mg of MgO to a beaker. Partially neutralize with NH_4OH."

Line 5, change to read, "add 10 cc of a saturated soln of NH_4 oxalate for each 50 cc of soln." Lines 10, 11, delete "To the filtrate add 2 cc of 10% HCl" and change to read, "evaporate the filtrate to a volume of approximately 100 cc and add 5 cc of a 10% citric acid soln." Line 13, delete "If the fertilizer does not contain soluble phosphoric acid." That with these changes the method be adopted as official (first action).

(3) That the section of this report labeled "*Volumetric Modification*" under Method 2 of this report and changed in line 1 to read, "Filter the precipitate of $MgNH_4PO_4$ from the Bartlett-Tobey method through an asbestos pad on a Gooch crucible" be adopted as a tentative method for acid-soluble magnesium.

[14] For report of Subcommittee A and action by the Association, see *This Journal*, **23**, 50 (1940).

(4) That the study of methods for acid-soluble, water-soluble, and active magnesia in fertilizers be continued.

(5) That the method entitled "Acid-soluble manganese in fertilizers and manganese salts," *This Journal*, **21**, 292 (1938), changed last year to include 25 cc of H_2SO_4, per liter of 0.0910 *N* $FeSO_4$, be further changed as follows: Line 11, delete entire line, "either as the dry salt, or more conveniently from a solution of known concentration." Line 12, change to read, "Heat below the boiling point." Line 16, delete the word "thick." That with these changes, the method be adopted as official (first action).

(6) That the colorimetric method for acid-soluble manganese published as Method 6 of this report be adopted as tentative.

(7) That methods for manganese in fertilizers be studied further.

REPORT ON POTASH

By O. W. Ford (Purdue University Agricultural Experiment Station, West Lafayette, Ind.), *Associate Referee*

In accordance with the recommendations of the Association, *This Journal*, **22**, 288 (1939), referee work was conducted this year both by questionnaire and by collaboration. To obtain information on the use of factor weights and factor pipets and the need for additional platinum solution concentrations the following questionnaire was sent to 68 control officials and commercial chemists:

Questionnaire on Potash in Fertilizers

(5) Many have urged that the use of factor weights and factor pipets be considered as an option in the determination of potash in fertilizers. If you consider the use of either or both to be an advantage, please check below and return.

a. I consider the use of a factor weight an advantage. Yes or No. If so, list weight and conditions of use.

b. I consider the use of a factor pipet an advantage. Yes or No. If so, list size and calculations.

(9) Many have indicated that there is a need for additional platinum solution concentrations beyond the 1 and 0.2 gram platinum in 10 ml. now provided. If such is your opinion, please check below and return.

a. A platinum solution concentration *should* or *should not* be provided between 1 and 0.2 gram in 10 ml.

b. A platinum solution concentration *should* or *should not* be provided lower than 0.2 gram platinum in 10 ml.

c. The present platinum solution concentrations are *adequate* or *not adequate*.

A summary of the replies to parts a and b of section (5) of the questionnaire appears in Table 1.

Of the five collaborators favoring a factor weight, one suggested the use of 1.9376 grams made up to 200 ml. instead of the official 2.5 grams to 250 ml. Another suggested that both a factor weight and a factor pipet

TABLE 1.—*Use of factor weights and factor pipets*

NUMBER OF QUESTIONNAIRES SENT OUT	NUMBER RET'D		NUMBER NOT REPLYING	NUMBER FAVORING FACTOR WEIGHTS A		NUMBER FAVORING FACTOR PIPETS B	
	MARKED	UNMARKED		YES	NO	YES	NO
68	43	4	21	5	35	4	34

might be an advantage if they were not too small. Of the four favoring the use of factor pipets three favored a pipet of 19.38 ml. capacity (C. M. Bible patent) or some multiple of it and they used it as shown in Table 2.

TABLE 2.—*Illustrating use of factor pipets*

POTASH IN MATERIALS	SOLUTION USED	SAMPLE REPRESENTED	TO CONVERT WEIGHT K_2PtCl_6 TO % K_2O DIVIDE BY
per cent	*ml.*	*gram*	
up to 4	58.15	0.5814	0.03
4 to 20	38.76	0.3876	0.02
above 20	19.38	0.1938	0.01

Although the number favoring the use of factor weights and factor pipets is relatively small compared to those opposing their use, there seems to be sufficient argument in their favor to consider them as options.

A summary of the replies to parts a, b, and c of section (9) of the questionnaire appears in Table 3.

TABLE 3.—*Need for additional platinum solution concentrations*

NUMBER OF QUESTIONNAIRES SENT OUT	NUMBER RET'D		NUMBER NOT REPLYING	PART A		PART B		PART C	
	MARKED	UNMARKED		YES	NO	YES	NO	YES	NO
68	42	2	24	4	35	1	37	34	4

Although most of the replies indicate that the present platinum solution concentrations are adequate and many indicate that the matter should be left to the discretion of the individual chemist four replies indicated that a concentration about midway between the two present concentrations should be permitted. Therefore it will be so recommended.

PLATINUM RECOVERY

In order to recommend one or more methods for the recovery of platinum, copies of the four methods presented at the 1938 meeting were sent to 14 different chemists with instructions as follows:

(7) Prepare at least 50 ml. platinum solution representing 5 grams of metallic platinum by each of the enclosed four methods, listing below remarks and choice of method or designating a method of your own choice (X).

a. I consider method 1) 2) 3) 4) or _____ satisfactory.
b. I consider that this should be entirely optional. Yes or No.

The response to this part of the work was not favorable. One direct reply was received. This favored Method 2, with a substitution of *aluminum* in place of *zinc* in order to get away from impurities introduced by zinc. Indirectly there were four other replies stating that the recovery of platinum should be left to the discretion of the individual chemist.

In view of the lack of direct response it will be recommended that the four methods reported in 1938 be held as tentative and that this study be continued.

PREVENTION OF FOAMING

In accordance with the recommendation that a collaborative study be made of some modification of the present official method to prevent foaming during the boiling of the sample, three samples of fertilizer and a small bottle of defoamer were sent to 14 different chemists with the following instructions:

(10) To prevent foaming during the preparation of the sample some have suggested the addition of a small amount of a defoamer. To check the effect of this defoamer, determine the potash on Samples 97, 98, and 99 as follows:

a. Prepare six different solutions on each of the above samples and determine the potash, using the regular method of preparation of solution (no defoamer added).
b. Prepare six different solutions on each of the above samples and determine the potash, adding 1 ml. of the defoaming solution (defoaming solution, *20* grams of diglycol stearate dissolved in[1]——) before the solution is heated.

List the individual determinations of each sample by each method and any comment pro or con.

K_2O value of Sample 97 is about 6.0%.
K_2O value of Sample 98 is about 20.0%.
K_2O value of Sample 99 is about 4.0%.
Please find enclosed small sample of defoamer.

It will be noted (Table 4) that the averages in 7 out of 9 cases indicate that slightly higher results were obtained when a defoamer was used, and in the other 2 cases the difference was not great enough to be significant.

Although a few collaborators preferred a different defoamer from the one tried, nearly all replies were to the effect that it was a decided help. One chemist considered that a better extraction was obtained because the material stayed down in the solution better during the boiling of the sample. Another chemist hesitated to report his results as the averages for the two procedures were suspiciously alike. He used two other defoamers to good advantage. These were 1 drop of mineral oil and tributyl citrate, but no definite concentration was mentioned in the latter case. Generally, the results indicate that a small quantity of defoamer does not seriously affect the potash determination, and in many cases is a

[1] 1 liter of mixture of equal parts benzol and 95% ethyl alcohol.

TABLE 4.—*Collaborative study on the effect of a defoamer**

ANALYST NUMBER	NUMBER OF ANALYSES	SAMPLE 97 HIGH	SAMPLE 97 LOW	SAMPLE 97 AVERAGE	SAMPLE 98 HIGH	SAMPLE 98 LOW	SAMPLE 98 AVERAGE	SAMPLE 99 HIGH	SAMPLE 99 LOW	SAMPLE 99 AVERAGE
					Use of a defoamer					
3	3	5.78	5.60	5.70	19.86	19.50	19.70	5.40	5.02	5.29
7	6	6.47	5.77	6.06	20.16	19.38	19.76	5.73	5.52	5.60
8	2	5.98	5.88	5.93	19.68	19.46	19.57	5.50	5.40	5.45
11	6	6.07	5.80	5.96	19.92	19.92	19.83	5.40	5.24	5.33
13	2	6.06	6.01	6.04	19.92	19.62	19.77	5.57	5.50	5.54
15	11	6.13	5.84	5.98	20.30	19.52	19.86	5.76	5.59	5.67
16	3	5.57	5.44	5.52	19.16	19.03	19.08	5.58	5.36	5.44
17	6	6.05	5.89	5.98	19.65	19.49	19.58	5.58	5.43	5.49
18	2	5.96	5.94	5.95	20.90	20.80	20.85	5.76	5.70	5.73
19	6	6.09	5.88	6.00	20.04	19.76	19.86	5.69	5.58	5.66
Average		6.02	5.81	5.92	19.96	19.63	19.79	5.59	5.43	5.52
					Without using a defoamer					
3	3	5.74	5.50	5.65	20.18	19.40	19.75	5.33	5.15	5.22
7	6	6.15	5.97	6.05	20.30	19.38	19.81	5.71	5.46	5.60
8	2	6.00	5.98	5.99	19.64	19.54	19.59	5.56	5.33	5.45
11	6	6.17	5.99	6.05	20.04	19.52	19.80	5.78	5.37	5.51
13	2	6.01	5.90	5.96	20.06	19.84	19.95	5.68	5.45	5.57
15	12	6.11	5.80	5.98	20.26	19.40	19.88	5.65	5.39	5.52
16	3	5.35	4.81	5.07	19.48	18.91	19.12	5.10	4.91	5.01
17	6	6.08	5.93	5.99	19.65	19.45	19.56	5.58	5.43	5.49
18	2	5.91	5.88	5.90	20.93	20.85	20.89	5.69	5.62	5.66
19	6	6.16	5.95	6.08	20.02	19.76	19.94	5.63	5.55	5.59
Average		5.96	5.76	5.87	20.05	19.60	19.83	5.56	5.36	5.46

* Defoamer concentration—20 grams diglycol stearate (tech.) in 1 liter of mixture of equal parts benzol and 95% ethyl alcohol. 1 ml. of defoamer used per determination.

decided help. Diglycol stearate will therefore be recommended to prevent foaming.

DISSOLVING AND REWEIGHING K_2PtCl_6 *VERSUS* FILTRATION AFTER IGNITION AND SOLUTION

In accordance with the recommendation that a study be made of the determination of potash by dissolving out the potassium chloroplatinate and reweighing in place of "filtration after ignition and solution," Sample 96 was sent to 14 chemists with the following instructions:

Prepare a sufficiently large composite solution from Sample 96 for twelve potash determinations as follows.

a. Make twelve potash determinations by the regular method, making no correction for residue.

b. Make twelve determinations of potash by dissolving out the K_2PtCl_6 and reweighing.

c. Make twelve determinations of potash by filtering after ignition and solution before precipitation of the potash with H_2PtCl_6.

List the twelve individual determinations of each sample by methods a, b, and c and indicate whether the ignitions were in silica or platinum dishes, and type of filter, whether asbestos padded Gooch or glass sinter. If latter list number and porosity and approximate final temperature of ignition in degrees C.

Total number of potash determinations, 36.

The results of the nine collaborators are given in Table 5. The high, low, and average potash results by Method A are all higher than the corresponding results by both Methods B and C. Taken as a whole there is a greater variation between the high and low results by Method A than by Methods B and C. Another result submitted by Chemist 1 after the averages had been made indicates a value of 24.06 for Method A and 23.66 for Method B, ignition being carried out in platinum at 750° C. This difference of .4 per cent is about what would be expected when Methods A and B are used on Sample 96. The results also indicate that there is very little difference between Methods B and C. The expressions of various chemists indicate that Method A is not safe for mixed fertilizers. Many contend that the use of either Method B or C would remove the objection to the present method both in regard to the water-insoluble residues that are often encountered and to the fact that the potash as determined by

TABLE 5.—*Collaborative results on potash*

Dissolving out the K_2PtCl_6 and Reweighing
versus
Filtration after Ignition and Solution
Sample 96

ANALYST NUMBER	NUMBER OF ANALYSES	METHOD A			METHOD B			METHOD C		
		HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE	HIGH	LOW	AVERAGE
1	12	23.74	23.64	23.79	23.44	23.34	23.38	23.58	23.42	23.51
2	12	24.06	23.84	23.94	24.06	23.63	23.81	24.04	23.84	23.95
5	12	23.40	23.20	23.31	22.96	22.80	22.88	22.96	22.76	22.87
6	12	23.70	23.35	23.52	23.80	23.30	23.61	24.15	23.80	23.99
8	6	23.88	23.42	23.58	23.34	23.24	23.31	23.38	23.26	23.33
9	12	23.74	23.44	23.61	23.48	23.20	23.32	23.42	23.16	23.26
12	12	24.03	23.92	23.96	23.92	23.76	23.85	24.07	23.92	23.98
14	12	23.98	23.82	23.90	23.72	23.58	23.65	23.90	23.81	23.85
20	8	23.08	22.30	22.77	22.38	21.80	22.19	22.30	21.63	22.03
Average		23.73	23.44	23.59	23.45	23.19	23.33	23.53	23.29	23.42
Max. Variation		.98	1.62	1.19	1.68	1.96	1.66	1.77	2.29	1.95

Method A. Regular method—no correction for residue.
Method B. K_2PtCl_6 dissolved out and Gooch reweighed.
Method C. Filtration of insoluble residue before precipitation with H_2PtCl_6.

TABLE 6.—*Effect of degree of fineness of grinding*

ANALYST NO.	NO. OF ANALYSES	1 MM. DEGREE OF FINENESS						½ MM. DEGREE OF FINENESS					
		SAMPLE HIGH	100X LOW	1 MM. AV.	SAMPLE HIGH	100Y LOW	½ MM. AV.	SAMPLE HIGH	101X LOW	1 MM. AV.	SAMPLE HIGH	101Y LOW	½ MM. AV.
2	12	20.50	19.46	19.82	19.81	19.03	19.43	49.00	48.08	48.57	48.98	48.16	48.36
4	12	19.38	19.24	19.28	19.14	18.98	19.07	48.23	48.05	48.14	48.26	48.11	48.15
5	12	19.64	19.44	19.50	19.60	19.44	19.48	48.76	48.52	48.61	48.76	48.60	48.68
6	12	19.56	18.70	19.12	19.10	18.70	18.89	48.46	46.30	47.64	48.72	48.58	48.63
8	6							48.96	48.68	48.81	48.88	48.60	48.75
9	12	19.88	19.56	19.70	19.88	19.44	19.69	48.56	48.16	48.31	48.76	48.32	48.43
10	10	19.20	19.12	19.17	19.44	19.17	19.32	48.16	47.44	47.77	48.07	47.70	47.83
12	12	19.65	19.50	19.55	19.57	19.42	19.47	48.53	48.22	48.37	48.60	48.34	48.46
14	12	19.78	19.24	19.58	19.44	19.10	19.25	48.66	48.02	48.37	48.50	48.24	48.40
20	12	19.42	18.95	19.13	19.16	18.68	18.98	48.22	47.28	47.81	48.48	47.26	47.89
Average		19.55	19.24	19.43	19.46	19.11	19.28	48.55	47.87	48.24	48.60	48.19	48.36
Stand. Dev.				26			26			39			31

Method A at present is not all water-soluble. In view of the reaction to the method as it now stands it will be recommended that potash in mixed fertilizers be determined by Method B.

1 MM. DEGREE OF FINENESS *VERSUS* ½ MM.

To determine the effect of the fineness of grinding on the uniformity of potash results two samples of fertilizer of 1 mm. and ½ mm. degree of fineness, respectively, were sent to 13 chemists with the following instructions:

(11) That a collaborative study be made of the degree of fineness of grinding, with a view to elimination of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the official potash determination.

1 mm. degree of fineness *versus* ½ mm.

Determine the potash by the regular method on Samples 100X, 100Y, 101X, and 101Y as follows:

a. Weigh twelve 2.5 gram samples from samples 100X and 101X and determine potash in each of the twelve solutions of each sample.
b. Weigh twelve 2.5 gram samples from 100Y and 101Y and determine the potash in each of the twelve solutions of each sample.

List each individual determination of each sample by Methods a and b.

K_2O value of sample 100X and 100Y is about 20%.

K_2O value of sample 101X and 101Y is about 50%.

Total number of potash determinations, 48.

Although the uniformity of results obtained by the 10 collaborators in the case of the two samples ground to pass ½ mm. over that of the two samples ground to pass 1 mm. was not so good as that reported by Ford, *This Journal*, **22**, 285 (1939), the trend is still in favor of grinding samples to pass ½ mm. sieves. Examination of the variations will reveal that they are less between 100Y and 100X than they are between 101Y and 101X. This was to be expected since all the chemists would determine the potash in 100Y and 100X by **43(a)**, while it is possible that some might have determined the potash in 101X and 101Y by **43(b)**, without testing to see if interfering substances were present. This would tend to produce higher potash values unless the potash was washed out and reweighed or dissolved and filtered before being precipitated. Notation of those making such corrections may be found in the comments by the collaborators. Many other chemists have reported that they are in accord with a recommendation to grind finer in the cases of samples that are hard to check. With this in mind it will be recommended that the official method for the preparation of the sample be changed to permit grinding finer than the 1 mm., now official.

COLLABORATORS

(1) Allen, H. R., Agr. Expt. Sta., University of Kentucky, Lexington, Ky.

(2) Batton, H. C., Swift & Co., Fertilizer Works, Baltimore, Md.
(3) Boyce, E. F., Agr. Expt. Sta., Burlington, Vt.
(4) Hand, W. F., Mississippi State Chem. Lab., State College, Miss.
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(6) Hoffman, A. E., Darling & Company, East St. Louis, Ill.
(7) Howes, C. Clifton, The Davison Chemical Corp., Baltimore, Md.
(8) Hughes, C. W. Agr. Expt. Sta., Lafayette, Ind.
(9) Ingham, R. E., F. S. Royster Guano Co., Macon, Ga.
(10) Jones, W. Catesby, Dept. of Agr. & Immigration, Richmond, Va.
(11) Kerr, A. P., Dept. of Agr. & Immigration, Baton Rouge, La.
(12) Koch, R. C., Swift & Co. Fertilizer Works, Hammond, Ind.
(13) Merwin, R. T., Agr. Expt. Sta., New Haven, Conn.
(14) Powell, R. D., Virginia-Carolina Chemical Corp., Richmond, Va.
(15) Smith, Richard M., Chemical Division, Agr. Dept., Tallahassee, Fla.
(16) Midgley, M. C., Agr. Expt. Sta., Pullman, Wash.
(17) Struve, Oscar I., Eastern States Coop. Milling Corp., Buffalo, N. Y.
(18) Thompson, S. K., Dept. of Agriculture, Charleston, W. Va.
(19) Trimble, C. E., The American Agr. Chemical Co., Carteret, N. J.
(20) Webb, H. J., Clemson Agr. College, Clemson, S. C.

COMMENTS OF COLLABORATORS

Analyst

1. Potash ignited at 650° C. in controlled muffle—filtered into a Gooch padded first with a circle of filter paper and then with asbestos.

2. Samples 96, 100X, and 100Y digested as in **43(a)** for mixed fertilizer; samples 101X and 101Y made up as directed in **43(b)** for potash salts.

5. Ignited in platinum—filtered into asbestos padded Gooch.

6. Ignited in Pyrex dishes until glass showed red—filtered into asbestos padded Gooch.

7. Samples of this type should be ground finer than 1 mm.

8. Ignited in platinum over Purdue burner—filtered into Jena bg3 glass sinters —corrected for water-insoluble residue. All samples digested as directed in **43(a)**.

9. Ignited in platinum—filtered into Gooch padded first with a circle of filter paper to prevent loss and then with an asbestos pad.

12. Ignited in silica at 650° C., filtered into asbestos padded Gooch. Prefer 5 gram sample made to 500 instead of 2.5 gram to 250 as specified in the present official method.

13. Results corrected for water-insoluble residue.

14. Ignited in platinum at 800°–900° C.—filtered into asbestos padded Gooch.

15. Defoamer should be used only when necessary.

16. Results corrected for water-insoluble residue.

17. Defoamer was a distinct help in digesting the samples involved in this work.

18. Ignited over Purdue burner—filtered into Jena bg3 glass sinter—results corrected for water-insoluble residue.

20. Ignited in platinum at 600°–700° C. over gasoline Meker—filtered into Jena glass sinters.

RECOMMENDATIONS[2]

It is recommended—

(1) That the words "or some factor weight" be inserted between "50 cc" and "aliquot," *Methods of Analysis, A.O.A.C.*, 1935, p. 30, **44(a),** "Mixed fertilizers," and that this line read: "Evaporate nearly to dryness a 25 or 50 cc. or some factor weight aliquot of solution" (official, final action).

(2) That the words "and a Pt soln containing equivalent of 0.5 g of Pt(1.05 g of H_2PtCl_6)" be inserted after "(2.1 g of H_2PtCl_6)", *Methods of Analysis, A.O.A.C.*, 1935, p. 29, **42(b),** and that this section then read: "A Pt solution containing equivalent of 1 g of Pt(2.1 g of H_2PtCl_6) and a Pt soln containing the equivalent of 0.5 g of Pt(1.05 g of H_2PtCl_6) in every 10 cc."(official, final action).

(3) That the study of the four methods of platinum recovery published in *This Journal*, **22,** 286–287 (1939), be continued.

(4) That diglycol stearate be recognized as a reagent in the preparation of the potash solution and that it be inserted in *Methods of Analysis, A.O.A.C.*, 1935, p. 29, be designated **42(c),** and read as follows: "*Defoaming soln.*—Dissolve 20 g of diglycol stearate tech. in 1 liter of equal parts of benzol and ethyl alcohol" (official, final action).

(5) That the words, "and 1 ml. of diglycol stearate when necessary to prevent foaming" be inserted after "soln" in the second line of *Methods of Analysis, A.O.A.C.*, 1935, p. 30, **43(a),** and that it then read as follows: "Place 2.5 g of sample in 250 cc volumetric flask, and add 125 cc of H_2O and 50 cc of saturated NH_4 oxalate soln, and 1 cc of diglycol stearate when necessary to prevent foaming" (official, final action).

(6) That the words, "If not, dissolve the K_2PtCl_6 with hot H_2O, reweigh, and make correction for water-insoluble residue" be included in the parentheses of the last line, *Methods of Analysis, A.O.A.C.*, 1935, p. 30, **44(a),** and that this line then read: "(The precipitate should be completely soluble in H_2O. If not, dissolve the K_2PtCl_6 with hot H_2O, reweigh, and make correction for water-insoluble residue)," (official, final action).

(7) That the words "1/50″ ($\frac{1}{2}$ mm) in diameter and sift" be inserted in line 4, *Methods of Analysis, A.O.A.C.*, 1935, p. 18, **2,** "Preparation of Sample—Official," in place of "1/25″ (1 mm) in diameter and sift" (official, final action).

(8) That studies of the solvent action of acid alcohol and alcohols on K_2PtCl_6 be continued.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to H. R. Kraybill for his counsel in connection with this work and to C. W. Hughes for assistance in making potash determinations.

[2] For report of Subcommittee A and action by the Association, see *This Journal*, **23,** 50 (1940).

No report on acid and base-forming quality was given by the associate referee.

REPORT ON CALCIUM, COPPER, ZINC, AND SULFUR

By GORDON HART, *Associate Referee*, and W. Y. GARY
(Department of Agriculture, Tallahassee, Fla.)

CALCIUM

Ten methods were tested. The first three varied only in the indicator used. The next three methods were the same as the first three, except that a double precipitation was made. All six methods are gravimetric. Method 7 is the tentative method for stock feed; it was tried with three different indicators. Method 8 comprises the precipitation of the calcium sulfate from the official magnesium determination, as oxalate, in the presence of citric acid. Method 9 is a volumetric method; the calcium is precipitated as oxalate from acetic acid. Method 10 follows the procedure outlined by J. B. Smith for collaborative work on the Bartlett-Tobey method, *This Journal*, **22**, 270 (1939). However, a 1 gram aliquot was used instead of the smaller portion used by Smith. The second precipitation was made as directed in Method 5 and correction was made for manganese.

The following methods, samples, and instructions were sent to the collaborators:

Sample 1, specially prepared, contains 15% CaO, 1% CuO, 1% ZnO, 5% free sulfur, and also magnesium, iron, manganese, nitrogen, phosphoric acid, potash, and fat-bearing organic matter. Sample 2 is a mixed fertilizer. Sample 3 is superphosphate. Sample 4 contains 0.40% CuO and 0.40% ZnO.

Run calcium on Samples 1, 2, and 3; run copper on Samples 1 and 4; and run sulfur on Samples 1, 2, and 3.

Report calcium as CaO, copper as CuO, zinc as ZnO, and Sulfur as S.

CALCIUM

REAGENTS

(a) *Methyl red.*—Dissolve 1 g. of methyl red in 50 ml. of 95% alcohol and dilute to 100 ml. with water. Filter if necessary.

(b) *Methyl orange.*—Dissolve 0.1 g. of methyl orange in 100 ml. of water.

(c) *Bromophenol blue.*—Dissolve 0.1 g. of bromophenol blue in 1.5 ml. of 0.1 *N* NaOH and dilute to 25 ml.

(d) *Ammonium oxalate.*—Dissolve 40 g. of NH_4 oxalate in 1 liter of water (practically saturated).

DETERMINATION

1. (a) Weigh 2.5 g. of fertilizer into a 250 ml. volumetric flask, add 30 ml. of HNO_3 and 10 ml. of HCl, and boil for 30 minutes. Cool, make to volume, mix, filter through a dry filter paper, and transfer a 100 ml. aliquot to a 400 ml. beaker. Add a few drops of methyl red. Add NH_4OH until the solution is yellow, then HCl until barely pink. Add 20 ml. of a saturated solution of NH_4 oxalate, adjust the solution to *p*H 5.0 (a faint pink color) by the addition of HCl (1+4), boil for a few minutes, cool, and again adjust the reaction to *p*H 5.0, adding more methyl red if

necessary. Stir thoroughly and allow the solution to stand until the precipitate settles. Filter through a 11 cm. Whatman No. 42 (or equal) filter paper and wash 10 times with hot water.

(b) Transfer the precipitate and filter paper to crucible with cover, ash at low heat until filter is completely ashed, then cover crucible and heat in blast or furnace to 1,000°–1,200° C.; cool in desiccator and weigh as CaO. Repeat ignition and weighing to minimum weight.

2. Repeat the above, using bromophenol blue, and neutralize just to the point where the indicator changes from yellow to green (not to blue).

3. Repeat the above, using methyl orange, and neutralize to the point where the color changes from pink to orange (not to yellow). NOTE: It may be necessary to add more methyl red and methyl orange indicator as the HNO_3 tends to oxidize them.

Repeat the foregoing precipitation.

Transfer the precipitate and the paper to the beaker in which the precipitation was made. Add 30 ml. of HCl (1+4) and digest 15 minutes on steam bath (or hot plate). Filter into 250 ml. beaker through filter paper pulp, using suction. Wash six times, using hot HCl (5+95). Repeat the precipitation as directed above and ignite. Weigh as CaO.

4. Repeat, using methyl red.

5. Repeat, using bromophenol blue.

6. Repeat, using methyl orange.

7. *Tentative method for calcium in stock feeds, Methods of Analysis, A.O.A.C.*, 1935, 347, 44.

Repeat, using bromophenol blue, adjusting *p*H to where color changes from yellow to green (but not to blue).

Repeat, using methyl orange, adjusting the color to where it changes from pink to orange (but not to yellow).

8. *Calcium precipitated from the official magnesium determination.*—

(a) *Ammonium oxalate-oxalic acid wash solution.*—Dissolve 2 g. of $(NH_4)_2C_2O_4.H_2O$ and 1 g. of $H_2C_2O_4.2H_2O$ in H_2O and dilute to 1 liter.

(b) Weigh 2 g. of sample into a 200 ml. volumetric flask, add 10 ml. of HCl and 30 ml. of HNO_3, boil gently for 30 minutes, cool, and dilute to volume with H_2O. Pipet an aliquot containing not more than 30 mg. of MgO into a 250 ml. beaker, add 6 ml. of H_2SO_4 (1+1), remove the cover, and evaporate until white fumes appear. Cool slightly, wash down the inside surface of the beaker with a jet of water, and again evaporate until fumes of H_2SO_4 appear. Cool, add 10 ml. of water, stir thoroughly, and digest on the steam bath for 10–15 minutes. Remove from the steam bath, add 100 ml. of 95% alcohol, stir so that the $CaSO_4$ is well dispersed throughout the liquid, and allow to stand for 2 hours or longer. Filter by means of suction through a tight plug of filter paper pulp, using a Gooch crucible, and wash 5 times with 5 ml. portions of 95% alcohol containing 1 ml. of H_2SO_4 per 100 ml.

Transfer the precipitate and paper pulp to the beaker in which the precipitation was made, using a little HCl (1+4) to wash out the crucible. Dilute the solution to 25–30 ml. with HCl (1+4), and digest on the steam bath 10–15 minutes. Filter into a 250 ml. beaker through a mat of filter paper pulp, using suction, and wash well with hot HCl (5+95). Add 50 ml. of a saturated (at room temperature) solution of NH_4 oxalate and 10 ml. of 20% citric acid solution. Dilute to 200 ml. and heat to 80°–90° C. Add 2 drops of bromophenol blue indicator solution and then NH_4OH to the first distinct indicator color change that remains on stirring the solution. When the precipitate settles the solution should be green and not a distinct blue. Digest on the steam bath for 1–2 hours, stirring 2 or 3 times. Allow to cool to room temperature and settle. Filter through an 11 cm. No. 42 Whatman or similar paper.

TABLE 1.—*Collaborative results on calcium*

COLLABORATORS	METHOD 1 METHYL RED	METHOD 2 BROMO-PHENOL BLUE	METHOD 3 METHYL ORANGE	DOUBLE PRECIPITATION METHOD 4 METHYL RED	DOUBLE PRECIPITATION METHOD 5 BROMO-PHENOL BLUE	DOUBLE PRECIPITATION METHOD 6 METHYL ORANGE	METHOD NO. 7 STOCK FEEDS A.O.A.C., P. 347, 44 METHYL RED	METHOD NO. 7 STOCK FEEDS A.O.A.C., P. 347, 44 BROMO-PHENOL BLUE	METHOD NO. 7 STOCK FEEDS A.O.A.C., P. 347, 44 METHYL ORANGE	METHOD 8 CALCIUM SULFATE PPT. FROM OFFICIAL MG.	METHOD 9 ACETIC ACID	METHOD 10 1939 MODIFIED BARTLETT-TOBEY	MODIFIED BARTLETT-TOBEY—VOLUMETRIC
					Sample 1								
W. Y. Gary and	14.98*	14.85*	14.62*	14.72*	14.68*	14.43*	—	—	—	—	—	—	14.84†
Gordon Hart	15.34	15.23	14.93	14.84	14.80	14.54	14.92	14.92	14.84	14.63	14.51	14.88[1]	15.23
Thornton & Co.	15.63	15.46	15.44	15.35	15.28	15.39	14.86	—	—	14.72	14.84	15.11[2]	—
E. J. Dessyck	15.25	15.30	15.24	14.53	14.84	14.72	14.67	14.92	14.93	14.80	14.76	15.03*	14.80
G. S. Fraps (Smith, Analyst)	16.26	16.10	16.07	—	—	—	15.50	—	—	15.53	15.31	—	—
C. A. Butt and A. O. Hollman	15.32	15.29	15.10	15.02	15.03	15.18	14.94	14.98	15.06	14.96	15.12	14.81*	—
Southern Analytical Lab.	16.07	15.91	15.77	15.83	15.25	15.75	15.37	15.23	15.09	15.30	14.66	—	—
O. I. Struve	—	—	—	—	—	—	—	—	—	—	—	14.50[3]	—
Average	15.64	15.55	15.43	15.11	15.04	15.12	15.08	15.01	14.98	14.99	14.86	14.87	—
					Sample 2								
W. Y. Gary and	15.15*	15.07*	15.06*	15.13*	15.04*	15.00*	—	—	—	—	—	—	15.02†
Gordon Hart	15.21	15.12	15.12	15.15	15.06	15.02	15.00	14.92	14.92	15.33	14.59	14.99[4]	15.14
Thornton & Co.	15.33	15.64	15.66	15.11	15.14	15.21	14.86	—	—	14.64	15.12	15.09[5]	—
E. J. Dessyck	15.34	15.30	15.26	15.20	15.18	15.16	15.10	15.18	15.08	15.62	15.33	15.52*	15.10

* Corrected for Mn.
† Double ppt.
[1] Corrected for 0.0012 g. of Mn.
[2] Corrected for 0.0004 g. of Mn.
[3] Corrected for 0.17% Mn.
[4] Corrected for 0.0003 g. of Mn.
[5] Corrected for 0.0002 g. of Mn.

TABLE 1.—*Continued*

COLLABORATORS	METHOD 1 METHYL RED	METHOD 2 BROMO-PHENOL BLUE	METHOD 3 METHYL ORANGE	DOUBLE PRECIPITATION METHOD 4 METHYL RED	DOUBLE PRECIPITATION METHOD 5 BROMO-PHENOL BLUE	DOUBLE PRECIPITATION METHOD 6 METHYL ORANGE	METHOD NO. 7 STOCK FEEDS A.O.A.C., P. 347, 44 METHYL RED	METHOD NO. 7 STOCK FEEDS A.O.A.C., P. 347, 44 BROMO-PHENOL BLUE	METHOD NO. 7 STOCK FEEDS A.O.A.C., P. 347, 44 METHYL ORANGE	METHOD 8 CALCIUM SULFATE PPT. FROM OFFICIAL MG.	METHOD 9 ACETIC ACID	METHOD 10 1939 MODIFIED BARTLETT-TOBEY	MODIFIED BARTLETT-TOBEY—VOLUMETRIC
G. S. Fraps (Smith, Analyst)	15.59	15.64	15.67	—	—	—	15.89	—	—	16.06	15.99	—	—
C. A. Butt and A. O. Hollman	15.21	15.28	15.16	15.04	15.00	15.08	15.04	15.17	15.28	15.56	15.55	15.00*	—
Southern Analytical Lab.	15.55	15.15	14.50	14.80	15.01	15.45	15.23	15.37	15.02	15.32	15.37	—	—
O. I. Struve	—	—	—	—	—	—	—	—	—	—	—	15.18[8]	—
Average	15.37	15.36	15.23	15.06	15.09	15.18	15.18	15.16	15.07	15.50	15.32	15.15	
						Sample 3							
W. Y. Gary and Gordon Hart	26.99* 27.00	26.01* 26.01	24.97* 24.97	26.48* 26.48	26.19* 26.19	26.40* 26.40	— 26.27	— 26.19	— 26.22	— 26.51	— 25.86	— 26.35[7]	26.18† 26.18
Thornton & Co.	26.03	26.18	26.12	25.34	25.45	25.25	25.79	—	—	26.12	26.04	25.76*	—
E. J. Deszyck	26.39	26.35	27.07	26.22	26.12	26.44	26.44	26.59	26.58	26.10	27.19	27.16*	26.10
G. S. Fraps (Smith, Analyst)	27.46	27.56	27.51	—	—	—	28.04	—	—	28.04	—	—	—
C. A. Butt and A. O. Hollman	26.67	26.45	26.61	26.49	26.62	26.43	26.08	26.27	26.30	27.14	26.89	26.43*	—
Southern Analytical Lab.	27.52	25.96	28.08	28.23	25.64	26.12	24.52	24.55	24.23	28.50	25.61	—	—
O. I. Struve	—	—	—	—	—	—	—	—	—	—	—	26.68[8]	—
Average	26.84	26.42	26.73	26.55	26.01	26.13	26.19	25.90	25.86	27.07	26.32	26.47	

[6] Corrected for 0.04% Mn.
[7] Corrected for 0.0001 g. of Mn.
[8] Corrected for 0.006% Mn.

Loosen the precipitate sticking to the beaker with a rubber policeman, wash out the beaker with NH_4 oxalate-oxalic acid solution and wash the paper and precipitate 5 more times with the same wash solution. Transfer the paper and precipitate to a platinum crucible and ignite at a low temperature until the paper is completely oxidized. Cover the crucible and heat (at about 1,200° C.) over a hot flame for at least 30 minutes. Cool in a desiccator containing H_2SO_4 and weigh as CaO. Repeat the ignition and weighing until a minimum weight is obtained.

9. Weigh 1 g. into a 250 ml. volumetric flask, dissolve in 25 ml. of HCl, dilute to 250 ml., and take an aliquot of 25 ml. (0.1 gram). Add 10 ml. of acetic acid, heat, add 15 ml. of saturated NH_4 oxalate, heat to boiling, add ammonia to slight excess, using litmus paper, acidify with acetic acid, and let stand in warm place until precipitate has settled. Filter precipitate, wash with hot water, punch hole in paper, and wash back into the beaker in which precipitation was made; wash paper and funnel with small portions of dilute H_2SO_4 (5 ml. in 100 ml. water), add the rest to the dilute H_2SO_4, heat to 70° C. or above, and titrate with standard $KMnO_4$. Calculate to CaO.

The following suggestions were also included:

The Referee is attempting to make calcium dove-tail with J. B. Smith's magnesium methods. To do this it is necessary to make the first precipitation as near as possible as he does.

The copy of his method has two minor changes; namely the size of the aliquot, and a larger quantity of ammonium oxalate. (See Smith's report, p. 247.) Please make the calcium determination by this method and also determine the manganese in the calcium and correct for same.

Methods 1 to 6 were tried to determine whether a single or double precipitation was better and which indicator worked best. The basic method was taken from Research paper RP 1095, National Bureau of Standards, by James I. Hoffman and G. E. F. Lundell, pp. 616–617. Bromophenol blue gave the most satisfactory results, a *p*H of 3 to 4, when carefully followed. The manganese was determined by Gary and Hart in the calcium oxide in all three samples run by these six modifications of the method. Sample 1 contained considerable manganese as shown by the corrected results indicated by an asterisk, even after a double precipitation. Samples 2 and 3 did not have any added manganese; the quantity present was that naturally occurring in the materials. Gary and Hart also made further tests on Sample 1 by dissolving the calcium oxide from the double precipitate of these three samples and bringing them to the *p*H of 3 to 4 with bromophenol blue with ammonium hydroxide. No precipitate formed, indicating that the manganese present was in the form of the oxalate. Method 7 gave surprisingly good results on Sample 1. Method 8 should work well, but the analysts differed widely on it. Method 9 gave slightly low results and different analysts did not check well with it. Method 10 average was almost exactly theoretical.

SUMMARY

Of the first six methods Method 5, double precipitation with bromophenol blue indicator, appears to work best and possibly can be worked

out with a manganese correction to give good results. Method 7 appears to give good results unless there is more than 0.50 per cent manganese in the sample.

Method 10 is very similar to Methods 4, 5, and 6 and will work equally well since it supplements the Bartlett-Tobey method on which Smith is working. It should be tried out further, both gravimetrically and volumetrically. In the gravimetric methods, when the calcium oxide ash is brown, a correction must be made for manganese.

RECOMMENDATIONS

It is recommended that the tentative method for stock feed, as outlined in this report, be adopted as tentative for fertilizers (bromophenol blue indicator to be given preference).

It is also recommended that Method 10, as outlined, be studied further, collaboratively, and that the calcium procedure from the Bartlett-Tobey method, as outlined by J. B. Smith, be studied volumetrically.

COPPER

Method 1, electrolytic, and Method 2, volumetric, were used.

Two samples, No. 1 and No. 4, were prepared to contain 1.00 and 0.40 per cent copper oxide, respectively. They also contained manganese, zinc, iron, calcium, and magnesium, in addition to nitrogen, phosphoric acid, and potash.

Method 1.—This Associate Referee did not have any success with Method 1 because other elements besides copper were plated on the platinum dish and also owing to lack of time. However, two collaborators who reported on this method did check closely the known quantity of copper present.

Method 2.—Six collaborators reported on this method. The maximum variation was 0.10 per cent on Sample 1 and 0.06 per cent on Sample 2. The following criticism of this method was received:

> The method calls for additions of a measured amount of a standard copper solution to the unknown sample solution, if the copper oxide present is less than 0.01 gram. This is open to two objections. In the first place the analyst does not know before the completion of the analysis whether the sample solution contains more or less than 0.01 g. In the second place, the addition, to an unknown sample, of a quantity of the constituent to be determined, in order to bring the quantity within the range of accuracy of a given procedure, is not desirable.

If there is sufficient cupric oxide present in the unknown to show a light blue color, it is not necessary to add any standard copper solution. If the color is not present, either the copper solution must be added or an indicator must be used to show the approximate *p*H of the solution. Any indicator used will interfere with the final end point of the titration. The addition of a small quantity of the standard copper solution when the copper is not present in sufficient quantity is the simplest procedure.

Method 2 was taken from Scott and developed by E. J. Raudenbush and W. Y. Gary. Raudenbush used it on copper from 1933 to 1937. Gary further developed it from 1937 to 1939. The method gives accurate results for copper in mixed fertilizers.

TABLE 2.—*Collaborative results on copper*

COLLABORATOR	METHOD 1		METHOD 2	
	SAMPLE 1	SAMPLE 4	SAMPLE 1	SAMPLE 4
W. Y. Gary and Gordon Hart	x	x	1.006	0.408
Thornton & Co.	x	x	0.99	0.35
Frank D. Lundstrom	x	x	0.98	0.39
G. S. Fraps (J. F. Fudge)	x	x	0.993	0.402
C. A. Butt and A. O. Hollman	0.90	0.36	1.08	0.41
G. E. Grattan (C. V. Marshall)	0.945	0.41	1.04	0.41
Average	0.922	0.385	1.015	0.395

RECOMMENDATIONS

It is recommended that Method 2 be adopted as tentative, with a view to final adoption. The method will be published in *Methods of Analysis, A.O.A.C.*, 1940, to issue about July 1.

It is also recommended that electrolytic methods for copper be studied.

ZINC

Two methods were tried on zinc, collaboratively on two samples. The samples, No. 1 and No. 4, were the same as used for copper. Sample 1 contained 1.00 per cent zinc and Sample 4, 0.40 per cent zinc. Method 1 is the tentative method for zinc in gelatin, *This Journal*, **22**, 84 (1939). Method 2 is the method used in this laboratory. Both of these methods can be used for the determination of copper, and if copper is not present, the methods are still applicable. Method 1 does not seem to be adaptable to fertilizers. The results are erratic, probably due to the large precipitate that forms when the solution is made alkaline with ammonia, occluding some of the zinc.

TABLE 3.—*Collaborative results on zinc*

COLLABORATOR	METHOD 1		METHOD 2	
	SAMPLE 1	SAMPLE 4	SAMPLE 1	SAMPLE 4
W. Y. Gary and Gordon Hart	0.60	0.472	0.832	0.396
Thornton & Co.	0.91	0.50	0.88	0.44
F. D. Lundstrom	0.82	0.39	0.80	0.36
G. S. Fraps (J. F. Fudge)	0.455	0.245	0.775	0.405
C. A. Butt and A. O. Hollman	0.88	0.40	0.87	0.39
Average	0.635	0.403	0.381	0.398

Method 2 is more promising. However, 83.10 per cent of the zinc was recovered in one sample and 99.50 per cent in the other sample. Further study of this method showed that part of the zinc was occluded in the copper sulfide, but when the copper was precipitated out at a lower *p*H, the zinc was not occluded and 100 per cent zinc was recovered.

ZINC

Indicator.—Dissolve 0.1 g. bromophenol blue in 1.5 ml. of 0.1 *N* NaOH and dilute to 25 ml.

Special wash solution.—0.1 *M* formic acid solution (4 ml. of 23.6 *M* formic acid per liter) saturated with H_2S.

1. Boil the filtrate and washings from the H_2S precipitate of Cu until all H_2S is removed. Add 1 ml. of HNO_3 and continue the boiling until the volume is reduced to approximately 25 ml. Add 10 ml. of HN_4Cl (200 g. per liter), make definitely alkaline with NH_4 hydrate, heat nearly to boiling, and filter into a 100 ml. Erlenmeyer flask. Wash with warm alkaline NH_4Cl soln containing 50 g. of NH_4Cl and 25 ml. of NH_4 hydrate (sp.gr. 0.90) per liter. Neutralize the filtrate and washings with acetic acid, add 0.5 g. of Na acetate and sufficient glacial acetic acid to make an excess of 2 ml. for each 50 ml. of soln. Warm the mixture on the steam bath and saturate with H_2S. Allow to stand in a warm place for approximately 30 min. Filter through a small paper and wash thoroughly with warm acetic acid (1+1) saturated with H_2S. If the filtrate is turbid, return to flask, add a few drops of saturated $HgCl_2$ soln, shake, and filter again. Ignite in a tared Pt crucible at a dull red heat until completely ashed, then a few minutes at bright red heat. Weigh as ZnO, and report as zinc (Zn).

2. Evaporate the combined filtrate and washings from the CuS precipitation in a 250 ml. Erlenmeyer flask until the solution is clear and measures 85 ml. or less. Cool, and add 10 ml. of 20% citric acid solution. Add 2 drops of bromophenol blue and dilute, if necessary, to 95 ml. Add NH_4OH to the first color change of the indicator. Fit the flask containing the Zn solution with a two-holed rubber stopper and glass tubes, one of which almost touches the bottom of the flask and the other just extends through the stopper. (Several samples and the wash solution may now be connected in series.) Pass a rapid stream of H_2S (approximately 8 bubbles per second) through these solutions for at least 30 minutes. Clamp the connecting tubes and allow to stand until the precipitate settles (about 1 hour should be sufficient). Filter through a No. 42 Whatman or similar paper. Use a rubber policeman to loosen the precipitate and wash out the flask onto the paper with a stream of the prepared wash solution from a wash bottle. Wash the paper and precipitate five more times with small quantities of the wash solution, keeping the funnel covered with a watchglass as much as possible. The filtrate may become turbid due to oxidized sulfur. Place the paper and precipitate in a cleaned, ignited, and weighed porcelain crucible. Ignite at a low temperature until the paper is oxidized, then at 900° C. for one hour. Cool in a desiccator and weigh as ZnO.

RECOMMENDATIONS

It is recommended that Method 2 be further studied collaboratively; also that other methods for zinc be investigated.

SULFUR

One method was tried for total sulfur and free sulfur and two methods for sulfate sulfur. Samples 1, 2, and 3 were sent out. Sample 1 contained

5.00 per cent free sulfur and 4.90 per cent combined as sulfates, making a total of 9.90 per cent sulfur. Sample 2 was a mixed fertilizer and Sample 3 a superphosphate.

The average for total sulfur on Sample 1, Method 1, is close to theoretical, yet the collaborators do not agree closely, there being 0.47 per cent variation. Free sulfur ran too high when the Soxhlet extractor was used, indicating that some of the sulfate sulfur was extracted. Methods 1 and 3 agree fairly well on the average on Samples 2 and 3, but the collaborators differ too much on these samples.

TABLE 4.—*Collaborative results on sulfur*

COLLABORATOR	METHOD 1				METHOD 2		METHOD 3	
	SAMPLE 1 (TOTAL)	SAMPLE 1 (FREE S)	SAMPLE 2	SAMPLE 3	2	3	2	3
G. Hart	9.94	5.16	6.40	11.52	6.21	11.38	6.40	11.68
Thornton & Co.	9.80	5.31	6.06	11.36	6.19	11.36	6.58	11.80
O. I. Struve	9.77	5.103	6.31	11.045	6.206	11.125	6.327	11.842
G. S. Fraps	x	x	x	x	6.25	11.40	6.84	12.08
C. A. Butt and A. O. Hollman	10.24	x	6.64	11.82	6.35	11.37	6.57	11.90
W. C. Jones	9.81	x	6.42	11.59	x	x	x	x
W. B. Byers	10.24	4.28*	6.78	11.58	6.28	11.45	6.60	11.88
Average	9.97	5.19	6.42	11.586	6.244	11.354	6.553	11.695

* Not included in average. This sample was extracted with a Walker extractor, not a Soxhlet.

The following instructions and methods were submitted:

SULFUR

Determine free sulfur on Sample 1 only. Use Methods 2 and 3 on Samples 2 and 3 only. Use Method 1, total sulfur (free and combined) on all three samples.

TOTAL SULFUR (FREE AND COMBINED)

1. Weigh 1 g. of sample into 250 ml. beaker; add 10 ml. of a saturated solution of Br in CCl_4; cover with a watch-glass and allow to stand 15 minutes. Add 15 ml. of HNO_3; cover and allow to stand 15 minutes. Evaporate on hot plate to about 5 ml.; add 20 ml. of HCl and boil gently to about 5 ml.; add 50 ml. of water. Filter on close filter paper into 200 ml. volumetric flask, wash with 2% HCl and hot water. Take 50 ml. aliquot in 400 ml. beaker. Add 1 drop of methyl red; neutralize with NH_4OH. Add HCl, dropwise, to acid, then 1 ml. in excess. Dilute to 150 ml.; heat to boiling; lower flame to just boiling. Add hot 10% BaCl, dropwise, to slight excess. Digest with low flame until supernatant liquid is clear, about 1 hour. Filter on Whatman No. 40 or Munktell No. 00, 11 cm. filter paper. Wash 10 times with hot water. Ignite in a weighed crucible, and weigh as $BaSO_4$. Calculate to sulfur (S).

FREE SULFUR

Extract 1 g. of sample with CS_2 in a Soxhlet, allowing the extraction thimble to drain at least 12 times. Transfer the extract to a 250 ml. beaker. Evaporate off CS_2 in a draft at room temperature. Place in drying oven at 60°–70° C. for 20 minutes. Then proceed as directed above, but use whole sample instead of aliquot.

SULFATE SULFUR IN FERTILIZER MATERIALS

2. *Sulfates in acid phosphate, ammonium sulfate, kainit, nitrate of soda, and muriate of potash or mixed fertilizer.*—Heat 2.5 g. with 200 ml. of water and 10 ml. (or more) of HCl, nearly to boiling for 30 minutes. Filter, and wash with hot water up to 500 ml. Take 50 ml. (0.25 g.) for acid phosphate, ammonium sulfate, or kainit, and 100 ml. (0.5 g.) for nitrate of soda or muriate of potash, or mixed fertilizer, acidify with HCl and heat to boiling. Lower the flame and add BaCl in slight excess. Digest with a low flame until the precipitate settles and the supernatent liquid is clear, 1 hour or longer. Filter on a Whatman No. 40, 11 cm. filter paper and wash at least 10 times with hot water. (The precipitate is liable to crawl, and care should be taken that it is all washed out of the beaker into the filter.) Ignite in a weighed crucible, add a small crystal of NH_4, and ignite gently. Weigh, and report as sulfate sulfur (S) to 0.01%.

3. Weigh 2 g. of fertilizer into 200 ml. flask, add 30 ml. of HNO_3 and 10 ml. of HCl, digest 10 minutes, add 100 ml. of water, and boil gently for 30 minutes. Cool, make to volume with water, shake thoroughly, and filter on dry filter paper. Take 25 ml. aliquot in 400 ml. beaker, add 100 ml. of water and 1 drop of methyl red, and neutralize with NH_4OH. Add HCl, dropwise, to acid, then 1 ml. in excess. Dilute to 150–175 ml., heat to boiling, lower flame just to boiling and add hot 10% BaCl, dropwise, to slight excess. Digest with low flame until supernatant liquid is clear, about 1 hour. Filter while hot on Whatman No. 40 or Munktell No. 00, 11 cm. filter paper. Wash 10 times with hot water. Ignite in a weighed crucible and weigh as $BaSO_4$. Calculate to sulfur (S).

The Referee recommends further study.

RECOMMENDATIONS

It is recommended—

(1) That methods for the determination of sulfur in fertilizers be studied.

(2) That the work be divided and another associate referee be appointed to work on copper and zinc.

QUANTITATIVE SPECTROSCOPIC ANALYSES OF IMPORTANT TRACE ELEMENTS IN MIXED FERTILIZERS

By E. H. MELVIN, R. T. O'CONNOR, O. R. WULF, and C. H. KUNSMAN
(Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, Washington, D. C.)

(Abstract of a paper presented before the Association of Official Agricultural Chemists, Inc., Washington, D. C., October 30, 1939.)

A method has been developed for the simultaneous spectroscopic determination of boron, manganese, and copper in mixed fertilizers. In the samples analyzed these elements were present in amounts below 0.1 per cent.

Standards have been prepared that have the average composition of mixed fertilizers, but which have different known quantities of the minor

elements ranging from 0.0004 per cent to 0.4 per cent. An internal reference element, in this case beryllium, was used in this work. It is present in the standards and is introduced into the samples to be analyzed in the constant amount of 0.05 per cent. The average deviation of spectroscopic determinations made on these standards was about 16 per cent, which indicates the present accuracy of the analyses for these trace elements in mixed fertilizers.

Boron was present in the fertilizers analyzed to the extent of 0.004–0.04 per cent; copper, 0.001–0.017 per cent; and manganese, 0.005–0.11 per cent.

The desirability of the use of an internal reference element, as in the present instance the element beryllium, and the importance in the direct current arc excitation of the complete burning of the sample were discussed.

REFERENCES

(1) A Method for the Quantitative Determination of Boron, Manganese, and Copper in Mxied Fertilizers, Proc. of the 7th Summer Conference (1939) on Spectroscopy and its Application. John Wiley & Son, New York, January, 1940.

(2) A Method for the Quantitative Determination of Boron, Manganese, and Copper in Mixed Fertilizers, to be published in *Ind. Eng. Chem.*

(3) Other publications to follow.

MONDAY—AFTERNOON SESSION

REPORT ON EGGS AND EGG PRODUCTS

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

Since the last meeting of the Association the Secretary of Agriculture, in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act, has promulgated, effective January 1, 1940, definitions and standards of identity for liquid, frozen, and dried whole eggs and egg yolks (*Federal Register*, Vol. 4, No. 138, pp. 3374–3378, July 20, 1939). Thanks in large measure to the splendid work of L. C. Mitchell and his associates the Association finds itself with adequate methods of analysis for eggs and authentic data on the composition of eggs to make possible the effective enforcement of these standards. In sad contrast is the situation with respect to the estimation of the egg content of foods, the control of which is, after all, of even more importance to the ultimate consumer.

It is to be expected that legal standards of identity will be promulgated in the near future for numerous products of which eggs constitute an essential ingredient. There is urgent need for the development of methods to be used for establishing as well as enforcing such standards. The pro-

mulgation of the standards for eggs should lend impetus to the prosecution of the necessary work. Several years ago the Referee initiated development of a method for estimating the egg content of noodles based on a determination of the cholesterol content, which it was planned to extend to other food products. Reports of progress on the work were made at the 1935 and 1936 meetings. The Referee regrets exceedingly that the pressure of other duties has prevented him from advancing that work. It is only in the past few weeks that he has had opportunity to devote an appreciable amount of time to the problem. Accordingly, as Associate Referee on Unsaponifiable Constituents and Fat, he is again not prepared to submit a report, but sincerely hopes that time will be made available for him to continue the work on the method which has shown so much promise of successful application.

L. C. Mitchell, Associate Referee on Detection of Decomposition and on Added Glycerol, Sugar, and Salt, has submitted an interesting report of preliminary work on the use of the volatile acid number as a means of detecting decomposition in egg yolks. This method shows promise and the work should be continued. The term "base number" as used in the Associate Referee's report should be more clearly defined. The Referee approves the recommendations made by the Associate Referee.

H. A. Lepper, former Referee on Eggs and Egg Products, in his report at the 1935 meeting, recommended, for reasons set forth, deletion of the tentative method for the determination of unsaponifiable matter in eggs from *Methods of Analysis, A. O. A. C.*, prior to the last revision. Through an oversight this specific recommendation did not appear in the final list of recommendations. The Referee concurs in the opinion that the method should be dropped and recommends its deletion from the forthcoming revision of *Methods of Analysis.*

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative method for the determination of unsaponifiable matter (*Methods of Analysis, A. O. A. C.*, 1935, sec. **13**, p. 300) be dropped and deleted from the forthcoming revision of *Methods of Analysis, A. O. A. C.*

(2) That the study of methods for the determination of cholesterol and fat by acid hydrolysis be continued.

(3) That the method for the determination of chlorine adopted as official (first action) last year, *This Journal*, **22**, 77 (1939), be adopted as official (final action).

(4) That the method for the determination of dextrose and sucrose adopted as official (first action) last year, *Ibid.*, be adopted as official (final action).

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **23**, 63 (1940).

(5) That studies on methods for the determination of added glycerol be continued.

(6) That study of chemical methods for the detection of decomposition be continued.

No report on unsaponifiable constituents and fat was given by the associate referee. See preceding report of the referee.

REPORT ON DETECTION OF DECOMPOSITION IN EGGS AND ON ADDED GLYCEROL, SUGAR, AND SALT IN EGGS

By L. C. MITCHELL (U. S. Food and Drug Administration, Mineapolis, Minn.), *Associate Referee*

DETECTION OF DECOMPOSITION

At the 1935 meeting, Associate Referee Callaway reviewed the work of the Association on methods for the detection of decomposition in eggs, *This Journal*, **19**, 201 (1936).

During 1936 and 1937 comparative collaborative studies were made of the official method and of a rapid method for the determination of acidity of the ether extract, which work resulted in the adoption by the Association of the rapid method, as a tentative method, *This Journal*, **21**, 70 (1938).

At the 1936 meeting, Tubis, *This Journal*, **20**, 159 (1937), submitted a study of the Bandemer and Schaible absorption method for the determination of ammonia nitrogen in eggs,[1] in comparison with the present tentative aeration method. Subsequent collaborative studies of the method during 1937 and 1938 indicate that the absorption method is preferable to the aeration method, *This Journal*, **21**, 179 (1938) and **22**, 298 (1939), for this determination.

Last year the Associate Referee recommended that additional chemical methods for measurement of decomposition in eggs be sought.

Since the actual process of decomposition in eggs, which are composed chiefly of proteins and lipids, is a chaos of numerous activities involving many types of micro-organisms and probably yielding a large number of end-products and by-products, it is not likely that any one chemical method can be developed to detect decomposition in all of its variations. In regard to the decomposition of proteins as a class, R. A. Gortner,[2] states that they are hydrolyzed by fungi and bacteria to their constituent amino acids, which are then acted upon by the micro-organisms to yield either bases or acids; that as a rule aerobic organisms cause the

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[1] *Ind. Eng. Chem., Anal. Ed.*, **8**, 201 (1936).
[2] Outlines of Biochemistry, 2nd ed., (1938) pp. 549–550.

elimination of nitrogen with the formation of acids while anaerobic organisms cause the elimination of carbon dioxide with the formation of bases; and that the formation of acids and amines usually proceeds simultaneously, the preponderance of one or the other being determined by the particular type of organism that is involved.

Eggs are also high in lipids, both simple (fat or oil) and compound (lecithin). These substances are likewise subject to certain changes such as hydrolytic with liberation of fatty acids, or oxidative with formation of aldehydes, ketones, acids, and perhaps other compounds.

It appears, therefore, that during the process of decomposition of eggs, acidic and basic compounds are probably liberated or formed simultaneously. Accordingly, the Dyer method for the identification and determination of volatile fatty acids as standardized by Clark and Hillig and used by them in evaluating spoilage in canned fish, *This Journal*, **21**, 684, 688 (1938) may be of value in detection of decomposition in eggs.

Total nitrogen was determined on an aliquot of the sample prepared for the estimation of volatile fatty acids with a view to ascertaining whether any soluble basic (nitrogenous) compounds not precipitable by the phosphotungstic acid were present and whether such information would have any diagnostic value in the detection of decomposition.

This report covers some preliminary exploratory work on six samples of laboratory separated yolks in advanced stages of putrefaction. The formic acid number and the volatile acid number were determined according to the method of Hillig and Clark, *This Journal*, **21**, 694 (1938), except that the volume of the prepared sample was made to 500 ml. instead of 250 ml., as given in the method. The percentage of lipids, the acidity of the lipids after purification and also directly, and the volatile acid number on the aliquot used for the direct acidity were also determined. The lipid solution was prepared according to method 11, *Methods of Analysis, A.O.A.C.*, 1935, 299. A 10 gram sample and 10 ml. aliquot were used for estimation of the lipids, and separate 25 ml. portions were used for the determination of acidity of the lipids. The results obtained are given in Table 1. Those for formic acid, volatile acid, and base numbers were determined by William Horwitz of the Minneapolis Station. As soon as the absorption cells are available, work on ammonia nitrogen will be undertaken, as this determination merits further study.

A study of the results indicates that the volatile acid number may have diagnostic value in the detection of decomposition, inasmuch as good yolks have little or no volatile acids, while yolks in advanced stages of decomposition have appreciable quantities.

TABLE 1.—*Some comparative results on normal and putrefactive yolks*

SAMPLE NO. INV.	CONDITION OF YOLKS			FORMIC ACID NO. MG./100 G.	VOLATILE ACID NO. ML. $N/100$ PER 100 G.	BASE NO. ML. $N/100$ PER 100 G.	LIPIDS			
	ODOR	AFTER MIXING					LIPID %	ACIDITY		VOL. ACID, NO. DIRECT ALIQUOT ML. N/100 PER 100G.
		CONSISTENCY	COLOR					PURIFIED ALIQUOT ML. $N/20$ PER 1G. LIPID	DIRECT ALIQUOT	
48146-D	Rancid Cheesy	Thick Paste	Egg Yellow	6.0	28.0 30.9	3314	33.40	40.5	41.8	—
48195-D	Sulfidic Sour, Gas	Liquid	Olive Green	1.7	326.8	3178	34.05	6.7	12.0	372.9
48449-D	Vinegar Cheesy Gas	Liquid	Orange Yellow	8.3	263.6	1260	34.10	6.1	8.2	—
48624-D	Sauerkraut Sour	Liquid	Sl. Greenish Yellow	20.2	364.2	5280	35.70	10.4	18.2	—
48627-D	Rancid, Sour Musty	Gelatinous	Olive Green	9.2	1699.4	12314	36.45	24.4	45.8	1627.6
48629-D	Ammoniacal, Fecal Gas	Liquid	Iron Gray	40.0	602.6	6310	34.90	8.7	13.3	—
74509-D	Normal Storage	Liquid Eggs	Normal	—	0.0	606	32.55	3.1	3.1	0
74510-D	Normal Fresh Eggs	Liquid	Normal	—	0.0	1012	34.60	2.8	2.8	0

RECOMMENDATIONS[3]

It is recommended—

(1) That the official method for the determination of chlorine, modified to include added salt and adopted as official (first action) last year, *This Journal*, **22**, 77, be adopted as official (final action).

(2) That the official method for the determination of dextrose and sucrose, modified to include the paragraph to correct for the error due to the volume occupied by the precipitate in samples containing added sucrose and adopted as official (first action) last year, *This Journal*, **22**, 77, be adopted as official (final action).

(3) That studies on methods for the determination of added glycerol be continued.

(4) That further work be done on the absorption method for the determination of ammonia nitrogen in eggs.

(5) That additional chemical methods for measurement of decomposition in eggs be sought.

REPORT ON PRESERVATIVES

By WILLIAM F. REINDOLLAR (State of Maryland Department of Health, Baltimore, Md.), *Referee*

Following the suggestion of the Committee on Recommendations, the Referee investigated the qualitative methods for the identification of saccharin. The two tests now official depend, in the first case, upon the sweet taste of the ether extract imparted by the compound, and in the second, upon its conversion to and identification as salicylic acid. As substances other than saccharin will produce a sweet taste, and as the latter test may yield a false reaction if the quantity of naturally occurring or added salicylic acid is too large to be completely destroyed, a more specific reaction is highly desirable. The method adopted for study combines features of several procedures described in the literature. It depends upon the extraction of saccharin from the foodstuff by an ether-petroleum ether mixture, its conversion to phenolsulfonphthalein by heating with a phenol-sulfuric acid solution, and subsequent identification by the red color developed when a diluted solution of the latter compound is made alkaline with sodium hydroxide. The reagents and procedure are described as follows:

METHOD

REAGENTS

(a) *Ethereal solvent.*—Mix equal volumes of ethyl ether and petroleum ether (b.p. 30°–60°).

(b) *Phenol-sulfuric acid.*—Dissolve pure colorless crystalline phenol in an equal weight of H_2SO_4.

[3] For report of Subcommittee C and action by the Association, see *This Journal*, **23**, 63 (1940).

PROCEDURE

Solid or semi-solid preparations.—Transfer 25 g. of the sample to a 100 ml. volumetric flask by means of a little hot water and add sufficient boiling water to make a volume of about 75 ml. Allow the mixture to stand for an hour, shaking occasionally. Then add 3 ml. of glacial acetic acid, mix thoroughly, add a slight excess (5 ml.) of 20% neutral Pb acetate solution, dilute to the mark with cold water, mix, allow to stand for 20 minutes, and filter. Transfer 60 ml. of the filtrate to a separator, add 5 ml. of HCl, and extract with 50, 25, and 25 ml. of the ethereal solvent. Wash the combined ethereal extracts once with 5 ml. of water, remove the major portion of the solvent, transfer to a 30 ml. beaker, and allow to dry at room temperature. Add 5 ml. of the phenol-H_2SO_4 reagent to the residue remaining after the evaporation of the solvent and heat for 2 hours at a temperature of 135°–140°. Cool, dissolve in a little hot water, and pour into about 250 ml. of water. Allow to stand for about 3 hours or overnight and filter, using a small quantity of filter-cel if necessary. Make alkaline with 10% NaOH solution and dilute to 500 ml. A magenta or reddish purple color develops if saccharin is present. A yellow, buff, or pale salmon shade is not significant. (If vanillin, which may be recognized by its odor, is present, it may be removed by extracting the ethereal residue several times with CCl_4.)

One part of saccharin is generally considered equivalent in sweetening power to 500 parts of sugar. On this basis, 80 p.p.m. of saccharin, equivalent to about 4 per cent sugar, was added to the foodstuffs tested. The materials examined were apple butter, apple butter containing 500 p.p.m. of vanillin, tomato ketchup, India relish, and ginger ale. In the case of ginger ale, the sample was extracted by the ethereal solvent without preliminary treatment. The colors developed are described below.

	Control	*Sample*
Apple butter	Pale straw	Deep magenta
Apple butter+500 p.p.m. vanillin	Salmon	Pale magenta
Tomato ketchup	Buff to peach	Deep magenta
India relish	Pale salmon	Magenta
Ginger ale	Nearly colorless	Deep magenta

The amount of saccharin represented in the final volume is 1.2 mg. Indications are that a much smaller amount will give a positive test. Vanillin interferes with the reaction and must be removed. Although saccharin is insoluble in carbon tetrachloride, some of it is lost, probably by mechanical removal, during extraction with this solvent. The semi-quantitative manner in which the test is applied permits the analyst to distinguish readily the color developed by saccharin from that occurring in the control.

RECOMMENDATIONS

It is recommended that the qualitative test for saccharin be studied further with a view to learning its applicability (a) to other foodstuffs, and (b) in the presence of other possible interfering substances.

No report on benzoate of soda was given by the associate referee.

REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (U. S. Food and Drug Administration, New York, N. Y.), *Referee*

The Committee on Recommendations requested further collaborative study of the quantitative estimation of ponceau SX in mixtures with ponceau 3R. With this purpose in view, the Referee sent out seven sets of samples consisting of five subdivisions each, and instructions to determine the dye mixtures by a submitted method. It is regretted that only four collaborators reported their results.

The samples were of the following compositions (based on $TiCl_3$ titrations):

Sample No.	Ponceau SX	Ponceau 3R	Total Color
	%	%	%
1	0.45	83.50	83.95
2	5.37	78.88	84.25
3	0.00	83.92	83.92
4	22.39	62.94	85.33
5	33.58	52.45	86.03

The collaborators reported the following results:

Sample No.	Ponceau SX	Ponceau 3R	Total Color
	%	%	%
O. L. Evenson, Food and Drug Administration, Washington, D. C.			
1	1.1	82.6	83.7
2	6.0	78.1	84.1
3	probably trace	82.2	82.2
4	23.4	62.1	85.5
5	33.9	52.5	86.4
S. S. Forrest, Food and Drug Administration, Washington, D. C.			
1	1.25	81.8	83.05
2	5.30	78.35	83.65
3	trace or none	84.35	84.35
4	22.2	61.5	83.7
5	33.0	51.1	84.1
J. L. Hogan, Food and Drug Administration, New York City			
1	0.60	84.05	84.65
2	6.60	78.24	84.84
3	0.00	84.67	84.67
4	22.21	63.28	85.49
5	32.30	53.64	85.94
L. Koch, H. Kohnstamm and Company, Brooklyn, New York			
1	1.08	83.37	84.45
2	6.60	78.50	85.10
3	0.00	84.65	84.65
4	24.00	62.65	86.65
5	35.70	50.55	86.25

The following comments and criticisms were submitted by the collaborators:

COMMENTS OF COLLABORATORS

O. L. Evenson.—Good check results were obtained. There may be a trace of Ponceau SX in Sample 3.

S. S. Forrest.—The method gives excellent check results. In Sample 3 the dye is apparently all destroyed, only a trace of color remaining, which clears up on the addition of a drop of $TiCl_3$. With this in mind the dye is reported as Ponceau 3R.

J. L. Hogan.—The end points of the titer were found very satisfactory and especially sharp in the treated samples. Nevertheless considerable variations were noted in the amount of $TiCl_3$ solution required to reduce equal portions of similarly treated dye solution. The maximum difference for untreated samples was 0.11 ml. approximately 0.1 *N* $TiCl_3$ and 0.19 ml. for treated samples.

Sample 1 may be off a little because the titer for Ponceau SX was very small. This value is so close to the end of the line on the graph that small errors in making the correction might figure into very considerable percentage error for Ponceau SX.

L. Koch.—The $TiCl_3$ end points after oxidation of the dye mixture were very satisfactory, the complete disappearance of color being easily observed by the addition of 1 drop. It is suggested, however, that a semi-micro buret be used for the titration of small quantities of Ponceau SX, e.g. 1–5%. Also an enlarged graph for more accurate interpretation of the true titer value, especially where small quantities of Ponceau SX are involved.

DISCUSSION

To arrive at a better understanding, the results are presented in table form below, expressed in percentage:

Sample 1	*Theoretical*				
Ponceau SX	0.45	1.10	1.25	0.60	1.08
Ponceau 3R	83.50	82.60	81.80	84.05	83.37
Total color	83.95	83.70	83.05	84.65	84.45
Sample II	*Theoretical*				
Ponceau SX	5.37	6.00	5.30	6.60	6.60
Ponceau 3R	78.88	78.10	78.35	78.24	78.50
Total color	84.25	84.10	83.65	84.84	85.10
Sample III	*Theoretical*				
Ponceau SX	0.00	probably trace	trace or none	0.00	0.00
Ponceau 3R	83.92	82.20	84.35	84.67	84.65
Total color	83.92	82.20	84.35	84.67	84.65
Sample IV	*Theoretical*				
Ponceau SX	22.39	23.40	22.20	22.21	24.00
Ponceau 3R	62.94	62.10	61.50	63.28	62.65
Total color	85.33	85.50	83.70	85.49	86.65
Sample V	*Theoretical*				
Ponceau SX	33.58	33.90	33.00	32.30	35.70
Ponceau 3R	52.45	52.50	51.10	53.64	50.55
Total color	86.03	86.40	84.10	85.94	86.25

The results reported by the collaborators indicate that good results can be obtained by this method. The majority of the results vary less than 1 per cent from theoretical, and only in isolated cases are they slightly above that figure, while in two instances the difference is slightly over 2 per cent. The suggestion proposed by some of the collaborators to enlarge the graph is not considered practicable by the Referee since its size would prove unwieldy. The results show that 1 per cent and even less can be determined by that chart.

Since this collaborative work was undertaken chiefly to determine amounts of ponceau SX ranging from 0.5 to 35 per cent, it is suggested that it be continued with increased percentages of ponceau SX.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be continued on the quantitative determination of ponceau SX in the presence of ponceau 3R.

(2) That investigational work be continued on the quantitative separation and determination of tartrazine and sunset yellow FCF in mixtures.

(3) That investigational work be undertaken to separate and determine quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

(4) That the following changes be made in *Methods of Analysis, A.O.A.C.*, 1935.

Chapter **XXI**, p. 245, **16(b)**: Add the phrase, "and dry carefully, and if the color turns purple the presence of annatto is confirmed," after the words, "Dry the filter and add a drop of $SnCl_2$ soln."

Chapter **XXIII**, p. 290, **87**, read "HCl" instead of "HCl (1+2)."

REPORT ON METALS IN FOODS

By H. J. WICHMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The Referee and local associate referees have revised Chapter XXIX, Metals in Foods, of *Methods of Analysis, A.O.A.C.* in preparation for the fifth edition. Most of the revisions concern changes in language that are intended to clarify the methods without changing the principles. Some of the changes require recommendations by the Referee, but no special prior collaboration appeared to be necessary. These changes will be discussed later in the special sections. As a result of recommendations of the associate referees some of the methods have been made tentative. No reports are offered by the associate referees on the determination of selenium and fumigation residues in foods.

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, 23, 71 (1940).

ANTIMONY

The Referee had hoped that he would be able to call attention to the publication of a paper on the determination of micro quantities of antimony based on the principles of the Cassil-Wichmann arsenic method, *This Journal*, **22**, 436 (1939). These hopes have not been fulfilled. Last year's recommendation for work on the determination of antimony is therefore repeated.

ARSENIC

The associate referee endeavored to combine a further study of the sample preparation of arsenic samples with the Cassil-Wichmann method of arsenic determination. He had no opportunity to study the molybdenum blue method proposed last year for quantities of arsenic trioxide up to 10 micrograms. The Klein-Vorhes xanthate-molybdenum blue method, *This Journal*, **22**, 121 (1939), has produced good collaborative results for quantities of arsenic from 100 to 800 micrograms, but it has not been tested for smaller quantities. The Referee believes that a combination of the Cassil-Wichmann system of arsenic isolation with a molybdenum blue colorimetric determination should be successful for 1–10 micrograms of arsenic and hopes that the idea can be tested during the coming year. Some quick and accurate method for the ultramicro range of arsenic is needed. A number of chemists are working on the determination of minute quantities of arsenic, and the Referee hopes that some of these investigations will be completed during the year.

The associate referee presents some collaborative results that may be classified as fair, but they are not up to the standard anticipated by the authors of the Cassil-Wichmann method. Some unexpected difficulties appeared. The Referee believes that these have been overcome to a great extent by the associate referee since the meeting. Both referees consider that a further collaborative study should now produce some satisfactory results.

The Referee apologizes for his inability to present a quick, reliable, and accurate method for both minute and large quantities of arsenic that could be substituted for the somewhat antiquated Gutzeit method. He believes it unwise, however, to make any recommendations for adoption of any new arsenic methods at this time. He therefore recommends further study of arsenic methods.

COPPER

The associate referee submitted an abstract of the work he has done on the determination of 25 micrograms of copper in the presence of 10–25 micrograms of cobalt and nickel and 10–100 micrograms of bismuth. He reports that 10 micrograms of the three contaminants had little effect on the results for copper by visual or photoelectric filter photometry. If larger quantities of these interfering metals are present, some measures

for their removal must be provided or, in the case of bismuth, its effect determined by complexing out the copper. The associate referee found that the maximum deviations of either visual or photoelectric filter photometry were small, but that the maximum deviations of the former were two to three times that of the latter. No average deviations are given.

It is unfortunate that the associate referee did not submit a detailed description of a copper method that could have been made the basis for some recommendation. The formulation of such method, therefore, must be left to the next associate referee.

FLUORINE

The previous associate referee, *This Journal*, **21**, 208 (1938), called attention to the small plus errors of the original Willard and Winter thorium nitrate titration and also of the back titration modification introduced by Allen (private communication) and presented by Dahle *et al.; This Journal*, **21**, 459 (1938). This error persists even when pure fluorides and perchloric acid are used. As it amounts to only a relatively few micrograms per 150 or 200 ml. distillate, it is not of much concern in the determination of fairly large quantities of fluorine but it does become serious in the determination of the smaller quantities. The previous methods could not even detect it. The Referee and Dahle, then associate referee, thought the error might be due to fluorine derived from the glass apparatus. The associate referee shows that the error can be due to fluorine from glass only in very slight degree, and that the greater part of the blank must come from some other source.

Another interesting point is the announcement by the associate referee that a new fluorine method suitable for micro as well as larger quantities of fluorine will soon be published. It depends on the bleaching effect of fluorine on the red aluminon complex of aluminum. It seems suitable for the photometer as well as for Nessler tubes. If it can be adapted to the "strip" solution obtained in the determination of lead on apples, it may prove to be the faster and cheaper method for fluorine so badly needed in the fruit industry. The Referee hopes that the thorium "back titration" method and the new aluminum method can be developed during the coming year, so that there may be two widely differing methods for use in the problems of sample preparation and determination of small quantities of natural fluorine in foods, feeds, and biological materials. Comparable results by these methods on the same sample would prove very convincing.

LEAD

Certain deletions and revisions in the present tentative and official lead methods are advisable and necessary.

In paragraph **16(a)** is a description of the treatment given to lead dithizonate extracts prior to electrolytic determination. The Referee recommends that Perlman's method, *This Journal*, **20**, 622 (1937), of treating the extract with 110 ml. of 1 per cent nitric acid, filtering, and electrolyzing a 100 ml. aliquot be added as an alternative procedure. The associate referee checked this procedure and finds that it is practicable and quite accurate. Former fears that enough chlorine would be liberated to vitiate the results seem to be unfounded. This finding of fact by two independent observers appears to be a sufficient substitute for collaborative work.

The Referee recommends that paragraph **25(b)**, which was intended to insure the complete removal of tin before the colorimetric dithizone determination, be eliminated as it is no longer necessary. At the time the 1935 edition was issued apprehension existed in regard to the possible reduction of stannic tin to stannous tin by the ammoniacal citrate-cyanide solution. It has since been shown that this reduction may occur only when the tin in solution exceeds 50 milligrams. Paragraph **25(a)** describes a method that will reduce the amount of contaminating tin to much less than 50 milligrams.

Paragraph **26(a)** describes the bismuth-iodide-ethyl acetate method for removing the major part of a bismuth interference. This method has not become popular among analysts. They generally prefer the Willoughby separation at *p*H 2.0 for small quantities of bismuth as given in **26(b)**. Paragraph **26(c)** describes a procedure for handling large quantities of bismuth, as in the determination of lead in bismuth compounds. It is therefore recommended that **26(a)** be eliminated.

Paragraphs **31** and **32** were written when the greatest interest in the quantity of lead on apples and pears was centered at .018 gr./lb. The greatest sensitivity in the Nessler tube dithizone method is found at the beginning and at the end of the range, not in the center. Solutions were therefore arranged so that the quantity of lead of greatest interest was placed a little below the top of the range. At present most interest is shown in a lead load of .025 gr./lb., therefore the top of the range is now .027 gr./lb., and .025 gr./lb. is in the sensitive part. The Referee requests authority from the Association to make this change.

The associate referee's report deals with a collaborative examination of two maple sirups by a modified Perlman[1] procedure. The modifications include a stronger solution of hydrochloric acid to make sure of the solution of water-insoluble lead, a larger sample, and possibly a more rational system of taking an aliquot. The use of stronger acid gave zinc an undesired opportunity to react with dithizone and be extracted with the lead because the complexing cyanide combined with other complexes set free from the sugar. A weaker acid is therefore indicated. The associate

[1] *Ind. Eng. Chem., Anal. Ed.*, **10**, 134 (1938).

referee recommends repetition of the collaborative work with a view to adoption of a tentative method next year.

MERCURY

The report of the associate referee discusses concentration of mercury by metallic zinc and a final titration with dithizone, which is the reverse of the Fischer-Leopoldi[2] two-color titration method. The associate referee's results on the final titration were few in number but of surprising accuracy. No collaborative results are reported. He indicates that he has at least temporarily abandoned the idea of determining mercury by photometric methods because of unexplained fluctuations in the readings when the mercury solutions are exposed to the intense light within the photometer. The Referee agrees to the changes the associate referee proposed for the tentative method, not entirely on the basis of the results submitted, but because the fundamental ideas are similar to those tested and published by Fischer. The associate referee should now be in a position to submit his method to extensive collaboration as a step in the process of making it official.

ZINC

The tentative method for zinc, which specifies that the sample taken for analysis shall contain a minimum of 2 milligrams of zinc, was formulated when a milligram was considered a micro quantity. To obtain 2 milligrams of zinc, a 50 or 100 gram sample of low zinc material might be required, or, if only a 10 gram sample is available, it would necessitate 200 p.p.m. of zinc to provide the 2 milligrams of zinc. Such a state of affairs is unsatisfactory at this time, and therefore the Association enlisted the services of the biochemists in developing suitable methods. Holland and Ritchie submit a micro method that determines 100 micrograms of zinc as a possible maximum. They sent one sample of finely ground polished rice and one of seed rye to collaborators, and ten sets of results are reported. On the rice sample, the zinc varied from 18.3 to 26.2 p.p.m. and on the rye sample, from 24.3 to 51 p.p.m., with averages of 22.5 and 40.5 p.p.m., respectively. No information is available on the true content of zinc nor on the quantity of possibly interfering metals such as copper, cadmium, cobalt, nickel, or lead. Some of these metals may have been present in quantities approximating that of the zinc. The collaborative work therefore consisted of determining the "spread" among different analysts working with the same sample. In the Referee's opinion the spread was more than it should be for a satisfactory method, and he hopes that further effort will diminish it. A micro method for zinc should appear in the new edition of *Methods of Analysis* in addition to the one now tentative so that analysts may have an opportunity to select the method most suitable for materials to be analyzed. Therefore it is

[2] *Z. Anal. Chem.*, **103**, 241 (1935).

recommended that this method be adopted as tentative, with the understanding that tentative status does not necessarily carry a 100 per cent endorsement of the Association. It is also recommended that collaborative work be continued for another year in an effort to improve the method. Next year's work should include the collaborative analysis of at least one synthetic ash containing known quantities of zinc as well as interfering metals, such as copper, cadmium, lead, cobalt, nickel, and bismuth. Probably such a sample should be of a slightly acid nature and be distributed in carefully cleaned Pyrex bottles to prevent contamination before analysis.

RECOMMENDATIONS[3]

It is recommended—

(1) That the first three recommendations made by Committee C last year with reference to arsenic and antimony be continued.

(2) That studies on micro methods for the determination of copper be continued.

(3) That studies on the determination of fluorine be continued.

(4) That changes recommended by the Referee in this report with respect to changes in the lead methods be adopted and that studies on the determination of lead be continued.

(5) That the changes in the present tentative method for mercury recommended by the associate referee be adopted and that intensive collaborative work on the modified method be undertaken.

(6) That studies on the determination of selenium be continued.

(7) That the method for the determination of zinc proposed by the associate referee be adopted as tentative and that it be subjected to further collaborative study.

(8) That the study of the determination of hydrocyanic acid be continued.

REPORT ON ARSENIC

By C. C. CASSIL (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

At the 1938 meeting of the Association of Official Agricultural Chemists, Cassil and Wichmann proposed a rapid volumetric micro method for determining arsenic, *This Journal*, **22**, 436 (1939). This method has a range of 5–500 micrograms of arsenious oxide and was found to have an accuracy of 99.5 per cent and a standard deviation of 0.85 per cent. In addition to its accuracy, this procedure has the advantage over other procedures that not more than 10 minutes is required for a determination. Since the method appeared to be far superior to the present official Gut-

[3] For report of Subcommittee C and action by the Association, see *This Journal*, 23, 64 (1940).

zeit method and had the advantages stated over other arsenic micro methods, the Associate Referee decided to submit it to collaborative study.

Three arsenic solutions and a sample each of shrimp and tobacco were sent to collaborators to be analyzed for arsenic. The samples of shrimp and tobacco served to check, not only the method of determination, but also the sample preparation with perchloric acid as recommended by Cassil, *This Journal*, **20**, 171 (1937). The following directions were sent to each collaborator:

DIRECTIONS FOR THE ANALYSIS OF A.O.A.C. SAMPLES

The analyst should first familiarize himself with the method by running a sufficient number of known quantities of arsenic through the generator and the titration procedure before continuing with the unknown samples. It is believed that the directions given in the reprint, previously sent to you, are ample for the arsenic determination. If micro burets are not available, use 10 ml. burets graduated to 0.05 ml.

Sample 1, labeled "Arsenic solution (low content)," contains arsenate equivalent to 5–50 micrograms of As_2O_3 per 10 ml. Use 10 ml. aliquot per determination, titrate with 0.001 *N* iodine (p. 438 of reprint), and report as micrograms of As_2O_3 per 10 ml.

Sample 2, labeled "Arsenic solution (medium content)," contains arsenate equivalent to 50–250 micrograms of As_2O_3 per 10 ml. Use 10 ml. aliquot per determination, titrate with 0.005 *N* iodine and report as micrograms of As_2O_3 per 10 ml.

Sample 3, labeled "Arsenic solution (high content)," contains arsenate equivalent to 250–500 micrograms of As_2O_3 per 10 ml. Use 10 ml. aliquot per determination, titrate with 0.01 *N* iodine, and report as micrograms of As_2O_3 per 10 ml.

Sample 4 (shrimp) and sample 5 (tobacco) are to be treated as follows:

Place 10 gram sample in 800 ml. Kjeldahl flask, add 10 ml. of water to prevent spontaneous combustion, and then introduce a mixture of 20 ml. of H_2SO_4 and 15 ml. of HNO_3, or add the HNO_3 first followed by the H_2SO_4. As soon as the initial reaction has subsided, apply heat from a burner and add more HNO_3 in small portions from time to time as the material begins to turn brown or darken. After the third addition of HNO_3, add 10 ml. of 60% $HClO_4$ to the solution and continue to heat. Usually the solution clears or turns yellow (owing to liberation of Cl) very shortly, but if carbonization occurs after the addition of the $HClO_4$, a small portion of HNO_3 will clear the solution. Both shrimp and tobacco contain materials that are refractory to acid digestion, but they can be completely digested if an additional 5 ml. of 60% $HClO_4$ is added and the mixture is boiled for 30 minutes. Make the digested water-white solution to a definite volume and use a suitable aliquot for the 5 minute arsine evolution and iodine titration as directed in the reprint. Run each sample in duplicate and report results as p.p.m. of As_2O_3.

RESULTS

The results obtained by eight collaborators and the Associate Referee are given in Table 1. The figures for the arsenic solutions are averages based on two or three determinations, and those for shrimp and tobacco are averages of two determinations and two digestions. The precision (or duplication) obtained by each collaborator is within the limits stated by Cassil and Wichmann. Some of the recoveries, however, are not so good

as those shown in the development work, but in general they are a definite improvement over the empirical procedures previously used for small quantities of arsenic.

The Associate Referee believes that the next set of collaborative samples will make an even better showing, because certain improvements were developed after the collaborators' results and suggestions had been received.

TABLE 1.—*Collaborators' analyses on three arsenic solutions and a sample each of shrimp and tobacco*

COLLABORATOR	ARSENIC SOLUTIONS			SHRIMP	TOBACCO
	NO. 1	NO. 2	NO. 3		
	microgram As_2O_3 per 10 ml.			p.p.m. As_2O_3	
	Arsenic added				
	30.0	150.0	375.0		
	Arsenic found by collaborator				
American Can Company,					
O. F. Ecklund	28.0	149.5	371.5	14.0	35.1
California Department of Agriculture	31.1	145.0	367.7	15.6	32.7
U. S. Department of Agriculture:					
Food and Drug Administration					
A. K. Klein	29.8	148.9	361.3	15.3	38.1
D. M. Taylor	28.1	142.0	376.0	15.2	40.5
P. A. Mills	26.9	151.0	372.5	14.9	41.3
Bureau of Entomology and Plant Quarantine					
L. Koblitsky	33.7	154.3	376.8	10.3	29.3
J. E. Fahey	26.1	136.8	325.5	13.6	31.3
C. C. Cassil	29.1	148.0	372.0	15.4	34.8
H. D. Mann	—	155.0	374.0	13.4	36.8
Average	29.1	147.8	366.4	14.2	35.5
Average per cent recovered	97.0	98.5	97.7	—	—

MODIFICATION OF METHOD AND PRECAUTIONS

Experiments have shown that the method can be definitely improved by the omission of the potassium iodide, reagent (g). There is sufficient iodide in the standard iodine solutions (25 grams per liter), and when the potassium iodide is added in this manner there is a simultaneous oxidation of the mercury arsenides and the formation of the mercury iodide complex. This modification not only saves time in the preparation of reagent (g) and the quantity of potassium iodide, but the oxidation is faster and more complete, and much less trouble (if any) is encountered in the sticking of the arsenides to the resin tube. If the potassium iodide is added separately, the mercury particles tend to agglomerate and thus become more difficult to oxidize. Potassium iodide reagents from two sources were

used in making two standard iodine solutions, and these gave an average recovery of 99.1 per cent of arsenic on seven determinations each. These recovery tests were made to show that the potassium iodide of reagent grade appears to be sufficiently pure, when used as stated above, without the necessity of recrystallization, which was previously required.

Potato starch forms small granules that absorb iodine, making the solution titrated appear blue even after the end point has been reached. For this reason reagent (k) should be made from a good grade of soluble starch instead of potato starch.

The several granulated zincs that have been tried for the evolution of hydrogen and arsine in this method give satisfactory results. Some of these zincs tend to remain on the bottom of the flask or float with a spongy ball of reduced tin below the surface of the liquid, whereas others tend to float with a spongy mass of reduced tin at the surface of the evolution solution. Although all arsenic reduced to trivalent form is completely liberated from the solution in 5 minutes or less by the various types of granulated zinc used, it appears more desirable to keep the zinc-tin mass below the surface of the liquid so that the generated atomic hydrogen can obtain better contact with the solution and at the same time afford better stirring action. If the floating type of zinc is used, about 1 ml. of 1 per cent lead acetate solution will prevent it from floating on top of the evolution solution. It now seems desirable to specify 20-mesh granulated zinc in place of 20- or 30-mesh, since the larger particles have suffcient surface area and are less likely to float with the spongy tin.

Stannous chloride reduces arsenious oxide, at least partially, to insoluble metallic arsenic in a moderately concentrated acid (1+1 HCl or more concentrated); and metallic arsenic is much more difficult to reduce to arsine by the action of atomic hydrogen than is the soluble arsenious oxide. Consequently low recoveries are likely to be obtained if the stannous chloride is added to the evolution solution before the final dilution (80–90 ml.) is made.

Standard ground-glass joints are not always gas tight. Obviously when gas is lost through imperfect ground-glass joints, the recoveries are certain to be low. Such leakage can be obviated by grinding the joints with fine emery dust; if the leaks are not too large, the apparatus can be hermetically sealed with a small quantity of stopcock grease.

Atomic hydrogen produced by the action of acid on zinc, under the conditions of the method, is not capable of reducing arsenate to arsine quantitatively. The reduction of arsenate to arsenite by potassium iodide is a function of acid concentration, temperature, and time. This rapid volumetric method allows no additional time for the reduction of arsenate to arsenite. When the required amounts of acid and potassium iodide are added to an aliquot of arsenate solution not exceeding 50 ml. before final dilution, complete reduction and recoveries are obtained. If

the aliquot exceeds 50 ml. before the addition of these reagents, a loss of 2–10 per cent may result due to incomplete reduction. One collaborator reported that he did not have this difficulty with 75 ml. aliquots when he allowed time for reduction as given in the official Gutzeit method. Since it is seldom necessary to use an aliquot greater than 50 ml., the above facts do not place a limitation of any consequence on such a flexible method.

The perchloric acid procedure described is necessary for materials such as shrimp, tobacco, dyes, etc., since they cannot be completely destroyed by the ordinary sulfuric-nitric acid digestion. If the procedure is followed as directed, there is a considerable saving in time and reagents, but some analysts do not like to use perchloric acid because of the alleged danger connected with such a digestion. However, the perchloric acid may be added after the usual nitric-sulfuric acid digestion appears to be complete. To make sure that the digestion is complete by either procedure, it may be necessary, even after the solution is taken to white fumes, to add two additional 5 ml. portions of perchloric acid (60%) and heat to fuming each time. A sure test for complete digestion is the failure to obtain a dark or black appearance of the zinc in the arsine-generating flask after the reagents are added.

RECOMMENDATIONS[1]

It is recommended that further collaborative study be done on this method, since these results are not so good as those reported by Cassil and Wichmann and because certain improvements have been made.

REPORT ON COPPER

By DAVID L. DRABKIN (Medical School, University of Pennsylvania, Philadelphia, Pa.), *Associate Referee*

The following samples were subjected to analysis by several collaborators:

1. 0.025 mg. Cu in dilute HCl.
2. 0.025 mg. Cu plus 0.01 mg. Co, 0.01 mg. Ni, and 0.01 mg. Bi in dilute HCl.
3. 0.025 mg. Cu plus 0.01 mg. Co, 0.01 mg. Ni, and 0.10 mg. Bi in HCl.
4. 0.025 mg. Cu plus 0.025 mg. Co, 0.025 mg. Ni, and 0.10 mg. Bi in HCl.

The determination in all cases was by means of the colored diethyl dithiocarbonate complexes extracted by 10 ml. of iso-amyl acetate, and the use of the photometric procedure suggested by the Associate Referee, *This Journal*, **22**, 320 (1939).

Upon Samples 1 and 2, three collaborators obtained the following results:

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 23, 64 (1940).

	Maximum deviation (%)
Visual filter photometry	5.2
Photoelectric filter photometry	2.1

Upon Sample 3, results were unacceptable by the direct procedure (i.e. involving no separation of copper from contaminants), but were good by the Associate Referee's method of difference, with cyanide, *Ibid.*

	Maximum deviation (%)
Visual filter photometry, and cyanide separation	7.1
Photoelectric filter photometry, and cyanide separation	1.9

These results verify the effectiveness of the use of cyanide in correcting for contamination by bismuth, and also confirm the fact that contamination with small quantities of nickel and cobalt is of little moment, *Ibid.*

Good results upon Sample 4 were obtained only after copper was separated from the cobalt and nickel by treatment with hydrogen sulfide in acid solution by the technic (Method I) suggested by Coulson, *Ibid.*, **19**, 219 (1936) and subsequently corrected for contamination by bismuth by the cyanide method.

It is the opinion of the Associate Referee that during the next few years objective or photoelectric methods will largely replace so-called colorimetric methods. In view of this possibility lists of filters have been prepared, so that the users of different instruments may have no trouble in choosing filters. It has been verified that, aside from sufficient total transmission, the main consideration is the wave-length location of the maximum transmission of a filter, so that glass composites such as the Corning colored glasses, or Wratten dye-impregnated gelatin filters, may be used interchangeably.

The Associate Referee believes that the method is ready for tentative adoption by the Association.[1]

REPORT ON ZINC[2]

By E. B. HOLLAND and W. S. RITCHIE, *Associate Referees*

For further study of a colorimetric method for the determination of zinc in foodstuffs two samples, with granulated zinc for the standard, were submitted with detailed directions to various collaborators. The response as usual was restricted by the pressure of other duties. Sample 1 was polished rice and Sample 2, seed rye. Both samples were substantially air dry and ground in a 1 mm. sieve.

Some operators experienced difficulty in ashing the rice, which yielded a compact ash that retained a portion of its zinc unless boiled. The rye gave a light bulky ash that was more easily extracted. The analytical results are shown in the table.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 23, 64 (1940).
[2] Contribution No. 357, Massachusetts Agricultural Experiment Station, Amherst, Mass.

The details of the method, which was adopted as tentative, will be published in the new (1940) edition of *Methods of Analysis, A.O.A.C.*, to issue about July 1st, 1940.

The collaborators seemed to acquire the general technic more readily than was expected, but their control was faulty. Attention may be called to several features of the work that merit consideration.

(1) The ash solution should be heated to boiling to insure complete solution of the zinc. Digestion on a steam bath was indefinite and proved inadequate.

(2) The dithizone should be carefully prepared and added in small portions to the proper tint in the first and third extractions.

(3) Many operators can not control consecutive visual readings of the colorimeter within ±0.20 and different individuals show a wider range than this on the same solution. A photoelectric instrument is preferable.

Summarized results

	Sample 1	Sample 2
	p.p.m.	*p.p.m.*
R. W. Barton, Mead Johnson & Co., Evansville, Ind.	24.4	31.4
L. N. BeMiller, Mead Johnson & Co., Evansville, Ind.	18.7	31.2
E. J. Miller, Michigan State College, East Lansing, Mich.	21.0	51.0
L. V. Taylor, American Can Co., Maywood, Ill.	24.5	49.4
W. O. Winkler, U. S. Food and Drug Adm., Washington, D. C.	26.22	24.31
R. A. Caughey,* Agricultural Expt. Station, Amherst, Mass.	18.33	41.84
W. H. Hastings, Agricultural Expt. Station, Amherst, Mass.	22.32	44.54
W. H. Hastings, solution boiled	24.69	45.53
V. F. Coutu, solution boiled, Agricultural Expt. Station, Amherst, Mass.	22.06	45.52
Av.	22.47	40.53
P. A. Clifford,† U. S. Food and Drug Adm., Washington, D. C.	25.3	39.6

* By the original method.
† Received too late to be included in average.

REPORT ON FLUORINE

By P. A. CLIFFORD (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The Willard and Winter method[1] for titration of fluoride with thorium nitrate, as modified by several investigators, has proved very satisfactory for the larger quantities of fluorine, but efforts to adapt this procedure to micro quantities (< 50 micrograms) have not been so successful. There are several reasons for this.

The mechanism of the Willard and Winter titration involves the addition of a standard thorium nitrate solution to a solution of fluoride, in the presence of alizarin, untill all the fluoride present is bound up as insoluble thorium fluoride. At this point further addition of thorium solution results in the formation of a purple thorium-alizarin lake, and under optimum conditions of *p*H and with a thorium solution of sufficient strength this end point is very sharp.

[1] *Ind. Eng. Chem., Anal. Ed.*, 5, 7 (1933).

Adaptation of this general procedure to the micro determination of fluorine has necessitated the use of a very dilute standard thorium solution (commonly about .0004 *N*) and even when the titration is conducted in very small volume the end point is not so sharp. McClure[2] reports an average of 0.36 ml. of standard .0004 *N* thorium solution, equivalent to approximately 2.0 micrograms of fluorine, consumed merely in developing the thorium-alizarin lake to a satisfactory end point. The Associate Referee has attempted to check photometrically the rate of development of this end point lake as follows:

Twenty micrograms of F_2 as NaF was measured into each of a series of 10 ml. volumetric flasks, 0.25 ml. of a 0.01% solution of alizarin red was added, and the solutions were brought to the transition point of the indicator by a trace of dilute (0.01 *N*) HCl. Next 0.25 ml. of the Hoskins-Ferris buffer mixture and the indicated quantities (Table 1) of an approximately 0.0004 *M* solution of $Th(NO_3)_4$ (0.25 g. of $Th(NO_3)_4 \cdot 12H_2O$ per liter) were measured into the flasks. After dilution to mark the mixtures were filled into a 2 inch cell and read with a photometer and monochromatic filter centered at 514 mu. (This wave-length point is about optimum for tracing the development of the reddish-purple thorium-alizarin lake.)

Scale readings and descriptions of the colors are given in Table 1.

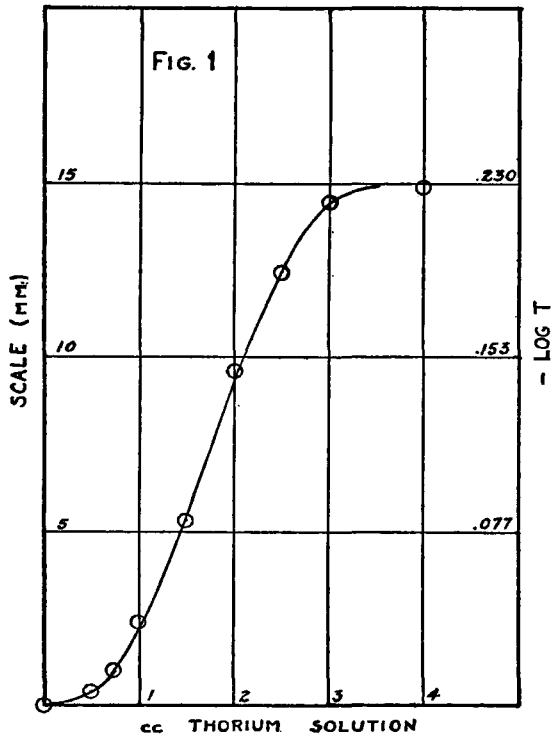
TABLE 1.—*Scale readings and colors*

$Th(NO_3)_4$ SOLUTION	SCALE READING	APPEARANCE
ml.		
0.0	0.0	Greenish yellow
0.50	0.4	Greenish yellow, paler
0.75	1.0	Tannish yellow
1.00	2.4	Pinkish tan
1.25	—	Stronger pinkish tan
1.50	5.3	Tannish pink
2.00	9.7	Pink
2.50	12.4	Stronger pink
3.00	14.4	Purplish
4.00	14.8	Purplish

Scale readings are plotted in Figure 1, and the curve shows that under these conditions the lake develops slowly but progressively before the equivalence point (about 0.75 ml.) is reached, and more rapidly thereafter. Visual changes are apparent almost from the start and are quite noticeable at the equivalence point. The relative appearances of the colors were not altered appreciably when the titrations were conducted in small volumes in vials, with less indicator and buffer. It is noted that considerable excess thorium, with this dilute solution, is required to bring the color to the "incipient pink" usually taken as an end point. In this case the operator would probably choose an end point at, or a little above, 1.0 ml. of thorium.

[2] *Ibid.*, 11, 171 (1939).

Thus, the end point is apt to be sluggish for the micro titration, and these figures point to the fact, not often stressed, that the titration with very dilute thorium solutions does not involve an end point in the usual



sense of an abrupt color change after the equivalence point is reached, but rather an equilibrium condition between fluorine and thorium, and indicator and thorium.

If conditions are rigidly standardized and titrations carried to the same end point, accurate results can be obtained, but it is sometimes difficult to reproduce this end point even when a reference titration vial is used. The reasons for this may be quite obvious. The end point is produced by a lake, and lakes are apt to assume different appearances, depending upon their degree of dispersion as affected by agitation, slight differences in *p*H, and excess of dissolved salts.

When a fluorine distillate is evaporated the perchloric acid, always present, must be neutralized. This introduces a variable amount of extraneous salt and is responsible for the plus "neutralization error" discussed by Dahle *et al.*, *This Journal*, **21**, 470 (1938). This may be due to

*p*H disturbances, but it is quite possibly due to partial coagulation of the end point lake by the excess salt. On the other hand, McClure reports losses of fluorine when evaporations are carried out in glass or porcelain instead of in platinum.

It would thus seem to be very desirable to eliminate the necessity for neutralization and evaporation of a fluorine distillate. Dahle, the previous referee, has indicated such a method as a variation of the "back-titration" procedure (*loc cit.*). The various steps are as follows:

1. Distil the ashed sample from $HClO_4$, using the precautions for obtaining a low-acid distillate, and make the distillate to volume, usually 150 ml.

2. Titrate an aliquot portion (at least 40 ml.) with 0.05 *N* NaOH, using alizarin red as indicator. To another 40 ml. of distillate, contained in a 50 ml. Nessler tube (preferably glass-stoppered) add enough 0.05 *N* acid to make the total acidity in the tube equal to 2.00 ml. of 0.05 *N* acid. Either standard 0.05 *N* HCl or $HClO_4$, which are interchangeable for the purpose, may be used.

3. Add 1 ml. of 0.01% alizarin red indicator, mix, and add $Th(NO_3)_4$ solution of approximately .0004 *M* strength until the transition point of the indicator, an "incipient pink" or "tannish pink," appears. Make to volume. (Care must be taken to develop the indicator color to this intermediate shade; if the end point is taken too yellow, over titrations in the back titration will result, and if the end point is taken too red, the analyst is apt to under-titrate.)

4. To a "blank" Nessler tube, matched for height with the "sample" tube, add about 40 ml. of water and 2.00 ml. of 0.05 *N* acid. Next add and mix in exactly the same quantity of indicator and thorium solutions used in the "sample" tube. Back-titrate with standard fluoride solution (1 ml. = .01 mg. of F) until the colors match, making nearly to volume before adding the last few drops of solution. Allow all air bubbles to escape before making the final color comparison, and check the end point by adding 1–2 drops of excess fluoride. A distinct "over-bleach" should develop.

Nessler tubes (100 ml.) with 4.00 ml. total of .05 *N* acid and 2.00 ml. of indicator may be used. In this case a 75 ml. aliquot (half of total distillate volume of 150 ml.) is convenient. The back-titration can be successfully applied even when the acidity of the distillate is considerably beyond the optimum 2.00 ml. of .05 *N* acid per 40 ml., although the upper limit is about 8.00 ml. In these cases the acidity of the "blank" tube is adjusted to an equal figure. The Associate Referee considers that this procedure is the best modification of the thorium micro titration because it effectively eliminates salt or neutralization errors.

Dahle and other investigators have noted an apparent fluorine content of the distillate from pure freshly boiled perchloric acid. The Associate Referee has found this "distillation blank" to vary from 2 to 7 micrograms of fluorine per 150 ml. of distillate. For a time it was thought that this blank was actual fluorine leached from the glassware of the still during distillation, but repeated evaporation and re-distillation of the same distillate did not cause this bank to mount.* Some substance, possibly chlorine from the perchloric acid, is developed during the distillation and

* More careful work has subsequently shown that a small portion of this blank is actually fluorine

interferes by altering or partially bleaching the color of the end-point lake. It was impossible to eliminate this "distillation blank," which is believed to be the only factor limiting the accuracy of the back-titration procedure.

DETERMINATION OF FLUORINE WITH ALUMINUM AND AURINTRICARBOXYLIC ACID (ALUMINON)

Fluoride combines with aluminum under certain conditions to form a complex anion, probably AlF_6'''. Therefore it is prominently mentioned as an interference in most colorimetric methods for aluminum. During the past year a method has been developed that utilizes the measure of this interference with a standard colorimetric aluminum method to estimate fluorine itself.

Aluminum combines with aluminon to form a highly colored reddish-purple lake. The reaction is conducted in a medium strongly buffered at about *p*H 4.5, and the colors with known quantities of aluminum and a definite quantity of the dye are used as standards in the estimation of unknown quantities of aluminum. Fluorine prevents full development of this lake color and with a properly chosen ratio of aluminum to dye this "bleaching," expressed as photometric density ($-\log T$), is proportional to the amount of fluorine present. Various ranges of fluorine concentration may be covered by varying the quantities of aluminum and dye. The colors produced are entirely reproducible, and are quite stable if a stabilizer such as gelatine is used. The Associate Referee intends to use the method, in conjunction with the back-titration procedure, in the analysis of a large number of samples of biological material. An application of the method to the determination of fluorine directly in apple "strip" solutions has given encouraging preliminary results. Details should be fully worked out by next year.

It is recommended that work on fluorine be continued.

REPORT ON LEAD

By P. A. CLIFFORD (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Work this year was confined to a collaborative study of the determination of lead in maple sirup. The rapid colorimetric dithizone method of Perlman[1] has proved very attractive to chemists engaged in the routine examination of this product for lead. Several objections have been made to his procedure: (1) The use of a rather small (15 gram) sample, which may not be representative in cases where the sirup contains considerable sediment and which may be difficult to weight out with accuracy on ordi-

[1] *Ind. Eng. Chem., Anal. Ed.*, **10**, 134 (1938).

nary trip scales; (2) the somewhat awkward way of taking the aliquot and the difficulty, sometimes experienced, in withdrawing a clear 11 ml. aliquot after the preliminary dithizone shake-out because of emulsion formation; and (3) the necessity for working up another sample for a repeat determination if the range is exceeded, as 10 of the 11 ml. of "strip acid" is used in the color development.

Two samples of sirup were sent out for study. Sample A was a commercial sirup of rather poor quality, with high zinc content but testing low in lead; and Sample B was a portion of the same sirup to which had been added pure lead nitrate equivalent to .016 grain/lb.

TABLE 1.—*Net results on collaborative samples of maple sirup (grain/lb.)*

COLLABORATOR*	REAGENT BLANK	SAMPLE A	SAMPLE B	B—A	ERROR
1	.001	.003	.021	.018	+.002
		.003	.020	.017	+.001
2	.001	.002	.018	.016	.000
		.002	.018	.016	.000
3	.000	.000	.020	.020	+.004
		.000	.020	.020	+.004
4	.0005	.0039	.0195	.016	+.000
		.0040	.0200	.016	+.000
5	.002	.006	.020	.014	−.002
		.006	.020	.014	−.002
6	.000	.001	.018	.017	+.001
		.001	.018	.017	+.001
7	.001	.003	.019	.016	.000
		.003	.018	.015	−.001

* The collaborators were: 1, M. D. Voth, Food & Drug Adm., Boston; 2, G. Kirsten, Food & Drug Adm., New York; 3, D. W. Williams, Food & Drug Adm., San Francisco; 4, L. Greathouse, Washington, D. C.; 5, C. J. Tressler, New York Agr. Exp. Sta., Geneva; 6, S. Berman, Food & Drug Adm., Buffalo; 7, P. A. Clifford, *Associate Referee.*

Collaborators were requested to analyze these samples by a modified procedure designed to eliminate the cited objections to Perlman's method. A 50 gram sample was specified, and the preliminary acid treatment was with 10 ml. of concentrated hydrochloric acid added directly to the sirup in the centrifuge bottle, as it was thought that the diluted acid used by Perlman might not dissolve all insoluble lead. The concentration of the ammonia-cyanide-citrate reagent was doubled so that excessive volumes would not be required to neutralize this strong acid for the preliminary dithizone shake-out, but amounts of reagents were kept roughly in the

ratio of the different sample weights of 15 and 50 grams. Aliquots were changed to allow the use of ordinary transfer pipets; viz., 10/20×10/25. The directions for standard preparation were retained as these aliquots represent the same weight of sirup in the final color comparison. The collaborative results are shown in Table 1.

COMMENTS OF COLLABORATORS

In nearly all cases collaborators noted the extraction of zinc in the first dithizone shake-out. Collaborator 1 could not always match colors because of this interference and was compelled, except in one case, to re-extract a portion of the acid strip solution. Collaborator 2 noted that zinc came out in the first extraction but was repressed in the final color development. He noted a yellow "off" shade in the "backing" tubes and complains that the sorting value of the original Perlman technic is largely lost because of the interference of zinc in the first dithizone shake-out. Collaborator 3 repressed the zinc interference in the first shake-out with 2–3 grams of solid potassium cyanide and noted that the sample colors seemed paler than those of the standards. Collaborators 4, 5, and 6 also noted zinc coming out in the first extractions. Collaborator 6, in addition, noted poor color matches with Sample B, but a satisfactory match at .019 grain/lb. after an additional extraction. He objected to the two strengths of acid and of ammonia-cyanide-citrate, and to the use of a larger sample as impairing the speed of the procedure. The volume of strong dithizone in the preliminary shake-out was stated to be too small for general application because of excessive emulsion formation with certain sirups.

With Perlman's unmodified method Collaborator 1 got .003 and .020 grain/lb. on Samples A and B and noted no color interference in the first shake-out. With the ashing-electrolytic procedure, *Methods of Analysis, A.O.A.C.*, 1935, 379, **14, 17, 18,** he obtained respective results of .002 and .019 grain/lb. Collaborator 2 got .003 and .019 grain/lb. with the unmodified colorimetric method. Collaborator 5 got .003 and .019 with his own method.

Most of the collaborators noted approval of the different system of taking an aliquot and of the use of the larger sample. The chief complaint was of the interference of zinc.

DISCUSSION OF RESULTS

Recoveries are sufficiently close to theoretical in most cases to demonstrate the general reliability of the Perlman technic. The suggested modification apparently does more harm than good because of zinc interference and the appearance of "off" shades in the color development. These difficulties were not noted in the unmodified procedure and are apparently due to the use of the larger sample and to the more drastic "mushing." The strong hydrochloric acid apparently partially decomposes the sugar

and liberates groups that react with cyanide, thus permitting zinc to be extracted. The use of strong hydrochloric acid was probably a mistake, as the diluted acid of Perlman appears just as efficient in dissolving lead.

The study should be repeated after these troubles are overcome with a view towards tentative adoption of the method. It is recommended that work on lead in foods be continued.

REPORT ON MERCURY

By W. O. WINKLER (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The work this year consisted largely of a consolidation of the work done in the past. It was directed mainly to the preparation of the solution for the determination of mercury, and to the concentration of mercury with powdered zinc. The investigation resulted in a more accurate method of titration both for very small quantities (0–15 micrograms) and for larger quantities (0-250 micrograms) and in a better understanding of the difficulties encountered in the photometric method.

CONCENTRATION WITH ZINC

It was shown in the report of the Associate Referee two years ago, *This Journal*, **21**, 220 (1937), that mercury could be completely removed from a slightly acid solution by replacement precipitation with powdered zinc. The quantity of zinc required (3 grams) contributed 12–14 micrograms of mercury. By preparing a smooth coat (filter strata) of zinc on a No. 3 porosity fritted glass filter crucible (Ace), the Associate Referee found that it was possible to remove mercury completely from the pure solutions (slightly acid HCl), using only 0.8 gram of zinc. This quantity of zinc contributed only 3.2 micrograms of mercury. Time has not permitted the investigation of the removal of mercury from organic solutions by this method. Some results obtained on pure solutions are given in Table 1.

TABLE 1.—*Recovery of Hg from slightly acid solutions by filtration through zinc dust (micrograms)*

Hg ADDED	Hg CONTRIBUTED BY ZINC*	TOTAL Hg PRESENT	Hg FOUND	DIFFERENCE BETWEEN Hg FOUND AND PRESENT†
micrograms				
11.32	3.2	14.5	13.5	−1.0
14.53	3.2	17.7	17.7	−0.0
21.28	3.2	24.5	23.8	−0.7
45.54	3.2	48.7	48.1	−0.6

* The Hg content of the Zn may not be uniform and the figure may therefore be slightly different in each sample.

† Any error in determination or reading of buret in adding the Hg is also present in the result. The content of Hg in the Zn if not entirely uniform would also contribute to the difference between Hg present and Hg found.

The results show that the procedure is worthy of further study.

ISOLATION AND PREPARATION OF THE MERCURY SOLUTION

In the present tentative method, *Methods of Analysis, A.O.A.C.*, 1935, 395, 4, the mercury is concentrated from the original acid extract of the food (after the latter has been oxidized) by a preliminary extraction with dithizone. These dithizone extracts are then either evaporated and the dithizone residue oxidized, or the dithizone is oxidized directly while in solution in the solvent (CCl_4 or $CHCl_3$) by shaking with a warm oxidizing solution. A much more satisfactory procedure for accomplishing this is by transferring the mercury from the dithizone-solvent phase to the aqueous phase. This can be done as indicated in the report of 1937 by shaking with an acid thiosulfate solution. Destruction of the sodium thiosulfate with potassium permanganate then releases the mercury from the thiosulfate complex. Excess permanganate is reduced with hydroxylamine hydrochloride, which produces a solution very stable to dithizone and ready for determination. The transfer can be made without loss with dilute nitric acid in place of sulfuric acid, which was formerly used.

It is proposed, therefore, to treat the dithizone extracts obtained as indicated above in the following manner in place of the present A.O.A.C. procedure:

Place the dithizone (CCl_4) extracts in a separatory funnel (125–250 ml.), add 40 ml. of distilled water, 1 ml. of HNO_3 (1+19), and 5 ml. of 1% $Na_2S_2O_3$. Close the funnel and shake vigorously for 30 seconds. Allow the layers to separate and draw off the CCl_4 layer. Filter the aqueous layer through a wet plug of cotton in a short-stemmed funnel into a 500 ml. Erlenmeyer flask to remove any CCl_4 droplets. Wash the separatory funnel with 2 portions of water (about 5 ml. each) from a wash bottle and then use these portions to wash the short-stemmed funnel by allowing it to run down the sides of the latter. Add 1 ml. of HNO_3 (1+1) and 6 ml. of a saturated solution of $KMnO_4$ and place an air condenser about 2.5 feet long held by a rubber stopper in the flask and then place the flask on the steam bath for 8–10 minutes. (Before preparing the air condenser, boil the stoppers in distilled water.)

Remove the flask from the steam and cool to 35° or below. Then add slowly (dropwise) a 10% solution of $NH_2OH \cdot HCl$ while shaking with gentle rotation until the solution is clear. Add the last few drops very slowly and rotate the flask to dissolve any particles on the side. Then add 0.4–0.5 ml. of the $NH_2OH \cdot HCl$ solution in excess. Place a thermometer in the flask and warm on the steam bath to just 60° C. Remove the flask and the thermometer from the bath and cover for 1 minute, then cool the flask under a tap of cold running water. The solution is now ready for the determination.

DETERMINATION OF MERCURY

The work of the actual determination has resulted in increased sensitivity of the titration procedure and in a clearer diagnosis of some of the difficulties encountered in the photometric determination. For very small quantities (0–15 micrograms) a comparative titration was devised; it is the reverse of the one reported by the Associate Referee two years ago, *This Journal*, 21, 225 (1938), and is a modification of H. Fischer's method.[1]

[1] *Z. Anal. Chem.*, 103, 241 (1935).

The details of the method will be published in *Methods of Analysis, A.O.A.C.*, 1940, to issue about July 1st, 1940.

Results obtained by the method are given in Table 2.

TABLE 2.—*Results by modified method*

Hg PRESENT *micrograms*	Hg FOUND *micrograms*
11.6	11.6
6.5	6.5
4.4	4.4
3.1	3.1

The quantities of mercury were measured out with a 10 ml. buret graduated to 0.05 ml., from a standard solution containing 10 micrograms per ml. Readings were estimated to .005 ml. The results are considered to be remarkable as they are as accurate as it was possible to read the buret used. The determinations were made with regular 125 ml. Pyrex pear-shaped separatory funnels.

TITRATION OF LARGER QUANTITIES

Quantities of mercury of 16–250 micrograms may be titrated with dithizone of the same strength (10 mg. per liter). For quantities greater than 250 micrograms, an aliquot of the final mercury solution can be taken or a stronger dithizone solution used. The titration should be made similarly to the present tentative A.O.A.C. method, that is, by adding a 3 ml. portion of dithizone, shaking, and drawing off the extract after each second addition until an excess of dithizone has been added. From this point the procedure differs somewhat from the present A.O.A.C. method.

Since the quantity of mercury remaining in the funnel at the last can be determined with considerable precision by the comparative method discussed in this report, and the mercury equivalent of the dithizone when saturated can be determined accurately, the procedure should give accurate results on fairly large quantities of mercury.

PHOTOMETRIC DETERMINATION

It was stated in last year's report that there was a rise in the photometric reading of the dithizone containing mercury shortly after it was placed on the photometer. This rise occurred when a filter centered at 610 millicrons (No. 61) was used, but with a No. 49 filter there appeared to be a drop in the reading. It was suggested last year that the readings be made after equilibrium had been reached and the reading came to a maximum. However, this procedure does not appear to be advisable because the change is not always the same and readings of standards are often out of line. There appears to be a shift in the mercury-dithizone equilibrium, probably from keto to enol form under the influence of a strong light. Further work will be necessary to determine whether this difficulty in the photometric method can be overcome.

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative method for the determination of mercury be changed to conform to the findings of the work done this year.

(2) That collaborative work be done on the titration method.

No report on selenium was given by the associate referee.

No report on fumigation residues in foods was given by the associate referee.

REPORT ON FRUIT AND FRUIT PRODUCTS

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

No reports were received on the subjects of electrometric titration of acids and polarimetric methods for jams and jellies.

The subject of the determination of ash in fruit products was studied by Associate Referee R. A. Osborn and 10 collaborators. The report includes ash results obtained on a master solution of a strawberry preserve by three procedures, direct ash, carbonated, and sulfated ash. Based on the good agreement obtained by the analysts, the Associate Referee discusses the merits of the three procedures from the standpoint of manipulation and serviceability. Although no definite preference for any one of the three procedures is voiced, it appears that the Associate Referee prefers the direct ash in the case of products yielding an alkaline ash, such as fruit products.

The Referee agrees with the Associate Referee's views regarding the three methods of ash determination and concurs in the recommendations made by him.

H. Shuman submitted a paper on the volumetric determination of P_2O_5 in fruit products. He states that the presence of sulfates and silicates tends to give high results and suggests an over-all blank to correct for these interferences. After such a blank had been introduced 8 analysts reported well agreeing results on a synthetic P_2O_5 solution and a sugared grape juice. The results on the synthetic solution are somewhat high, about 0.9 mg. on the average on 19 samples.

As in the past, the Referee has devoted much time to methods for determining fruit acids. Although considerable progress has been made, much still remains to be done before a final report can be written regarding the determination of isocitric and inactive malic acids.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 23, 64 (1940).

Associate Referee H. Gerritz has introduced several modifications into the Zinzadze procedure for the determination of P_2O_5 in fruit products, thereby greatly improving the accuracy of the method presented last year. Collaborative results show that the volumetric method gives appreciably higher results than the colorimetric method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of electrometric titration of acids be continued.

(2) That the study of polarimetric methods for fruit products be continued.

(3) That the study of P_2O_5 in fruit products be continued.

(4) That the study of ash in fruit products be discontinued.

(5) That the study of acids in fruit products be continued.

No report on electrometric titration of acids was given by the associate referee.

No report on malic, isocitric, and lactic acids was given by the associate referee.

REPORT ON ASH IN FRUIT PRODUCTS

By R. A. OSBORN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The determination of ash and of ash constituents, such as potash (K_2O) and phosphate (P_2O_5), in fruits and fruit products and the comparison of such data with the corresponding average values obtained from the analysis of authentic fruits are of general interest in the estimation of fruit content, *This Journal*, **21**, 502 (1938). This report contains a detailed description of the collaborative study made of three methods of ash determination during the past year. Data are also given on the volumetric determination of phosphate in samples of sulfated ash.

Eleven chemists analyzed portions of the same sample solution of a strawberry and sugar mixture that was prepared according to *Methods of Analysis, A.O.A.C.*, 1935, 319, 2(C_1). Aliquots of the freshly prepared sample solution were sent to each analyst in clean Pyrex, glass-stoppered bottles, and they were analyzed upon receipt for (1) normal ash (two 100 ml. portions); (2) carbonated ash (normal ash previously obtained); and (3) sulfated ash (two 100 ml. portions of the sample solution). The Associate Referee pointed out that greater care must be exercised in weighing the normal ash since it is more hygroscopic than the sulfated ash, and also

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 23, 71 (1940).

that danger of mechanical loss in the sulfated ash determination can be avoided if overheating does not occur during the preliminary carbonization.

The following directions for the ash determinations were sent to the collaborators:

(1) *Ash (normal).*—At 525°C., *This Journal*, **21**, 504 (1938). Determine the ash of the sample solution as directed in *Methods of Analysis, A.O.A.C.*, 1935, 321, **9**, and 336, **8**, with the temperature of ashing not over 525°C. When excessive swelling or foaming occurs, add 2 or 3 drops of ashless olive oil as provided, *Ibid.*, 465, 8 and 9. Regardless of the appearance of the ash, moisten with water and without refiltering, dry on the steam bath and hot plate, and ash in the muffle. When the ash is free from carbon, place in a desiccator for at least 30 minutes, using one desiccator to each platinum dish and an efficient desiccant. Obtain the approximate weight, reheat in the muffle furnace at 525°C., cool as before, place the approximate weights on the balance pan before removing the dish from the desiccator, and weigh accurately and quickly. If constant weight has not been obtained (two weighings differ by more than 1 mg.), reheat, cool, and reweigh.

(2) *Carbonated Ash.*—Add 5 ml. of 10% $(NH_4)_2CO_3$ (reagent grade) solution to the ash (normal), stir thoroughly, evaporate on the steam bath, and carefully dry on a hot plate to avoid spattering. Heat in a muffle furnace for 30 minutes at a temperature not exceeding 275°C. Cool for 30 minutes in a desiccator, using one desiccator for each dish. Obtain the approximate weight, then reheat at a temperature not above 275°C. for at least 15 minutes, cool, and weigh accurately and quickly. If constant weight has not been obtained (two weighings differ by more than 1 mg.), reheat, cool, and reweigh.

(3) *Sulfated Ash.*—To 100 ml. of sample solution add 5 ml. of a mixture of 20% reagent grade $(NH_4)_2SO_4$ and 10% reagent grade H_2SO_4 in distilled water and evaporate to dryness in a platinum dish on a steam bath. Holding the dish by the edge with a pair of crucible tongs, heat over a moderate free flame until charring is complete. Use care to avoid spattering and luminescence of the platinum while charring and protect from drafts while burning. Place the dish in a controlled muffle at 525°C. until almost carbon free. Remove from the muffle, cool and add 5 ml. of 10% H_2SO_4 solution, wetting all portions of the ash. Heat moderately over a free flame until heavy fuming of SO_3 ceases and place in the muffle at 600°C. until a carbon-free ash is obtained. Cool in an efficient desiccator and weigh promptly after reaching room temperature. If a practically carbon-free ash is not obtained after heating 30 minutes at 525°C., resulfate and heat for 30 minutes at 600°C.; again resulfate, and heat 30 minutes at 600°C. before weighing. If appreciable quantities of carbon remain at the time of the final resulfating, low results may be obtained due to formation of sulfides rather than sulfates.

COLLABORATIVE RESULTS

Table 1 contains the collaborative results. Those obtained with each of the three ashing procedures are in good agreement, and in general, the duplicate determinations by each of the three methods agree closely. An evaluation of the three procedures may be obtained by a comparison of the results of one analyst with those of the other ten.

The average carbonated ash value is approximately 7 per cent greater than the normal ash value. The average sulfated ash value is approximately 18 per cent greater than that of the normal ash. On the basis of

TABLE 1.—*Collaborative results on sample solution of strawberry and sugar mixture*

ANALYST	STATION*	PROCEDURE 1 ASH	PROCEDURE 2 CARBONATED ASH	PROCEDURE 3 SULFATED ASH
			per cent	
J. L. Hogan	New York	.206	.225	.258
		.208	.222	.262
C. A. Wood	New York	.206	.220	.257
		.206	.219	.257
E. H. Wells	Chicago	.206	.217	.247
		.203	.219	.236
F. J. McNall	Chicago	.211	.227	.244
		.206	.224	.237
J. T. Field	St. Louis	.231	.245	.265
		.229	.243	.263
Samuel Alfend	St. Louis	.231	.243	.263
		.229	.242	.263
H. W. Gerritz	San Francisco	.199	.216	.255
		.202	.219	.250
H. M. Bollinger	San Francisco	.239	.245	.255
		.217	.225	.253
H. J. Wichmann	Washington, D. C.	.207	.216	.274
		.208	.218	.274
N. J. Menard	Washington, D. C.	.193	.213	.261
		.198	.218	.258
R. A. Osborn	Washington, D. C.	.203	.225	.261
		.205	.227	.263
Average		.211	.226	.257

* U. S. Food and Drug Administration.

the tabulated data, no particular one of the ash procedures appears to have an advantage over the others from the standpoint of reproducibility. The determination of carbonated ash involves one more step than that of normal ash. In addition, care must be exercised to avoid heating above 275°C., or some of the carbonates may be decomposed. The ash results are slightly higher and the ash is slightly less hygroscopic than in the case of normal ash. The majority of the analysts preferred the ordinary ash procedure even though this ash is more hygroscopic and must be weighed immediately, particularly on humid days.

A means to eliminate the difficulty of weighing a hygroscopic ash was suggested by H. J. Wichmann of this Administration and was used by the writer. Special lipless platinum dishes were obtained, and light alloy lids that fit tightly to the inner circumference of the dishes were made on a lathe from a cylindrical bar of the alloy. A projection from the center of the lid affords a means for manipulation of the lid with a pair of tongs. The lids were numbered to correspond with the numbers on the platinum dishes. Once the lids were placed, the dishes, lids, and hygroscopic contents could be weighed on humid days without the slightest increase in weight.

The determination of phosphate (as P_2O_5) in sulfated ash samples of fruit products and in corresponding ordinary ashes was also studied. Three authentic jam samples were analyzed. After the ash had been obtained the phosphate was determined volumetrically by treating the ash with hot dilute nitric acid, filtering, neutralizing with ammonium hydroxide, precipitating with ammonium molybdate reagent, filtering on an asbestos Gooch, washing, dissolving the precipitate in an excess of standard alkali, and back titrating with standard acid to the phenolphthalein end point as directed in *Methods of Analysis, A.O.A.C.*, 1935, 21, **12.** The results are given in Table 2. It will be observed that the sulfated ash samples must receive special treatment before the addition of the ammonium molybdate reagent. Apparently, the sulfated ashing temperature of 600°C. does not result in volatilization of phosphate but rather results in a change into meta or pyro phosphate.

TABLE 2.—*Phosphate (P_2O_5) in ash of fruit jams (whole sample)*

JAM	STRAWBERRY		CHERRY		RASPBERRY	
	mg./100 grams of sample					
		Av.		Av.		Av.
Ordinary ash	32.8		26.9		29.4	
525°C.[1]	33.0	32.0	27.6	27.3	30.2	29.8
Sulfated ash	13.7		10.3		14.0	
600°C.[1]	16.0	14.9	12.5	11.4	15.4	14.7
Sulfated ash	35.5		26.4		29.4	
600°C.[2]	34.5	35.0	26.5	26.4	29.6	29.5

[1] Ash treated with 50 ml. of hot HNO_3 (1+9), no more heating.
[2] Ash treated with 50 ml. of hot HNO_3 (1+9) and heating continued for 1 hour with addition of water at intervals to compensate for evaporation.

Considering the advantages and disadvantages of the three methods, the Associate Referee concludes that the normal ash determination is preferable. This conclusion is based on several fundamental considerations. Sulfating the ash changes the nature of the ash. The determination must be carried out at 600°C. or above in order to arrive at a constant weight within a reasonable time. At this temperature there may be

volatilization of some of the constituents, and also a change in the composition of the phosphates that causes the ash to be less valuable for the analysis of ash constituents. These objections do not apply to the normal ash, which is customarily used in the determination of the ash constituents. The chief objection to the normal ash determination made under controlled heat conditions is its hygroscopicity, but means for avoiding this difficulty are available. There are also many published authentic normal ash data that may be used in the interpretation of results of this determination.

The Associate Referee recommends the normal ash procedure.

RECOMMENDATIONS*

It is recommended—

(1) That the modifications of normal ash procedure described herein be included in the official method.

(2) That no further work be done on the subject of ash.

P_2O_5 IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS

VOLUMETRIC METHOD

By HARRY SHUMAN (U. S. Food and Drug Administration, Philadelphia, Pa.), *Associate Referee*

The volumetric method, *Methods of Analysis, A.O.A.C.*, 1935, 20, 10, offers a simple and convenient means of determining P_2O_5 on the ash of fruits and fruit products. Recent collaborative studies by Shuman, *This Journal*, **21**, 430 (1938), and by Gerritz, *Ibid.*, **22**, 131 (1939), however, have shown that results by this method vary appreciably.

Substances interfering in the volumetric method and either occurring in fruit ash or subsequently introduced are sulfates and silicates. Sulfates (as SO_4) occur normally up to a maximum of about 55 mg. per 100 grams in some fruits, the average value for all fruits being about 22 mg. per 100 grams.[1] Ross, *This Journal*, **13**, 203 (1930) and others have demonstrated conclusively that precipitation of phosphates at 45°–50° C. in the presence of sulfates yields high results. These studies, however, were made on relatively large quantities of phosphates and sulfates. Soluble silicates may be present in the ash, or introduced in neutralizing the nitric acid solution of the ash with ammonia.

Ammonium hydroxide nearly always contains small quantities of silicates derived from the bottles in which it is stored. The presence of silicates in the determination is denoted by the yellow color of the filtrate from the phosphomolybdate precipitate. Hillebrand and Lundell[2] state

* For report of Subcommittee D and action by the Association, see *This Journal*, **23**, 71 (1940).

[1] Unpublished data, U. S. Food and Drug Administration.

[2] Applied Inorganic Analysis, p. 562 (1929).

that silicates may be carried down by the precipitate as ammonium silicomolybdate. They, therefore, recommend the removal of silica by dehydration or with hydrofluoric and nitric acids before precipitation.

EXPERIMENTAL

Table 1 gives the results obtained with reagent-grade monopotassium phosphate twice recrystallized, ground, and dried to constant weight at 105° C. With the exception of the method of precipitation used in II, Table 1, the usual volumetric method was used with 10 grams of ammonium nitrate and 20 ml. of the molybdate solution. However, an overall blank was subtracted from the determinations.

TABLE 1.—*Influence of sulfates and silicates on recoveries of small quantities of P_2O_5 (expressed as mg.)*

PRESENT	FOUND					
	NO INTERFERING SUBSTANCES	6.0 MG. H_2SO_4 ADDED	Na_2SiO_3 ADDED A=5.0 MG. SiO_2 B=0.5 MG. SiO_2		10 ML. HNO_3 NEUTRALIZED WITH NH_4OH* (A AND B FROM DIFFERENT BOTTLES)	
			A	B	A	B
	I. Precipitation for 30 minutes at 45°–50° C.					
1.00	1.06–1.06	1.11–1.11	1.37	1.12	1.10	1.19
4.98	5.09–5.12	5.24–5.21	5.18	5.10	5.17	5.22
9.96	10.11	10.37				
19.92	19.96					
	II. Precipitation overnight at room temperature.					
1.00	1.07–1.07	1.07–1.04	1.32		1.22	
4.98	5.03–5.05	5.12–5.14	5.51		5.24	
9.96	9.99	10.02				
19.92	19.85					
	III. Precipitation by shaking 30 minutes (25°–30° C.)					
1.00	0.98–0.98	1.01–1.04	1.11	1.02	1.01	1.03
4.98	5.00–5.01	4.99–5.03	5.09	5.01	4.98	5.02
9.96	9.96	10.08				
19.92	19.85					

* Used in place of 10 grams of NH_4NO_3.

It is seen (Table 1) that small quantities of sulfates give slightly high results, particularly with precipitation at 45°–50° C. Small quantities of silicates have a like effect on the recovery of P_2O_5 when precipitation is made overnight or at 45°–50° C. The stirring method of precipitation shows negligible interference from sulfates and silicates.

COLLABORATIVE STUDY

It was believed that more concordant collaborative results than previously reported could be obtained for small quantities of P_2O_5 if several minor changes in the volumetric method were made. Collaboration was invited, and two samples were sent out as follows: Sample 1 was a solution

containing 2.85 mg. of P_2O_5 and 3.30 mg. of sulfuric acid per 10 ml., representing approximately the average quantities of P_2O_5 and twice the average quantities of sulfate in 15 grams of a jam or jelly. Sample 2 was a grape jelly prepared from equal weights of grape juice and cane sugar.

The essentials of the directions sent to the collaborators follow.

TABLE 2.—*Collaborative results*

COLLABORATOR	SAMPLE 1 (19.1 MG. PER 100 GRAMS)*				SAMPLE 2 (MG. PER 100 GRAMS)			
	METHOD OF PRECIPITATION			P_2O_5 BY OTHER METHODS	METHOD OF PRECIPITATION			P_2O_5 BY OTHER METHODS
	45°–50° C.	OVER-NIGHT	SHAKING OR STIRRING		45°–50° C.	OVER-NIGHT	SHAKING OR STIRRING	
J. W. Sanders, Jr.	21.4 21.5 21.2	21.6 21.7 21.4	21.3 21.4 21.1		14.0 14.1 13.9	13.8 14.4 14.2	13.5 13.6 13.9	
I. Schurman	20.4 20.3	20.3 20.2	20.1 20.3		13.8 13.7	13.9 13.8	13.4 13.5	
J. L. Hogan	19.2	19.4	19.1	19.4†	12.7	12.5	12.5	12.3†
H. W. Gerritz	20.0	20.0	19.8	18.8† 19.2‡	12.9	12.9	13.3	12.1† 12.7‡
A. A. Hochman	19.6 19.4	19.4 19.6	19.4 19.4		12.7	12.7	12.5	
H. W. Haynes	20.1 20.3	19.9	20.0		12.3 12.4	12.1 12.1 11.9	12.8 12.5	
J. D. Schuldiner	19.6 19.7	19.4	19.3		12.5 12.5	12.5 12.5	12.2 12.8	
H. Shuman	20.1 20.1	19.6 19.8	19.3 19.3		13.0 13.1	13.4 13.4	12.5 12.4	
Average	20.0	20.0	19.8		13.0	13.0	12.9	

* 10 ml. of Sample 1 assumed equivalent to 15 grams of a jam or jelly.
† Colorimetric method, Gerritz, *This Journal*, 22, 131 (1939).
‡ Gravimetric method as $Mg_2P_2O_7$.

Sample 1.—Pipet 10 ml. into a 250 ml. beaker, or flask, add 2 ml. of HNO_3 (1+1) and 10 grams of NH_4NO_3, dilute to 70–80 ml., heat in a water bath to 45°–50° C., and add 20 ml. of the freshly filtered molybdate solution, *Methods of Analysis, A.O.A.C.*, 1935, 19, 7(a). Allow to remain in the bath, stirring occasionally, for 30 minutes. Filter at once through a retentive 9 cm. filter paper. Wash the precipitating vessel and the filter with small portions of cold water, and finally with CO_2-free water until the filtrate from one filling yields a strong color with 1 drop of 0.1 *N* alkali and phenolphthalein. Transfer the filter to the precipitating vessel,

unfold the filter paper with the aid of a glass rod, and dissolve the precipitate in a small excess of 0.1 *N* NaOH. Dilute to about 50 ml. with CO_2-free water, add 6 drops of phenolphthalein, discharge the pink color with 0.1 *N* HCl, and complete the titration with 0.1 *N* alkali. 1 ml. of 0.1 *N* NaOH is equivalent to 0.3088 mg. of P_2O_5. Run a blank determination and subtract the alkali consumed from the sample analysis.

Repeat the determination as above, substituting the following procedures for the precipitation: (a) Allow to stand overnight at room temperature; (b) adjust to 25°–30° C. and by means of a shaking or stirring apparatus shake or stir for 30 minutes.

Sample 2.—Ash 15 grams as directed in *Methods of Analysis, A.O.A.C.*, 1935, 321, 9. Cover the dish with a watch-glass and dissolve the ash in 11 ml. of HNO_3 (1+9), warm on the steam bath, dilute with water, and transfer to a beaker or flask. Add 10 grams of NH_4NO_3, dilute to 70–80 ml., and complete the determination as directed for Sample 1.

The results of this collaborative study (Table 2) show less variation than do previously reported studies. Most of the results, as shown for Sample 1, are fairly close to the actual value.

The importance of an over-all blank is shown by the fact that several collaborators have reported figures as high as 0.30–0.36 ml. of 0.1 *N* alkali, the latter being equivalent to 0.7 mg. per 100 grams.

The Associate Referee wishes to express his appreciation for the cooperation given by the collaborators, all of whom, with the exception of A. A. Hochman of the Quartermaster Supply Office, Brooklyn, N. Y., are members of the U. S. Food and Drug Administration.

REPORT ON P_2O_5 IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS

By HAROLD W. GERRITZ (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

Last year the writer suggested a colorimetric procedure for the determination of P_2O_5 in fruits and fruit products, *This Journal*, 22, 131 (1939). The method depends on a wet-ashing procedure followed by the molybdenum blue phosphate determination devised by Zinzadze.[1] The method gave accurate results, but experience with it revealed certain difficulties resulting from small differences in salt concentration, volume and temperature at which color was developed, and time of heating. Also, under the conditions of that procedure, the color intensity was not a linear function of the P_2O_5 concentration in the lower ranges. It was suggested that these effects were caused by the high salt concentration resulting from the acid digestion.

Schricker and Dawson, *Ibid.*, 167, in a paper presented at the same time detailed a method for determining micro quantities of phosphates in soil

[1] *Ind. Eng. Chem., Anal. Ed.*, 7, 227 (1935).

solutions by modifying the Zinzadze procedure to adapt it to the conditions of their work. They studied the effect of varying the proportions and concentrations of the ingredients of the molybdenum blue reagent with a view to overcoming difficulties ascribed to relatively high concentrations of salts or buffers present in the solutions with which they were working. Schricker and Dawson found that increasing the concentration of sulfuric acid and decreasing that of molybdenum trioxide served to overcome salt effects, and a similar approach was adopted in the investigation here reported. The reagent recommended by them could not be used in fruit work because the concentration was suitable for a range only one-tenth as great as that encountered in fruit. In order to cover the greater phosphate range, it was found necessary to increase the reduction concentration.*

A modification of the procedure, designed to standardize the salt concentration, is recommended, and additional minor modifications and precautions dictated by experience in the routine use of the method are included in the revised procedure.

Results of this study indicate, as suggested by Schricker and Dawson,[1] that the color produced by the combination of phosphate and molybdenum blue does not appear to be a definite compound. The depth of color produced by a given concentration of phosphate seems to depend on temperature and time of heating. It also depends on the concentration of molybdenum pentoxide, of molybdenum trioxide, and of sulfuric acid, and thus, indirectly at least, on the salt concentration and on the volume.

EXPERIMENTAL

The use of the Zinzadze reagent as specified in the previous report, wherein the equivalent of 1 ml. of concentrated sulfuric acid, neutralized with potassium hydroxide, is present in each test solution, results in concentrations of constituents of the final 100 ml. of test solution† as follows: H_2SO_4, .25*N*; MoO_3, .00195 *M*; Mo_2O_5, 0.0010 *N*; and K_2SO_4, .18 *M*.

The reagent found most suitable by Schricker and Dawson gave the following concentrations in the final test solution: H_2SO_4, 0.36 *N*; MoO_3, .0016 *M*; and Mo_2O_5, .0004 *N*.

The first step was to increase the acidity of the test solution to .36 *N* by preparing a dilute reagent, mixing 10 ml. of Zinzadze's concentrated reagent with 10 ml. of 11 *N* sulfuric acid, and diluting to 100 ml.

Column 1 of Table 1 gives readings obtained on standards developed according to the previous procedure. Columns 2 and 3 give comparative data when the acidity was increased to .36 *N*. Inspection of this data shows that, with increased acidity, although a straight line is obtained up to about .5 mg. P_2O_5 and the blank reading is reduced, the desired range is not covered.

* Reduction concentration = concentration of Mo_2O_5 calculated from permanganate titration of the reagent.

† Test solution refers to the solution in the flask in which color is developed.

It appeared that the molybdenum pentoxide concentration of .0010 *N* was not sufficient in the presence of acidity of .36 *N* and therefore a second dilute molybdenum blue reagent was prepared with 11 ml. of the Zinzadze concentrated reagent and 7.8 ml. of 11 *N* sulfuric acid. This reagent gave concentrations in the diluted test solution of H_2SO_4, .36 *N*; MoO_3, .00215 *M*; and Mo_2O_5, .0011 *N*.

Results with this reagent are given in Column 4 of Table 1. This modification gave a linear function between photometer reading and P_2O_5 concentration up to .6 mg. Data in Table 1 are represented graphically in Figure 1.

TABLE 1.—*Concentrations are given in terms of test solution diluted to 100 ml. K_2SO_4—.18 M throughout*

	1	2	3	4
P_2O_5 mg./100 ml.	H_2SO_4 .25*N* Mo_2O_5 .0010*N* MoO_3 .00195*M*	.36*N* .0010*N* .00195*M*	.36*N* .0010*N* .00195*M*	.36*N* .0011*N* .00215*M*
	Photometer reading in millimeters			
0	27.9	13.1	12.9	14.0
.1	45.9	34.1	35.0	36.0
.2	64.9	55.8	55.7	57.2
.3	84.0	77.4	77.6	78.7
.4	104.1	98.5	99.1	100.3
.5	124.9	120.1	120.0	121.9
.6	145.8	135.0	138.5	143.1

To study the effect of additional increase of acidity and variation of molybdenum pentoxide, further mixtures of Zinzadze's reagent and sulfuric acid were made up. The results are shown in Table 2, and represented graphically in Figures 2A and 2B.

The data given under A were obtained in the absence of potassium sulfate; those reported under D were obtained in the presence of .18 *M* potassium sulfate to simulate conditions resulting from digest solution.

The data indicate that with increased acidity an increase in concentration of molybdenum pentoxide or molybdenum trioxide, or both, is necessary to obtain a linear function over the desired P_2O_5 range. Section B indicates that the presence of potassium sulfate has the same effect as reducing the acidity and thus points to the probability that it acts in the capacity of a buffer by reacting with the sulfuric acid of the molybdenum blue reagent to form acid potassium sulfate.

In preparing the reagents as described, it will be noted that the concentration of both the molybdenum trioxide and the molybdenum pent-

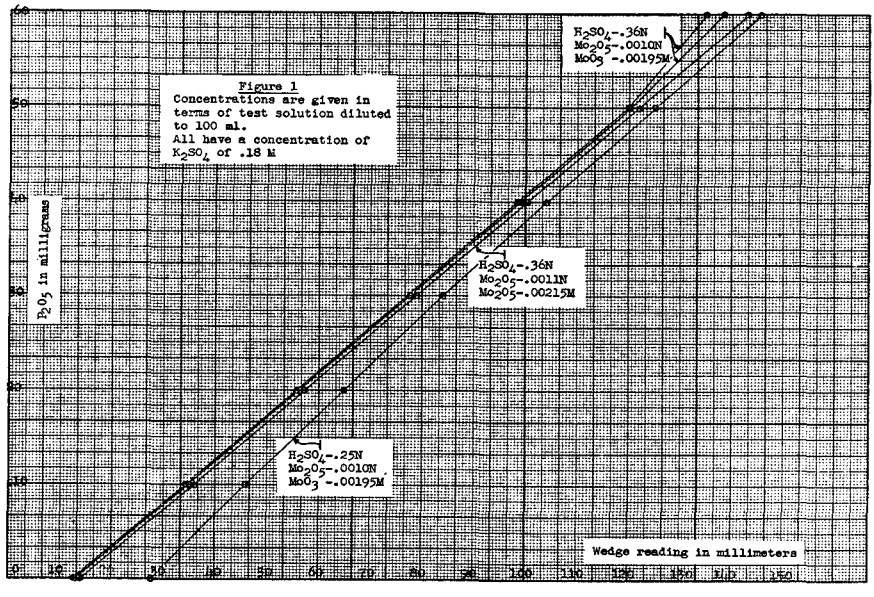


Figure 1
Concentrations are given in terms of test solution diluted to 100 ml.
All have a concentration of K_2SO_4 of .18 M
H_2SO_4-.36N
Mo_2O_5-.0010N
MoO_3 -.00195M
H_2SO_4-.36N
Mo_2O_5-.0011N
Mo_2O_5-.00215M
H_2SO_4-.25N
Mo_2O_5-.0010N
MoO_3 -.00195M
P_2O_5 in milligrams
Wedge reading in millimeters

FIG. 1

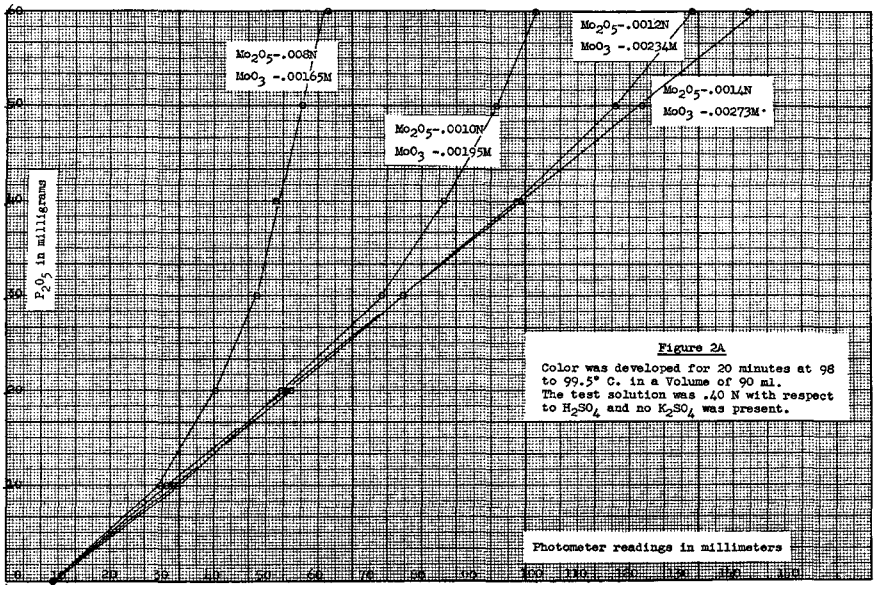

Mo_2O_5-.008N
MoO_3 -.00165M
Mo_2O_5-.0012N
MoO_3 -.00234M
Mo_2O_5-.0014N
MoO_3 -.00273M
Mo_2O_5-.0010N
MoO_3 -.00195M
Figure 2A
Color was developed for 20 minutes at 98 to 99.5° C. in a Volume of 90 ml.
The test solution was .40 N with respect to H_2SO_4 and no K_2SO_4 was present.
P_2O_5 in milligrams
Photometer readings in millimeters

FIG. 2A

TABLE 2.—*Color was developed for 20 minutes at 98°–99.5°C. in a volume of 90 ml. Concentration of H_2SO_4 was .40 N throughout*

	A				B		
					H_2SO_4 AND KOH EQUIV. TO .18*M*/K_2SO_4 WERE ADDED		
P_2O_5/mg.	MoO_3 .00165 *M* Mo_2O_5 .0008 *N*	.00195 *M* .0010 *N*	.00234 *M* .0012 *N*	.00273 *M* .0014 *N*	.00195 *M* .0010 *N*	.00234 *M* .0012 *N*	.00273 *M* .0014 *N*
	PHOTOMETER READINGS (MM.)						
0	9.0	9.1	9.1	9.2	10.2	15.0	24.8
.1	29.6	33.0	32.1	30.8	32.6	35.0	42.0
.2	40.1	53.0	54.8	53.9	54.8	56.5	61.3
.3	48.1	72.1	76.1	76.4	76.8	77.8	81.9
.4	52.0	84.2	98.3	99.0	98.1	100.1	102.6
.5	57.0	94.5	117.2	122.1	121.1	121.6	lost
.6	62.0	102.0	132.1	142.9	140.1	142.1	146.1

oxide was varied. The results obtained may therefore reflect, in part at least, the effect of variations of molybdenum trioxide.

To study the effect of independent variation of molybdenum trioxide a series of reagents was made up according to Schricker and Dawson's procedure. Two concentrated molybdenum blue stock solutions were prepared as follows:

I	II
H_2SO_4—.36 *N*	H_2SO_4—.36 N
Mo_2O_5—.11 *N*	Mo_2O_5—.11 *N*
MoO_3—.055 *M*	MoO_3—.55 *M*

By mixing the proper proportions of I and II, various concentrated reagents with ratios of MoO_3/Mo_2O_5 ranging from 1 to 5 were prepared. The sulfuric acid and molybdenum pentoxide concentrations remained constant. Color was developed in a series of standards, these molybdenum blue reagents being used. Results are given in Table 3 and represented graphically in Figure 3.

These results show that under the conditions imposed and for a concentration of .36 *N* sulfuric acid and .0011 *N* molybdenum pentoxide, the optimum concentration of molybdenum trioxide is about .0018 *M*. The concentration may be increased to .0022 *M* without greatly affecting the linear P_2O_5 color relationship. Either increasing or decreasing the latter concentrations, markedly, tends to shorten the range over which a linear relationship holds.

Increase of molybdenum trioxide beyond .0022 M results in effects similar to lower acidity, i.e., high blanks and a non-linear function in the lower and upper range. The conditions under which the results of Column 4 were obtained very closely approximate those for Column 4 of Table 1.

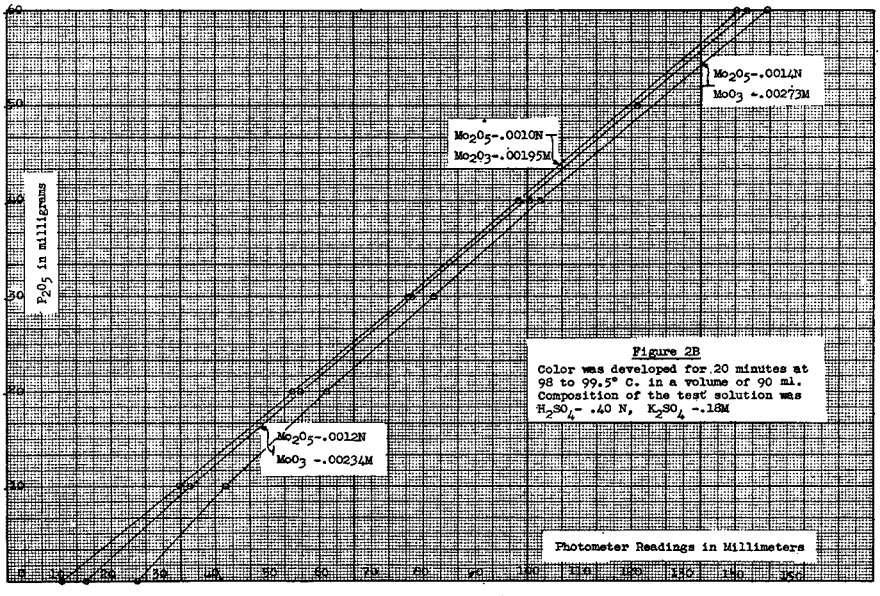


Figure 2B
Color was developed for 20 minutes at 98 to 99.5° C. in a volume of 90 ml. Composition of the test solution was H_2SO_4- .40 N, K_2SO_4 -.18M

FIG. 2B

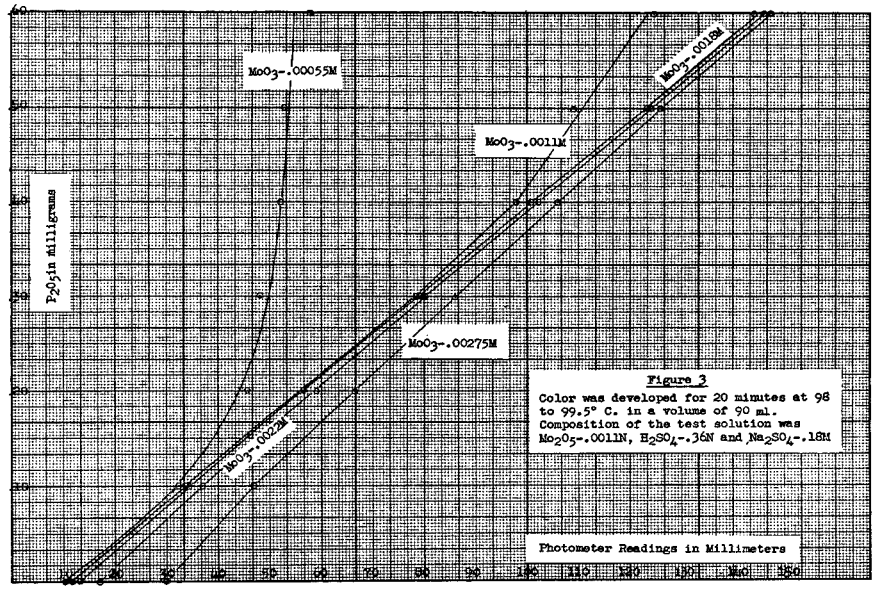


Figure 3
Color was developed for 20 minutes at 98 to 99.5° C. in a volume of 90 ml. Composition of the test solution was Mo_2O_5-.0011N, H_2SO_4-.36N and Na_2SO_4-.18M

FIG. 3

A difference exists, however, in that .18 *M* sodium sulfate rather than potassium sulfate was present in the latter instance because sodium hydroxide was used as the neutralizing agent. Both yield straight lines and the slightly higher results throughout in Table 4 (Col. 4) are thought to be due to differences in P_2O_5 content of the alkalies used. Apparently either sodium hydroxide or potassium hydroxide may be used as neutralizing agent so long as the same reagent is used in the standards.

TABLE 3.—*Color was developed for 20 minutes at 98°–99.5°C. in volume of 90 ml. Composition of the diluted test solution was* Mo_2O_5 *—.0011N,* H_2SO_4 *—.36N,* Na_2SO_4 *—.18M. The* MoO_3 *concentration was varied*

MoO_3	.00055*M*	.0011*M*	.0018*M*	.0022*M*	.00275*M*
$\frac{MoO_3}{Mo_2O_5}$	1	2	3.3	4	5
P_2O_5/MG.	PHOTOMETER READINGS (MM.)				
0	10.3	11.6	13.0	17.0	30.0
.1	32.4	33.4	34.6	36.7	46.8
.2	45.4	56.6	56.6	59.0	66.5
.3	48.2	78.5	78.7	80.1	86.0
.4	52.2	98.0	100.7	102.2	106.1
.5	52.8	109.0	123.3	124.1	126.4
.6	58.0	125.0	144.5	146.2	147.0

Results of the foregoing studies indicate that for the conditions imposed a concentrated molybdenum blue reagent should have the following composition: H_2SO_4, 36 *N*; Mo_2O_5, .11 *N*; and MoO_3, .18 *M*.

The method presented has advantages over the method previously described in that the molybdenum blue reagent prepared according to the technic of Schricker and Dawson entails much less difficulty than the preparation of Zinzadze's reagent; its stability has not been determined, but it is anticipated that it will keep indefinitely without deterioration as does Zinzadze's reagent. The modified reagent reduces the large apparent blank observed with Zinzadze's reagent, and its use results in a linear function between photometer reading and concentration of P_2O_5 when the Jena wedge is employed. A strictly linear function will not be obtained with the "B & L Smoke C" wedge for the reason that this wedge is not exactly neutral in the deep red.

MODIFIED METHOD

REAGENTS

(a) *Molybdenum blue.*—Place 9.78 grams of MoO_3 (99.5–100%) in a 500 ml. Kjeldahl flask, add about 150 ml. of concentrated H_2SO_4 (36 $N \pm 0.5$ *N*), and heat with gentle mixing until solution is complete. Cool to 150°C. Weigh, on a small watch-glass, 0.440 gram of very fine powder molybdenum metal (99.5–100%) and

transfer to the Kjeldahl flask by sliding the watch-glass down the neck of the flask. Keep at a temperature of 140–150° C. and mix vigorously until the Mo is dissolved (a few larger particles that do not dissolve readily may remain). Cool mixture, transfer to a 250 ml. volumetric flask, rinse the Kjeldahl with concentrated H_2SO_4, and transfer rinsings to the volumetric flask. Finally fill the volumetric flask to 250 ml. with concentrated H_2SO_4 and mix well. Dilute 10 ml. of this reagent with water and titrate with 0.1 *N* $KMnO_4$ to a pink color that persists for a minute. The reagent should be 0.11 *N*; if less than 0.109 *N*, add a calculated quantity of Mo and dissolve by reheating in a Kjeldahl flask to 150° C.

Preserve the deep green solution in glass-stoppered bottles, carefully avoiding contamination of any kind.

(b) *Dilute molybdenum blue.*—Pipet 10 ml. of Reagent (a) into about 60 ml. of distilled water in a 100 ml. volumetric flask. Mix, cool, fill to the mark with distilled water, and mix. This dilute reagent deteriorates with age and should not be used 8 or 10 hours after preparation.

The viscosity of the concentrated reagent requires a special technic in pipetting, as described by Zinzadze "use a pipet previously wet inside with water and washed down afterwards with a few ml. of water."

(c) *Sodium hydroxide solution.*—Phosphate- and arsenate-free. $3.6N \pm 0.05$ *N*. Should contain not over 0.0005% PO_4. (J. T. Baker's "Special sticks low in chlorine" has been found satisfactory.) Dissolve the NaOH in distilled water, using an arsenic-free Pyrex or porcelain vessel, cool, and titrate with standard acid. Preserve in a paraffin-lined container. Avoid leaving glass equipment in contact with this reagent for any extended period of time in order to minimize the possibility of dissolving arsenate or phosphate from the glass.

(d) *Normal sodium hydroxide.*—From reagent (c) prepare approximately normal NaOH. Preserve in an arsenic-free Pyrex or paraffined container fitted with a 1-hole stopper bearing a Pyrex medicine dropper.

(e) *Concentrated sulfuric acid.*—Reagent quality.

(f) *Approximately normal sulfuric acid.*—Transfer 30 ml. of reagent (e) to a liter volumetric flask, dilute to the mark with distilled water, and mix.

(g) *Concentrated nitric acid.*—Reagent quality, low in arsenic and phosphorus.

(h) *Perchloric acid.*—60%. Reagent quality.

(i) *Sodium alizarin sulfonate solution.*—Dissolve 0.20 gram of sodium alizarin monosulfonate in 100 ml. of distilled water and filter. Preserve in an indicator bottle.

(j) *Standard phosphate solution.*—0.05 mg. per ml. Dissolve 0.1917 gram of pure dry KH_2PO_4 in about 200 ml. of distilled water, add 10 ml. of normal H_2SO_4 and 6 drops of 0.1 *N* $KMnO_4$. Dilute to exactly 2 liters. According to Zinzadze this solution keeps indefinitely in a well-stoppered Pyrex bottle.

(k) *Glass beads.*—Boil a supply of small glass beads (2 or 3 mm. in diameter) in aqua regia, wash clean with distilled water, and dry.

PREPARATION OF SAMPLE

Transfer a portion of the sample containing 0.5–2.5 mg. of P_2O_5 to a 500 ml. Kjeldahl flask. For the determination of P_2O_5 on the water-soluble portion of fruits or fruit juices 25 or 30 ml. (3.75 or 4.5 grams) of the sample solution prepared according to 2(b) or (c), *Methods of Analysis, A.O.A.C.*, 1935, p. 319, is a convenient aliquot. (For jams and jellies 50 ml. of the prepared solution may be conveniently taken. If the sample has a low fruit content, a larger aliquot should be taken.) Add 5 ml. of H_2SO_4, Reagent (e), from pipet or buret, then add 10 ml. of HNO_3, Reagent (g) and 5 or 6 small glass beads. Place the flask on a digestion rack

over a free flame. (The flask should be protected from the flame by an asbestos mat with a hole of such size that the surface of the H_2SO_4 will be above the mat.) Boil over a moderate flame until darkening begins (avoid excessive charring). Add a few ml. of HNO_3 and again boil until slight darkening begins or until SO_3 fumes are evolved from a clear or amber solution. In the case of jams or jellies, 3 or 4 additions (about 5 ml. each) of HNO_3 may be necessary. Add to the hot flask 0.5 ml. of the $HClO_4$, and continue fuming for a few minutes. To avoid violent explosions of $HClO_4$ in the presence of organic matter do not add over 0.5 ml. at one time and then only after practically all organic matter has been removed with HNO_3 and do not fail to take all precautions advised in the use of $HClO_4$. When the digest is water clear or very slightly greenish yellow, cool somewhat and cautiously add 50 ml. of distilled water and boil to fumes to remove traces of HNO_3. Cool, add about 25 ml. of distilled water, transfer to a 100 ml. volumetric flask, mix, cool, make to volume, and mix thoroughly.

DETERMINATION

Transfer a 20 ml. aliquot of sample digest to a 100 ml. volumetric flask (Kohlrausch sugar flasks have been found convenient) marked at 70 ml. capacity. Add 20 or 25 ml. of distilled water, 3 drops of the sodium alizarin sulfonate solution and then add exactly 10 ml. of the 3.6 *N* NaOH solution. Adjust the acidity to just yellow by means of the normal H_2SO_4 and normal NaOH until a single drop of the normal H_2SO_4 just changes the color of the solution to yellow. Dilute to 70 ml. and mix by swirling. Place the flasks in a boiling water bath and bring to that temperature. With a pipet add exactly 10 ml. of the dilute molybdenum blue reagent, directing the stream into the solution—do not allow it to run down the side of the flask—mix by swirling, and continue to heat in the boiling water bath for exactly 20 minutes. Cool rapidly in cold water, dilute to volume, and mix.

It is very important that the standards and unknowns be heated at the same temperature. This may be accomplished by immersing the flasks in a boiling water bath wherein the water comes above the level of the solution in the flask. A simple water bath may be prepared by placing a ½-inch-mesh wire screen in the bottom of a 12 or 14 inch granite pan and filling the pan with water to such a depth that the liquid in the flasks will be below the level of the water. Place the pan on a stand and heat with a large Meker burner with flame so adjusted that it spreads over the bottom of the pan in such a manner as to keep the entire contents at a gentle rolling boil. Place flasks only around the periphery of the pan and weight with lead rings or otherwise support to prevent tipping. Keep the bath at a rolling boil throughout the heating period and add *boiling* water to the bath from time to time to keep the level of the water above the level of the liquid in the flasks. Keep a thermometer in the bath and do not permit a variation of more than 2° between the center and the edge of the pan. Determine the color intensity by means of the neutral wedge photometer, *This Journal*, **19**, 130 (1936), using a 1 inch cell, No. 66 filter, and Jena 0–2 neutral wedge. (Filter 66 is 4.5 mm. Corning dark pyrometer red No. 241. With "B & L Smoke C" glass wedge, use filter 65. Filter 65 is the same as 66 plus a half mm. of Jena BG18.)

The method covers a range up to 0.6 mg. of P_2O_5 in the final 100 ml. of solution. Prepare standards covering this range by placing 0, 2, 4, 6, 8, 10 and 12 ml. of the standard phosphate solution in 100 ml. volumetric flasks marked at 70 ml. capacity. Add 30 ml. of the normal H_2SO_4 and 3 drops of the indicator to each flask. Then treat the same as directed previously, beginning "add 10 ml. of the 3.6 *N* NaOH solution." Determine color intensity in the neutral wedge photometer. Make a large-scale graph of the standards, plotting mg. of P_2O_5 against photometer readings.

(A sheet of graph paper 20×36 inches with 10 lines to the inch is convenient.) By means of this plot, convert the sample photometer readings to mg. of P_2O_5 present in the final 100 ml. of solution. If preferred, the equation of the line may be calculated as described by Klein and Vorhes, *Ibid.*, 121, and the equation used in conversion.

NOTES

The photometer need be calibrated but once for each batch of reagents provided the adjustment is not altered and the temperature of the boiling water bath remains the same. It is advisable, however, to develop one or two standards with each batch of unknowns in order to detect any possible change of conditions.

It will be noted that standardization under these conditions automatically corrects for the blank on reagents, except HNO_3 and $HClO_4$. These reagents in the grade specified have not been found to contain significant quantities of arsenic or phosphorus. It is well, however, to determine the digestion blank on these reagents from time to time.

In the analysis of heterogeneous samples, such as lots of fresh fruit, for total P_2O_5, it may be necessary to digest a larger portion than specified above in order to minimize sampling and weighing error. In that case it is convenient to take double the above sample and double the amount of H_2SO_4 (10 ml.). Make the digest to 200 ml. and finally transfer a 20 ml. aliquot to a 100 ml. volumetric flask for color development. The amount of sample digested may be varied to suit the nature of the sample if the final aliquot taken for color development contains not more than 1 ml. of concentrated H_2SO_4 and not more than 0.6 mg. of P_2O_5.

INTERFERENCES

Iron, nitrate, and arsenic act as interferences in the development of the color by means of this reagent. Nitrates are not present in solutions prepared as described, and neither iron nor arsenic is ordinarily present in fruit or fruit products in sufficient quantity to constitute an interference. However, if the presence of excessive arsenic or iron is suspected, their interference may be prevented by a procedure used by Zinzadze. Follow the above procedure to the point, "adjust acidity to just yellow," after which add 10 ml. of exactly normal H_2SO_4 and then 10 ml. of 8% Na_2SO_4, and dilute to 70 ml. Heat in a boiling water bath for an hour. Then proceed as directed previously, beginning "add exactly 10 ml. of the dilute molybdenum blue reagent." Standards and blank, of course, must then be treated in exactly the same manner.

EXPERIMENTAL

In order to determine allowable variations in conditions imposed by the modified procedure, color was developed in a series of standards and conditions and concentrations were varied within reasonable limits. In order to save time and facilitate measuring, a solution of sodium sulfate was substituted for the equivalent of sulfuric acid and sodium hydroxide specified. Results are given in Table 4.

The method appears to allow reasonable variation in conditions, but it is recommended that the stated directions be adhered to as closely as possible since the varying of a number of factors at once might lead to unexpected disappointing results.

COLLABORATIVE RESULTS

Time available to the Associate Referee this year was spent in improving the method, and no formal collaborative samples were sent out.

TABLE 4.—*Except when otherwise stated color was developed for 20 minutes in a volume of 90 ml. at a temperature of 98°–99° C. and the composition of the test solution when diluted to 100 ml. was: Mo_2O_5—.0011 N, H_2SO_4—.36 N, MoO_3—.0018 M, and Na_2SO_4—.18 M. Flasks were cooled rapidly in cold water, and color was read about a half hour after making to volume.*

P_2O_5	1 AS STATED	2 AS STATED	3 COLOR DEVELOPED 25 MINUTES	4 SAME AS 3 BUT READ AFTER 20 HOURS	5 COLOR DEVELOPED AT 92°–93.5° C.	6 FLASKS COOLED AT ROOM TEMPERATURE	7 COLOR DEVELOPED IN 70 ML. VOLUME	8 Mo_2O_5—.0010*N*	9 Mo_2O_5—.0012*N*	10 5 ML. *N*-H_2SO_4 ADDED BEFORE MOLYBDENUM BLUE	11 SAME AS 10	12 Na_2SO_4—.145*M*	13 Na_2SO_4—.162*M*	14 Na_2SO_4—.195*M*
mg./100ml.	*Photometer Readings (mm.)*													
0	13.0	13.5	13.5	13.3	12.2	13.9	12.8	12.4	13.7	11.5	12.4	12.0	12.1	13.0
.1	34.6	34.8	35.2	34.3	34.1	34.9	34.1	34.3	34.6	33.8	34.4	33.9	33.9	35.0
.2	56.6	56.8	57.1	56.2	56.4	56.6	56.0	56.7	56.8	55.9	56.1	56.0	55.7	56.6
.3	78.7	79.0	79.0	76.9	78.6	78.5	78.2	79.0	79.0	78.0	77.7	77.5	78.0	79.0
.4	100.7	100.9	101.4	99.0	100.3	100.8	100.6	100.7	101.	99.8	99.8	99.8	99.9	101.4
.5	123.3	123.7	123.3	120.0	121.1	123.2	122.8	123.2	123.1	121.6	119.0	122.6	122.0	123.6
.6	144.5	144.9	146.1	142.2	137.0	145.0	144.6	143.0	144.5	140.9	139.0	143.9	143.5	144.9

However, through the courtesy of associates doing fruit work, some comparative results and comments were made available.

A sample solution of a preserve was sent to analysts by the Food Division (U. S. Food and Drug Adm., Washington, D. C.), W. B. White in charge, requesting certain analyses, among which was the determination of P_2O_5 by the above procedure and by the A.O.A.C. volumetric procedure. Results were compiled by R. A. Osborn (Table 5).

TABLE 5.—*Collaborative results*

U. S. FOOD AND DRUG ADM.	ANALYST	P_2O_5 VOLUMETRIC METHOD	P_2O_5 COLORIMETRIC METHOD
		mg./100 g.	*mg./100 g.*
New York Station	J. L. Hogan	12.1	11.7
		11.9	
	C. A. Wood	12.4	11.8
		12.3	
Chicago Station	E. H. Wells	13.2	11.2
		13.3	11.5
	F. J. McNall	14.3	11.7
		14.1	10.9
St. Louis Station	J. T. Field	12.9	10.9
		12.8	11.3
	Samuel Alfend	12.7	11.6
		12.3	11.7
San Francisco Station	H. W. Gerritz	12.9	11.1
		13.1	11.1
	H. M. Bollinger	14.0	11.1
		14.4	11.1
Washington, D.C.	N. J. Menard	12.9	11.8
		13.3	11.5
	R. A. Osborn	11.7	11.4
		12.9	10.7
Average		13.0	11.3

Two lots of water-pack peaches, three lots of apricot preserves, and one lot of pie-pack apricots were analyzed for P_2O_5 by the colorimetric and A.O.A.C. gravimetric methods. For the gravimetric procedure 400 ml. of sample solution was evaporated and ashed. Results are given in Table 6.

The following check results, together with comments, were taken from a report furnished by Samuel Alfend and J. T. Field, St. Louis Station, Food and Drug Administration.

> The revised method . . . appears to be a distinct improvement. The blank is lower and less variable, the range is greater, the reagent is much easier to prepare, and the standard curves . . . have become quite uniform. The curves are not straight lines, however, but steadily increase in slope. (We have a Bausch & Lomb glass wedge.)

TABLE 6.—*P_2O_5 by colorimetric and A.O.A.C. gravimetric methods*

ANALYST	METHOD	WATER-PACK PEACH FRUIT	WATER-PACK PEACH FRUIT	APRICOT PRESERVE	APRICOT PRESERVE	APRICOT PRESERVE	SOLID PACK APRICOT
		MG. PER 100 G. SAMPLE					
Donald A. Ballard	Colorimetric	43.8	46.2	30.7	34.2	24.5	63.0
Harold W. Gerritz	Colorimetric	44.0	45.5	30.4	34.2	24.3	63.1
	Gravimetric	43.0	45.0	30.4	32.6	23.9	62.4
		43.8	45.2	30.4	33.6	23.8	63.2

To check the wet digestion blank, a series of standards was carried through the wet digestion procedure and made to volume, and aliquots were run along with standards which had not been so treated. The values given (Table 7) are the averages of 3–5 photometer readings by each analyst:

TABLE 7.—*Results by Alfend and Field*

P_2O_5 STANDARD	WET DIGESTED			NOT WET DIGESTED		
	1ST RUN MM.	2ND RUN MM.	3RD RUN MM.	1ST RUN MM.	2ND RUN MM.	3RD RUN MM.
mg.						
ml. H_2SO_4-ml. HNO_3	5–15	5–15	5–15			
Blank	11.9	11.9	11.1	12.0	11.9	11.1
.1	35.5	35.9	36.0	36.1	35.9	36.4
.2	61.5	61.3	61.8	62.0	61.4	61.9
.3	89.6	88.6	89.0	89.2	88.6	88.7
.4	120.0	119.9	119.2	119.2	119.3	119.6

It appears that there is no significant blank introduced by the nitric and perchloric acids, and that there is no appreciable loss in the digestion. . . . A solution was prepared from grape jelly. . . . To test the effect of charring and excessive fuming during the wet ashing for the colorimetric method, eight 50 ml. aliquots were

TABLE 8.—*Results by Alfend and Field to show effects of charring*

ALIQUOT	CHAR	LOSS OF H_2SO_4	READING MM.	P_2O_5 MG./100 ML.
Blank-ashed	None	Prac. none	12.5	—
1	Heavy	2 ml.	41.8	1.21
2	Heavy	2 ml.	42.1	1.22
3	Moderate	Prac. none	46.0	1.38
4	Heavy	2 ml.	44.5	1.32
5	None	Prac. none	46.5	1.39
6	None	1 ml.	46.1	1.38
7	None	2 ml.	44.8	1.33
8	None	Prac. none	45.8	1.37
.1 mg. P_2O_5 Plus ash blank	None	Prac. none	36.6	—

wet ashed. Nos. 1, 2, 3, and 4 were allowed to char badly. Nos. 1, 2, 4, 6 and 7 were fumed until they had lost approximately 2 ml. of sulfuric acid. The results are shown in Table 8. The lowest results were obtained on those samples which were charred and fumed the most.

RECOMMENDATIONS[1]

It is recommended—

(1) That the colorimetric method discussed be adopted as tentative.

(2) That further collaborative work be carried on during the coming year with a view to testing the method on a wide variety of types of fruit product samples.

(3) That the volumetric method be placed in tentative status.

(4) That study of the volumetric method be continued.

REPORT ON VITAMINS

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

There will no doubt be new developments relating to vitamins for some time to come, making necessary revisions in the program of study of methods. New knowledge relating to the chemical nature of the vitamins, refinement in technic, and new legislation, all have a bearing on these studies.

The manner in which these problems change owing to progress through investigations is well exemplified by the study of vitamin K. A year ago some vitamin K preparations from natural sources had been used experimentally with sufficient promise to warrant the opinion that they would soon become important therapeutic agents. Very little was then known of the chemical nature of vitamin K, but during the year reports have been published on the isolation and identification of naturally occurring compounds and a number of compounds having vitamin K activity have been synthesized. An indication of the intense interest in this field is the fact that the June and July numbers of the *Journal of the American Chemical Association* carried a total of ten "Communications to the Editor" relating to the chemical nature of vitamin K.

The problem of biological assay has been simplified by the fact that a number of synthetic compounds can now be readily prepared and may serve as standards in the assay of products of unknown potency. The information now available concerning the chemical nature of vitamin K should make it possible to make chemical determinations on certain types of products.

Two recommendations concerning associate referees will be discussed briefly. One is that an associate referee be appointed on Vitamin C, and

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 23, 71 (1940).

the other is that no referee be appointed on technic and details of biological methods for Vitamin D carriers.

Chemical methods are now being used almost exclusively in the determination of the Vitamin C content of foods and in the examination of pharmaceuticals, and sufficient progress has been made so that the limitations of these methods are fairly well understood, particularly when they are applied to foods. Since a sufficient background has been developed, and it is believed that the problem can be approached with some definite assurance of success, a referee on vitamin C should be appointed.

Before the appointment of the present Referee on Vitamins there were two associate referees on the assay of vitamin D products for poultry, W. B. Griem on Biological Methods for Determination of Vitamin D Carriers, and L. L. Lachat on Technic and Details of Biological Methods of Vitamin D Carriers. Owing to the resignation of these men new referees were appointed during the year. It was difficult to make assignments for both of these referees that would not entail some degree of duplication. Collaborative work on this method is expensive and the number of labotories available for collaborative work is limited. After considering this matter with the Referee on Feeding Stuffs and the Associate Referees on Vitamins, the Referee decided that it would be preferable to have the collaborative work relating to vitamin D for poultry under the direction of one referee. Accordingly there is no report on technic and details of biological methods for vitamin D carriers, and it does not seem necessary to have a referee on this subject.

RECOMMENDATIONS[1]

It is recommended—

(1) That a referee on vitamin C be appointed.

(2) That no referee be appointed on technic and details of biological methods, vitamin D carriers.

(3) That the title of the referee, "Biological Methods for Determination of Vitamin D Carriers," be changed to "Vitamin D for Poultry."

(4) That the title of the referee, "Biological Methods for Vitamin B Complex," be changed to "Vitamin B_1," and the assignment changed accordingly.

The following recommendations of the respective associate referees are approved:

Vitamin A.—That continued studies of vitamin A determinations be made.

Vitamin B_1.—That a collaborative study be carried out to determine if the rat-growth methods of assay for vitamin B_1 content of foods have sufficient accuracy or if they may be improved by recently described modifications and by application of sulfite treatment.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 23, 54 (1940).

Riboflavin.—(1) That the various chemical methods for riboflavin be studied further and that fluorometric methods be considered.

(2) That the Snell-Strong bacteriological technic be further studied.

Biological Methods for Determination of Vitamn D Carriers.—That further work be done on this method to determine how apparent inconsistencies in results can be prevented.

Vitamin D.—That the method be revised so that the animals in the reference group receive in addition to the U. S. P. Reference Cod Liver Oil non-vitamin D skimmilk equal in quantity to the solids-not-fat in the volume of the milk fed the assay group.

Vitamin K.—No report.

REPORT ON VITAMIN A

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

Several important papers and collaborative reports on vitamin A determination were published during the past year. Therefore the Associate Referee will discuss briefly certain parts of these papers that seem to have the most important bearing upon the problem.

During the past two years vitamin A_2 has received much attention, especially by the British workers. According to Edisbury *et al.*[1] and Wolff[2] vitamin A_2 has about the same biological activity as has vitamin A, and indications are that it is quite similar to vitamin A in structure.

Gillam and his coworkers state[3] that the ultraviolet absorption maximum for vitamin A_2 extracts lies between 340 and 350 mu instead of between 325 and 328 mu, as is the case for vitamin A extracts. They also found higher ratios for 693/620 mu antimony chloride absorption values for the vitamin A extracts. Such a ratio varies for the fresh water fish oils, but it is usually about 2, whereas the same ratio for extracts from salt water fish livers is only 0.15–1.

According to Gillam *et al.* attempts to separate vitamin A_1 and A_2 as such have so far failed,[3] and Lederer and Rathmann[4] indicate that vitamin A_2 actually has two ultraviolet bands, one at 345 mu, the other at 280 mu. They also show that the 620 mu and 693 antimony trichloride absorptions have a mutual masking action, making it difficult to do much more than roughly estimate the relative quantities of A_1 and vitamin A_2 that may be present in an oil.

Embree and Shantz[5] may have discovered a solution to this problem. They found that after cyclization with alcohol hydrochloric acid an unsaponifiable vitamin A_1–A_2 mixture could be separated by a chromato-

[1] *Biochem. J.*, 32, 118 (1938).
[2] *Z. Vitaminforsch.*, 7, 227 (1938).
[3] *Biochem. J.*, 32, 405–16 (1938).
[4] *Ibid.*, 1252 (1939).
[5] *A.C.S. Abs. Div. of Biochemistry Communication*, Boston, Mass., Sept. 11, 1939.

graphic method, the vitamin A_2 being more easily adsorbed than the vitamin A_1. The cyclized vitamin A_1 and vitamin A_2 could then be separated and determined spectrographically. It appears that vitamin A_2 does not really present a very important complication from the practical standpoint. This may be because its distribution is limited largely to fresh water fish and also because the specific absorption of each of the vitamins at its respective maximum is essentially equivalent biologically to the other, which means that determination at either absorption maximum should produce a value not in serious error from the biological standpoint.[4]

The cyclized vitamin A may also occur naturally, as well as by treatment of vitamin A extracts with hydrochloric acid and alcohol. Embree[6] also reported upon cyclized vitamin A, which he believes to be the same product that has come to be known as spurious vitamin A. Cyclized vitamin A was first reported by a group of British workers, Pritchard, Williams, Edisbury, and Morton,[7] in 1932.

It is reported both by the earlier British workers, Castle, Gillam, Heilbron, and Thomson,[8] Heilbron, Heslop, Morton, Rea, and Drummond,[9] and then by Embree[6] that this is a naturally occurring product and not likely to be made by the vacuum distillation process used for preparing concentrates.

Embree proposed a formula based upon the relative absorptions at 326 mu and 368 mu that may be used for estimating the ratio of vitamin A and cyclized vitamin A present. This method could be checked biologically and therefore could be used to determine the lack of vitamin A potency or the quantity of spurious or cyclized vitamin A present.

The relative biological values of vitamin A and beta carotene have been investigated by Underhill and Coward.[10] They consider that 1 molecule of beta carotene can furnish but one biologically active molecule of vitamin A since biological experiments show that it has only one-half the activity of the purest vitamin A preparations or that of new ester preparations such as anthraquinone-2-carboxylate and vitamin A—2 napthoate.

The International Unit of Vitamin A is defined as the vitamin activity of 0.6 microgram of beta carotene. Beta carotene therefore contains 1,667,000 units of vitamin A per gram. The purest preparations of vitamin A indicate a potency of 3,000,000–4,000,000 units of vitamin A per gram.

Crystalline vitamin A has been reported by Holmes[11] to have an extinction coefficient as high as 2100, and a conversion factor around 2000 (usually accepted in this country) would then indicate a biological potency of approximately 4,000,000.

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[6] *J. Biol. Chem.*, **128**, 187 (1939).
[7] *Biochem. J.*, **26**, 1164 (1932).
[8] *Ibid.*, **28**, 1702 (1934).
[9] *Ibid.*, **26**, 1178 (1932).
[10] *Ibid.*, **33**, 594–600 (1939).
[11] *J. Am. Pharm. Assoc.*, **26**, 525–540 (1927).

However, there is insufficient evidence concerning the efficiency of the animal body in converting beta carotence to vitamin A to warrant much speculation as to whether 1 molecule of beta carotene provides 1 or 2 molecules that can be used by the organism.

Some interesting studies on the stability of vitamin solutions were reported by E. L. Smith and associates,[12,13] who found that stability was affected by the amount of organic peroxides present, which in turn occur in many natural oils owing to changes not readily under control. They showed also that concentrates were most susceptible to deterioration from such peroxides, the degree depending upon the amount present. Naturally occurring esters and the synthetic stearic esters had greater stability. The fact that heat alone may have deteriorative action was also noted. Their evidence led these workers to suggest a step-wise oxidation process for vitamin A, wherein a primary oxidation product was believed to be first formed, as was evident by a shift in the peak 328 mu absorption towards the shorter wave lengths, with considerable related absorption remaining at 328 mu. It was pointed out, however, that more severe oxidation could reduce all the 328 mu absorption to about 1.5 per cent of the original, thus indicating a further and final oxidation step.

Another paper by Smith and his coworkers[13] dealt with the action of light upon vitamin A solutions. Data were presented to show that about a 15 per cent decrease in the 328 mu absorption could occur by a five hours' application of north sky light. Larger decreases were caused by a mercury lamp. The most startling observations of this study, however, were those indicating a partial reversibility (as shown by the 328 mu absorption) by storage in decreased light or darkness subsequent to the exposure. This phenomenon was accounted for by the assumption that simultaneous photo chemical isomerism and slow destruction of the vitamin occurred.

Observations by Karrer, Morf, and Schöff[14] extending throughout the past nine years show the 620 mu absorption obtained with the antimony trichloride reaction to be associated with vitamin A_1 while the absorption at 693 mu indicates the presence of the vitamin A_2 previously mentioned. Its structure has been indicated and its characteristics are well known at present.[9]

Within the past two years there have been rather significant efforts on the part of several workers to revive the antimony tricholoride vitamin determination. In this connection Dann and Evelyn[15] found that good reliability could be expected if proper attention were given to the reagents and method, in which a rapid acting photoelectric colorimeter, such as the Evelyn, is used.

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[12] *Biochem. J.*, 33, 201 (1939).
[13] *Ibid.*, 207.
[14] *Helv. Chim. Acta*, 14, 1431 (1931).
[15] *Biochem. J.*, 32, 1008–1017 (1938).

Since the last A.O.A.C. meeting McFarlane and Sutherland[16] have also made studies with both the antimony trichloride colorimetric and spectrophotometric methods. They conclude that equal accuracy is possible by each method provided the precautions of Dann's method are observed and proper attention is given the saponification process.

McFarlane and Sutherland[16] also made some studies on the destruction of vitamin A by irradiation similar to those that have been conducted by Notevarp.[17] in an effort to find an alternative method. They found that under certain conditions of irradiation of a concentrate, where a light filter transmitting only 300–400 mu was used, only vitamin A is destroyed to any significant extent and that after the complete destruction of vitamin A only 10 per cent of the original 328 mu absorption remains. Other irradiation experiments of a preliminary nature were made, but they were not sufficiently complete to justify significant conclusions.

A collaborative report by the British worker, E. M. Hume,[18] of the Accessory Food Factors Committee appointed by the Lister Institute and Medical Research Council, has also appeared during the year. In this study it is pointed out that the results of 10 groups of workers in 1937 furnished the conversion factor 1570 after the statistical analyses had been completed. As a consequence the factor in round numbers was taken to be 1600. The U.S.P. Reference Cod Liver Oil was determined to have a potency of 3000 International units per gram by an earlier group of workers in America. The spectral absorption thus made necessary an approximate factor of 2000 in place of 1600 recommended by the British group. This discrepancy was then studied by the British workers with a fresh U.S.P. sample. The biological values obtained by 10 collaborators in British laboratories ranged from 1334 to 3270 International Units per gram. Six of the 10 values lay betweeen 2200 and 2600 units per gram. A weighted mean of 2619 International units per gram was obtained. The $E^{1\%}_{1cm.}$ value for the nonsaponifiable fraction of this particular oil was found to be 1.44. The conversion factor accordingly was taken to be 1820. On this basis the British workers indicate that previously reported conversion factors should be revised and suggest that a third collaborative experiment be undertaken when the pure A ester is available, but that until then they would still recommend the factor 1600.

The conversion factor close to 2000 is accepted almost universally in this country regardless of the fact that 1600 is the tentative accepted value of the English workers. The adherence to an approximate 2000 value is based upon repeated biological and spectrophotometric determinations such as the recent collaborative work by Barthen discussed next. However the factor cannot be considered to be definitely fixed.

Barthen and his coworkers compiled a collaborative report for the

[16] *Canadian J. Research*, **B16**, 421–431 (1938).
[17] *Biochem. J.*, **29**, 1645 (1935).
[18] *Nature*, **143**, 22–23 (1939).

American Drug Manufacturers Association. The purpose of this work was to compare a variety of ultraviolet light absorption methods with the biological method. Average biological values for the given oil samples were taken as the correct values for checking the accuracy of the light absorption methods. When extreme results were not discarded, and when no attempt was made to use correction factors, an average spread in the results was approximately 34 per cent. With the extremes removed the average spread of the absorption method was reduced to 20.16 per cent. It was also found possible to reduce the total average spread to 27.33 per cent by using a corrected unknown value conversion factor based upon one of the samples, which was the U.S.P. Reference Oil. The data in this report were further classified and given a statistical treatment in which unreliability of technic was indicated in those cases showing spread greater than 15 per cent. Conversion factors were corrected both by the unknown U.S.P. oil value and by average per cent deviation calculated for each laboratory. The average value of this corrected conversion factor was found to be 2064.

On the basis of his results Barthen suggested that the U.S.P. adopt a spectrophotometric method as an alternative for the biological assay, and that 75 per cent of the potency claimed by either assay be required. His summary also stressed the importance of repeated standardization and proper operation of the instruments and mentioned the need of making use of the saponifiable portion.

The Associate Referee believes that Barthen's results from the spectrophotometers should be given special mention. A spread of only -7.3 per cent to $+8.1$ per cent was obtained only when these instruments were used. These results seem to be fairly good when the lack of coordinated standardization in technic is considered. It has been claimed by various workers in this field that the photographic method of spectrophotometry can produce results accurate to within ± 2 per cent. However, the Associate Referee has found that if extraordinary precautions are not taken, a ± 5 per cent error may frequently be expected. This does not refer to other than spectrographic methods and does not include variables present owing to the method used in manipulating the sample.

The material that has been presented here, as well as past experience, indicates that the following points should be given attention in further work on vitamin A determination.

1. Apparatus of the spectrophotometric type should be standardized with potassium chromate in 0.05 *N* sodium hydroxide. A technic for standardization should be instituted.

2. The saponification procedure should be given specific consideration.

3. Details of technic should be made more evidence to coordinators of collaborative activity (a questionnaire is suggested).

4. Storage conditions and other matters relating to the deterioration of oils should be given consideration.

The Vitamin Committee of the U. S. Pharmacopoeia is planning new collaborative work on the vitamin A determination, and it is recommended that this Association continue its studies on this subject.

The saponification procedure used in this laboratory follows:

SAPONIFICATION PROCEDURE FOR OBTAINING NONSAPONIFIABLE PORTION OF FISH LIVER OILS FOR SPECTROPHOTOMETRIC VITAMIN A EXAMINATION

Boil 1 g. of the fish liver oil with 30 ml. of 95% alcohol and 3 ml. of 50% KOH for 20 minutes. Maintain the initial volume with a reflux condenser with a ground-glass joint (cork or rubber stoppers cannot be used), or by adding alcohol during the boiling. Cool, add 30 ml. of water, and extract with 30 ml. of diethyl ether in a separatory funnel. Make two additional extractions with two other 30 ml. quantities of diethyl ether. Combine all the ether extracts in a separatory funnel and pour 100 ml. of water without agitation through the ether layer. After allowing to stand 2 minutes, separate and discard this water. (*No subsequent aqueous portions should be separated until 2 minutes has been allowed for separation.*) Shake vigorously with 3 to 5 ml. of water. Discard aqueous portion. If a somewhat resistant emulsion forms in the aqueous portion, dilute with 100 ml. of water to eliminate or decrease this emulsion before discarding aqueous portion. Rinse with two additional 3–5 ml. portions of distilled water with vigorous agitation, and pour 100 ml. of water through the other solution without agitation following each of the agitated rinses. Pour 100 ml. of water through the ether portion as a final rinse. After separation of the final 100 ml. portion of water, make a test for the presence of soaps by acidulation with 10% HCl. Rinse further if appreciable turbidity is produced when the HCl is added. After the rinses are completed, allow the remaining ether extract to stand for 10 minutes. Discard any water that separates. Evaporate ether extract on a steam bath in a 100 ml. beaker to a volume of 3–5 ml., but never to dryness.

Dilute the ether extract to a convenient volume (50 ml. suggested) with a mixture of 90% absolute alcohol and 10% cyclohexane for the spectrophotometric examination.

REPORT ON VITAMIN D*

FEEDING OF NON-VITAMIN D SKIM OR WHOLE MILK WITH THE REFERENCE COD LIVER OIL

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

In two previous reports by the Associate Referee, *This Journal*, **19**, 248 (1936) and *This Journal*, **22**, 468 (1939), it was demonstrated that there was enhancement of the line response when skim milk was fed with the reference cod live oil. The report for 1938, *This Journal*, **22**, 468 (1939), included a recommendation that further studies be made of the feeding of skim or whole non-vitamin D milk with the reference oil in order to determine whether the oil and a quantity of one of these milks, equal to that of the vitamin D milk being assayed, should be used as a reference standard instead of the reference oil alone. Consequently a com-

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

munication was sent to collaborators to ask that collaborative studies be made in order to answer the question involved in the above recommendation. Collaborators were asked to feed non-vitamin D skim milk and if possible non-vitamin D whole milk with a sample of reference oil when routine vitamin D milk assays were being conducted with the usual reference oil group or groups. The Associate Referee furnished non-vitamin D whole milk (Klim), which repeated assays had shown to be either devoid of vitamin D or to contain only a very small quantity of the factor. The skim milk used in the tests which are reported below was furnished by the individual collaborators. With some exceptions, 8 to 10 rats were used for each test group.

The results of the feeding of a quantity of reference cod liver oil in comparison with the same quantity of the oil fed with a quantity of reconstituted skim milk are shown in Table 1. The quantity of skim milk

TABLE 1.—*Effect of feeding skim milk with the reference oil*

NAME OF LABORATORY	REFERENCE OIL ALONE		REFERENCE OIL PLUS SKIM MILK		DIFFERENCE IN RESPONSE
	QUANTITY FED U.S.P. UNITS	AV. RESPONSE	QUANTITY OF MILK *ml.*	AV. RESPONSE	
Michigan	3.25	0.60	7.1	0.65	0.05
Pennsylvania	3.8	0.68	9.0	0.58	−0.10
New Jersey	4.75	1.13	33.3	1.36	0.23
	4.75	0.91	11.6	0.93	0.02
Connecticut	5.0	0.66	12.0	0.90	0.24
	5.0	0.75	12.0	0.91	0.16
	5.0	0.72	12.0	0.78	0.06
New Jersey	5.7	0.78	40.0	1.05	0.27
	5.7	0.98	40.0	1.37	0.39

fed was equal in volume to that of vitamin D milk which would have been fed if the latter were being assayed according to the present tentative procedure, and the reconstituted skim milk contained the weight of solids-not-fat which would have been present in the sample of whole milk. In only one out of nine trials was the response less when skim milk was fed with the reference oil than when the reference oil was administered alone, and in this instance the response of the two groups was essentially the same. In four cases, including the one in which the response was less when the milk was added, there was essentially no difference in response between the groups with or without skim milk. These results, along with those of 7 trials previously reported, make a total of 16, in only one of which was the response with milk less than in the case of the oil alone.

When non-vitamin D whole milk was fed in the same manner so that the total solids of the supplement were equal to that which would have been contained in a sample of vitamin D milk actually under assay, the results shown in Table 2 were obtained. In all of the 16 trials a higher response occurred when milk was fed. The same result was obtained in the case of 7 trials previously reported, *This Journal*, **22**, 468 (1939), making a total of 23 trials in which there was a higher response in the case of the whole non-vitamin D milk fed with the reference oil.

TABLE 2.—*Effect of feeding non-vitamin D whole milk with the reference oil*

NAME OF LABORATORY	REFERENCE OIL ALONE		REFERENCE OIL PLUS WHOLE MILK		DIFFERENCE IN RESPONSE
	QUANTITY FED U.S.P. UNITS	AV. RESPONSE	QUANTITY FED *ml.*	AV. RESPONSE	
Nestlé's	3.0	0.88	20.0	1.08	0.20
Massachusetts	3.0	1.27	7.1	1.33	0.06
Michigan	3.25	0.60	7.1	1.00	0.40
Children's Hosp. Philadelphia	3.6	0.78	8.4	1.02	0.24
Pennsylvania	3.8	0.68	9.0	1.08	0.40
	3.8	0.68	24.0	0.80	0.12
Massachusetts	4.0	1.25	9.4	1.40	0.15
New Jersey	4.75	1.13	33.3	1.25	0.12
	4.75	0.91	11.6	1.04	0.13
Connecticut	5.0	0.66	12.0	0.83	0.17
	5.0	0.75	12.0	0.83	0.08
	5.0	0.72	12.0	0.88	0.16
New Jersey	5.7	0.68	40.0	1.20	0.52
	5.7	0.50	40.0	1.00	0.50
	5.7	0.78	40.0	1.28	0.50
	5.7	0.98	40.0	1.47	0.49

In the studies with both the skim and whole milk the volumes used at a given feeding level were calculated on the basis of vitamin D milk containing 135 or 400 U.S.P. XI units per quart.

When the differences in response for both the skim and non-vitamin D whole milks are arranged in accordance with the increase in the quantity of milk fed, there is a general tendency toward a greater difference in response as the quantity fed becomes larger. Although not marked there is

a tendency toward a stronger response in the case of the non-vitamin D whole milk particularly at the higher levels although this is not true in every individual case.

TABLE 3.—*Relation of the quantity of milk fed to the difference in response*

SKIM MILK		NON-VITAMIN D WHOLE MILK	
QUANTITY FED *ml.*	DIFFERENCE IN RESPONSE	QUANTITY FED *ml.*	DIFFERENCE IN RESPONSE
7.1	0.05	7.1	0.06
		7.1	0.40
		8.4	0.24
9.0	−0.10	9.0	0.40
		9.4	0.15
11.6	0.02	11.6	0.13
12.0	0.24	12.0	0.17
12.0	0.16	12.0	0.08
12.0	0.06	12.0	0.16
		24.0	0.12
33.3	0.23	33.3	0.12
40.0	0.27	40.0	0.52
40.0	0.39	40.0	0.50
		40.0	0.50
		40.0	0.49

In Table 4 it is readily apparent that the feeding of reconstituted skim milk without the reference oil, as compared with an equal quantity of non-vitamin D whole milk, results in a definitely lower response in the case of the skim milk levels.

TABLE 4.—*Effect of feeding non-vitamin D whole milk and skim milk without the reference oil*

QUANTITY FED *ml.*	RESPONSE
Non-vitamin D whole milk	
33.3	0.39
40.0	0.61
40.0	0.18
Total solids equivalent to 70 ml.	1.36
Total solids equivalent to 100 ml.	1.57
Skim milk	
33.3	0.0
40.0	0.0
Solids-not-fat equivalent to 70 ml.	0.38
Solids-not-fat equivalent to 100 ml.	0.44

Although the difference in the response, particularly at the lower levels in the case of skim milk and non-vitamin D whole milk, is not marked, the fact that when fed alone non-vitamin D whole milk shows a consider-

ably stronger response than the skim milk, leads to the conclusion that if milk solids are to be fed as a part of the reference standard it would be safer to use skim milk than whole milk, even though the latter contained only a very small amount of vitamin D. Non-vitamin D whole milk was tried with the hope that it might prove satisfactory for feeding with the reference oil, because a reference standard consisting of the reference oil and this type of milk would be more strictly comparable with the sample of vitamin D milk under examination than if skim milk were used, except that at lower feeding levels the fat intake of the reference group would be higher than that of the assay group.

The question may be raised with regard to the fat content of the reference oil and skim milk as compared with that of the sample of vitamin D milk. If the reference oil is diluted with a vegetable oil and fed during the assay period as 8 daily doses of 0.1 ml. each, the fat intake is approximately 0.7 gram. If 10 ml. of vitamin D milk is the quantity fed to the animals of the assay group, only 0.4 gram of fat is consumed, and therefore the animals of the reference group actually consume the greater quantity of fat. When it is necessary to feed as much as 40 ml. of the vitamin D milk undergoing assay, approximately 1.6 grams of fat will be consumed. Under these conditions the feeding of the reference oil solution at the 0.2 ml. level, for 8 days, affords an intake of approximately 1.4 grams. Therefore, the feeding of skim milk along with the reference oil actually provides for more nearly equal fat intakes by the reference and assay groups than if non-vitamin D whole milk were used.

In the laboratory of the Associate Referee it has been found convenient to reconstitute the skim milk and to feed it and the reference oil as separate supplements.

The results demonstrate that the feeding of solids-not-fat in the form of skim milk, in a quantity equal to that contained in the sample of milk under investigation, along with the calculated quantity of reference oil, should furnish a reference standard that is more comparable with vitamin D milk than is the reference oil alone.

The Associate Referee takes this opportunity to express grateful appreciation of the generous cooperation of the following collaborators: Rebecca B. Hubbell, Connecticut; C. R. Fellers, Massachusetts; C. A, Hoppert, Michigan; R. W. Titus, Nestlé's Milk Products, Incorporated. Marysville, Ohio; N. B. Guerrant, Pennsylvania; and Harold Stevens, Children's Hospital, Philadelphia.

It is recommended[1] that the method be revised so that the animals in the reference group receive in addition to the U.S.P. Reference Cod Liver Oil, non-vitamin D skim milk equal in quantity to the solids not fat in the volume of the milk fed the assay group.

No report on vitamin K was given by the associate referee.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 23, 54 (1940).

REPORT ON RIBOFLAVIN

By A. R. KEMMERER (Texas Agricultural Experiment Station, College Station, Texas), *Associate Referee*

Since this is the first report on riboflavin, a review of some literature on methods for its determination is included. Riboflavin is determined both biologically and chemically. Biological technics depend on the effect of small quantities of the vitamin on the growth of rats or chicks or bacteria. In this connection the bacteriological technic of Snell and Strong[1] is quite extensively used, while for the chemical estimation colorimetric and fluorometric procedures are used.

Charite and Khaustove[2,3] have developed a colorimetric method for riboflavin in animal tissues. The dried or wet tissue is extracted at 28°–36° C. with dilute methanol, and the clear extract is shaken with chloroform to remove carotenoid or other contaminating pigments. The yellow methanol solution of riboflavin is compared in a colorimeter with a standard 0.01 per cent potassium chromate solution. Another method for riboflavin in animal tissue was developed by Van Eekelen and Emmerie.[4] The tissue is extracted with 75 per cent methanol for 24 hours or boiled for 10 minutes and filtered. To the filtrate is added a few ml. of glacial acetic acid and then a few ml. of saturated potassium permanganate solution. After the mixture has stood for a few minutes, a small quantity of hydrogen peroxide is added. By this procedure contaminating impurities are oxidized but the riboflavin is not harmed. The quantity of riboflavin in the final solution is measured with a step photometer. All manipulations are carried out in red light to eliminate destruction of riboflavin by the short-wave lengths of light. In a later paper Emmerie[5] modified the method slightly so that it could be used for milk. In this procedure yellow standards are used instead of the step photometer for final estimations. Emmerie,[6,7] in some procedures, also adsorbed the riboflavin with lead sulfide, eluted with a water-pyridine-acetic acid mixture, and oxidized remaining impurities with potassium permanganate and hydrogen peroxide.

Some workers have adopted adsorptive technics for riboflavin determinations. Narasimhamurthy[8] extracts finely divided food stuffs with 20 per cent methanol containing sufficient hydrochloric acid to bring the acidity to 0.1 *N*. Then the riboflavin is adsorbed on fullers' earth in slightly acid solution and eluted with a mixture of methanol, pyridine and water. The resulting solution is further purified by precipitation of im-

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[1] *Ind. Eng. Chem., Anal. Ed.*, **11**, 346 (1939).
[2] *Comp. rend. acad. sci. U.S.S.R.*, **3**, 386 (1939).
[3] *Biochem. J.*, **29**, 34 (1935).
[4] *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, **5**, 77 (1935).
[5] Congr. intern. tech. chim. ind. agr. comp. rend. 5th Congr., **1**, 57 (1937).
[6] *Acta Brevia Neerland. Physiol. Pharmacol. Microbiol.*, **9–10**, 136 (1936).
[7] Nature, **138**, 164 (1938).
[8] *Indian J. Med. Research*, **24**, 1083 (1937).

purities with acetone. The riboflavin in the final solution is determined by measuring the amount of fluorescence. All manipulations are carried out in the dark or under the illumination of a dull red light. Neuweiler[9] and Bierry and Gouzon[10] also use adsorption by fullers' earth to determine riboflavin in food stuffs.

Chemical determinations of riboflavin in dairy products are used quite extensively in this country. Supplee and coworkers[11,12] report a simple method of estimation in which only a standard of pure riboflavin and an ultra violet light with a suitable filter are needed. The fluorescence of the unknown solution is compared with a series of standard solutions. These workers find that light destroys riboflavin. These same workers[13] in a later paper have applied their procedure to yeast, alfalfa, and other products. Weisberg and Levin[14] extract the riboflavin from solid dairy products by refluxing with methanol acidulated with acetic acid, in the presence of carbon dioxide and the absence of light. They remove impurities by adding acetone and then cooling at refrigerator temperature. In an alternative procedure they reflux the unknown with methanol only; adsorb the riboflavin on British fullers' earth; and elute with a mixture of pyridine, methanol, and water. In both procedures the quantity of fluorescence determines the quantity of riboflavin present. Sodium fluorescein solutions are used as standards. Witnah, Kunerth and Kramer[15] determine riboflavin in milk by precipitating the protein with trichloracetic acid and matching the fluorescence with a series of standard riboflavin solutions. Hand[16] also uses a fluorometric procedure for riboflavin in milk. Norris and coworkers[17] have developed a number of methods for riboflavin in milk and milk by-products and have lately extended them to other foods, but as these methods have not been published, a review of them will not be given here.

PLAN OF WORK

In the work this year the plan of the Associate Referee was to find or develop a simple chemical method for riboflavin that would be applicable to all kinds of material. A colorimetric procedure with a stable easily prepared standard seemed to meet the conditions more nearly than did a fluorometric procedure. A colorimetric procedure, though not so specific as a fluorometric procedure, does not require any special apparatus except a colorimeter. The colorimetric procedure outlined below and samples of dried skim milk and of yeast were sent out for collaborative study. This colorimetric procedure was a combination of the methods of Charite and

[9] *Hoppe-Seyler's Z. physiol. Chem.*, **249**, 225 (1937).
[10] *Documentation sci.*, **5**, 309 (1936).
[11] *J. Biol. Chem.*, **110**, 365 (1937).
[12] *J. Dairy Sci.*, **19**, 215 (1936).
[13] *Ind. Eng. Chem., Anal. Ed.*, **11**, 495 (1939).
[14] *Ibid.*, **9**, 523 (1937).
[15] *J. Am. Chem., Soc.*, **59**, 1153 (1937).
[16] *Ind. Eng. Chem., Anal. Ed.*, **11**, 306 (1939).
[17] Unpublished.

Khaustov and Emmerie and coworkers, cited previously. It worked fairly well in the laboratory of the Associate Referee and gave results that appeared to be quite reliable.

RIBOFLAVIN IN YEAST AND SKIM MILK

Place 10 g. of yeast or 20 g. of dried skim milk in a 200 ml. Erlenmeyer flask and add exactly 100 ml. of 40% methanol containing 4% acetic acid. Stopper the flasks tightly and place in an incubator at 35°–40° C. for 24 hours. Wrap each flask with paper to exclude the light. During the extraction period shake the samples well two or three times. After removing the samples from the incubator, filter but do not wash the residue. In filtering use ordinary laboratory light, but avoid direct sunshine. Pipet a 25 ml. aliquot of the filtrate, which may be slightly cloudy, into a 50 ml. volumetric flask. Add 4 ml. of 4% $KMnO_4$ solution, shake the mixture, and allow to stand 1 minute. Then add 4 ml. of 3% H_2O_2 and shake the mixture again to remove the color produced by the $KMnO_4$. If necessary add 1 or 2 ml. more of the H_2O_2. Dilute the solution to volume with pure methanol and filter. (This filtrate should be clear, and have a yellowish green fluorescence.) Estimate the riboflavin in this solution against .02% K_2CrO_4. (If proper equipment and standards are available, it may be estimated photometrically or fluorometrically.) For the colorimetric procedure place the riboflavin solution in one cup of the colorimeter and set this cup at a depth of 1, 2, 3, 4 cm., or any other convenient depth. Place the chromate solution in the other cup and adjust the depth until the color intensities match. This depth must be 4–18 mm. Make 10 colorimetric readings and take the average. To calculate the riboflavin in solution use the following equation:

$$C = \frac{d. + 4.4}{d_2 \times 1.4},$$

where d. is the mm. depth of .02% K_2CrO_4, d_2 is the cm. depth of the riboflavin solution, and C is the concentration of riboflavin in the solution (p.p.m.). To obtain the p.p.m. of riboflavin in the sample, multiply C by the ml. of solution (200) and divide by the grams of sample.

In addition to the above procedure the collaborators were requested to analyze the sample by any other procedure that they had used. Special emphasis was placed on the bacteriological method of Snell and Strong. The results of the collaborative study are given in Table 1.

COLLABORATORS

The Associate Referee appreciates the generous cooperation of the following collaborators in this study:

H. J. Prebluda, U. S. Industrial Chemicals, Inc., Baltimore, Md.
J. S. Andrews, General Mills, Inc., Minneapolis, Minn.
R. B. Hubbell, Agricultural Experiment Station, New Haven, Conn.
J. W. Clulow, Albers Bros. Milling Co., Seattle, Wash.
L. W. Conn, Sealtest, Inc., Baltimore, Md.
R. O. Brooke, Whitmore Research Laboratory, Malden, Mass.
L. C. Norris, Cornell University, Ithaca, N. Y.
M. D. Thaxter, California Packing Co., San Francisco, Calif.
H. J. Deobald, Allied Mills, Inc., Peoria, Ill.
V. O. Wodicka, Purina Mills, St. Louis, Mo.
E. P. Gundlack and W. B. Griem, State Depart. of Agriculture, Madison, Wis.

TABLE 1.—*Results of collaborative study on riboflavin (p.p.m.)*

ANALYST	SKIM MILK POWDER					YEAST				
	REFEREE'S COLORIMETRIC METHOD	REFEREE'S FLUOROMETRIC METHOD	REFEREE'S PHOTOMETRIC METHOD	OTHER CHEMICAL METHODS	BACTERIOLOGICAL METHOD	REFEREE'S COLORIMETRIC METHOD	REFEREE'S FLUOROMETRIC METHOD	REFEREE'S PHOTOMETRIC METHOD	OTHER CHEMICAL METHODS	BACTERIOLOGICAL METHOD
1	25.7				18.2	131.0				81.0
2	23.0				17.8	170.0				63.3
	20.5					128.0				
Av.	21.8					149.0				
3	30.6	15.5		18.6		268.0	16.9		56.2	
	28.0	14.3		18.5		242.0	15.0		57.6	
	39.3	12.4				276.0	11.6			
	26.6					234.0				
Av.	29.9	14.1		18.6		255.0	14.5		56.9	
4	20.2					124.0				
5	21.0					131.0				
6	29.0					162.0		29.0		
	25.0					160.0				
						160.0				
Av.	27.0					161.0				
7	21.0		16.0	19.0	19.0	107.0		107.0	53.0	52.0
8	15.7			15.6		216.5			80.0	
						202.0			24.1	
Av.	15.7					209.3				
9	17.6				14.5	137.0				62.0
	33.0					146.6				
	28.2					151.2				
Av.	26.3					144.9				
10	18.5		18.0			85.0		84.0		
	20.9		17.0			96.4		80.0		
Av.	19.7		17.5			90.7		82.0		
11	32.4			17.3		137.0			52.7	
12	20.0					162.1				
13	52.4					120.8				
	30.4					110.0				
	24.9					102.9				
	21.3					104.2				
	19.0									
						109.3				
Av.	29.6									
14					18.0					47.0
15	25.0					180.0				
	25.3					186.0				
Av.	25.2					183.0				
Mean of Averages	23.9	14.1	16.8	17.6	17.4	149.6	14.5	72.7	53.3	61.1

B. L. Oser, Food Research Laboratories, Inc., New York, N. Y.
F. M. Strong, Dept. of Biochemistry, University of Wisconsin, Madison, Wis.
W. L. Hall, U. S. Department of Agriculture, Washington, D. C.

COMMENTS OF COLLABORATORS

V. O. Wodicka.—The colorimetric method presented for determining the concentration of riboflavin in the sample entails considerable error. The technic of extraction and purification does not give a pure enough solution for colorimetric estimation from samples of yeast. This collaborator assayed the sample of dried skimmed milk by the regular procedure used in his laboratory, which is as follows: The dried skim milk is refluxed with 75% aqueous acetone, then chilled in the refrigerator, filtered, washed with 75% acetone, and the concentration of riboflavin in the solution measured fluorometrically. The results of duplicate analyses on the dried skim milk by this procedure were 18.6 and 18.5 p.p.m.

This collaborator also devised the following procedure for riboflavin in the yeast:

Reflux 1 gram sample of the yeast with 50 ml. of 2% acetic acid for 30 minutes. Cool the mixture to room temperature and add 5 ml. of 1 *N* NaOH. Then add 5 ml. of a 6% solution of takadiastase freshly prepared and incubate the mixture for 1½ hours at 100° F. Filter the mixture and take a 10 ml. aliquot. Add 0.2 ml. of 4% $KMnO_4$, stir the mixture and allow to stand for a minute or so. Add 5 drops of 3% H_2O_2 and stir the solution to give complete reaction. Stir in 30 ml. of acetone and allow the mixture to stand for 15–20 minutes to coagulate the flocculent precipitate that forms. Filter this solution and read the filtrate fluorometrically.

By this method results of 56.2 and 57.6 p.p.m. were obtained on the yeast sample. According to the collaborator the extraction and incubation are the result of unpublished research by Dr. Hennessy of Fordham University.

L. C. Norris.—Difficulty was encountered in matching the color of the unknown solution with the standard. It was also found that not all the riboflavin was extracted from the yeast by the fluorometric procedure commonly used. Oxidation, as a means of getting rid of interfering pigments, cannot be controlled, and interfering pigments cannot be removed by such a procedure without destroying some of the riboflavin. Unremoved contaminating pigments were thought to be the cause of the high values obtained. The samples were assayed by a procedure used in this laboratory. The value obtained for the dried skim milk was 17.3 p.p.m. and for the yeast 52.7.

E. P. Gundlack and W. B. Griem.—Variations in colorimetric readings gave large variations in the results. In the yeast sample interfering coloring substances were not all destroyed, giving high results, and the oxidizing treatment needs to be revised.

M. D. Thaxter.—Impurities that were found in the purified extract from yeast were soluble in petroleum ether, and had a fluorescence similar to riboflavin when subjected to irradiation in the near ultra violet. No such difficulty was experienced with the skim milk. A better extraction was obtained when the samples were shaken continuously. Also spectrophotometric analyses before and after and reduction by $Na_2S_2O_4$ gave results nearer the true results for yeast; namely 80 p.p.m. when continuous shaking was used.

R. O. Brooke.—It appeared that other pigments than riboflavin remained in the extract after oxidation. The samples were also assayed by the fluorometric method

used at Cornell University (unpublished) and by the Snell-Strong bacteriological procedure. Almost identical results were obtained. The Snell-Strong procedure has the great advantage in that interfering pigments do not complicate the problem. It is believed that it is practically impossible to extract riboflavin in a pure state from most feeds.

L. W. Conn.—The final color developed in the sample did not exactly match that of the standard. The standard was greenish yellow, while the samples had more of a slight straw color. Also there was some difficulty in oxidizing excess permanganate in the milk sample.

J. W. Clulow.—Difficulty was experienced in matching the solution color against the standard especially with the yeast extract. The reaction between the peroxide and permanganate was quite severe and tended to bubble over unless the peroxide was added very slowly.

R. B. Hubbell.—Difficulty was experienced in reading due in part to a slight brownish tint in the solution obtained from the yeast.

W. L. Hall.—Difficulty was experienced in matching the standard solution against the sample solution.

J. S. Andrews.—One of the big sources of error in the colorimetric method is the incomplete removal of non-flavin pigments. The matching of yellow color in such an instrument as the Duboscq colorimeter is a difficult and inaccurate process and there may be as much as 20–25% error in the colorimetric readings. The samples were also run by the Snell-Strong procedure, which has always given fairly reliable values.

H. J. Prebluda.—The Snell-Strong bacteriological procedure is recommended highly.

DISCUSSION OF RESULTS

From the data in the table and from comments of the collaborators it is readily seen that the proposed colorimetric procedure gave results that are high and that vary widely even in the hands of the same operator. The collaborators are of the general opinion that the method of extraction and purification are inadequate and that the final solution obtained, especially with regard to the yeast, contained other yellow and off-color pigments that greatly interfered with the final colorimetric comparison. Three collaborators used the photoelectric photometer without much better results. Most of the collaborators agree that fluorometric procedures are likely to give better results than colorimetic. The bacteriological method of Snell and Strong was studied by five collaborators and was well recommended. However, quite wide variations were obtained by the collaborators that used it. The results of this collaborative study look promising and indicate that a reliable chemical method for riboflavin is not an impossibility and that further work along this line should be undertaken.

RECOMMENDATIONS

It is recommended—

(1) That chemical methods for riboflavin be studied further and that fluorometric methods be considered.

(2) That the Snell-strong bacteriological technic be further studied.

REPORT ON CANNED FOODS

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

L. M. Beacham conducted collaborative work on the determination of chlorides and total solids in tomato products. No other collaborative work on methods of analysis for vegetables and vegetable products was done during the past year.

RECOMMENDATIONS[1]

It is recommended—

(1) That Beacham's recommendations on methods for tomato products be adopted.

(2) That, in Chapter **XXXV,** Vegetables and Vegetable Products, the sentences, "Without shifting the product, so incline the sieve as to facilitate drainage of the liquid. With the exception of very soft products, such as tomatoes, carefully invert by hand all pieces containing cups or cavities if they fall on the sieve with cups or cavities up. Cups or cavities in soft products may be drained by tilting the sieve, but no other handling of these products while draining is permissible," be added at the end of the first paragraph under **2**—Preparation of Sample—Official. Also change the first sentence of the second paragraph to read, "Allow the material on the sieve to drain 2 min., . . . "

(3) That the reference under **3**—Moisture—Official—be changed to refer to the method for total solids, for tomato products. (The present reference seems to be in error since it refers to a method of cold drying over sulfuric acid, although the Referee can find no indication that such a method was ever adopted by the Association for vegetables and vegetable products.)

(4) That Paragraph **20** under Tomato Products, Ash—Official, be changed to read the same as Paragraph **4,** Ash—Official.

(5) That the second paragraph under Paragraph **29,** Yeasts and Spores, be amended as follows: Place a period after "cover-glass" at the end of line 4 and substitute the sentence, "Discard any mount showing uneven distribution, absence of Newton's rings, or liquid that has been drawn across the moat and under the cover-glass," for the clause beginning "observing precautions to secure production of Newton's rings . . . " and ending with the words "supporting surfaces of the slide."

(6) That studies of methods for quality factors and fill of containers be continued.

(7) That collaborative studies on the method for total solids in tomato products be continued.

[1] For report of Subcommittee C and action by the Association, see *This Journal,* 23, 61 (1940).

REPORT ON TOMATO PRODUCTS

By L. M. BEACHAM (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

During the past year the practicability of determining the chlorides in tomato paste and purée by means of the official method for chlorides in tomato juice, *This Journal*, **20**, 78 (1937), was investigated. Since the purée filtered but slowly and the paste not at all, the procedure given in the note on the method was followed. In this procedure a weighed or measured quantity of paste or purée is placed in an Erlenmeyer flask, and 30–40 ml. of water, and sufficient nitric acid to destroy organic matter, are added. A measured quantity of standard silver nitrate is added, and the mixture is boiled until a clear supernatant liquid is obtained. The excess silver nitrate is titrated with standard ammonium thiocyanate. Numerous difficulties were encountered. The presence of great quantities of organic matter in the paste and purée made it difficult to obtain a clear solution. The precipitated silver chloride was colloidal and required prolonged boiling before a coagulated precipitate was obtained. When the excess silver nitrate was titrated with ammonium thiocyanate, the end point was very obscure, a condition that the addition of nitrobenzene did not overcome. Numerous modifications of the method were investigated, and the following method, which has been found equally applicable to tomato paste, tomato purée and tomato juice, was developed:

Weigh 5 g. of the tomato material and transfer with 80% C_2H_5OH to a 100 ml. volumetric flask. Add 80% C_2H_5OH to give a volume of approximately 50 ml. Shake well to get all the tomato material into suspension. Add 1 ml. of concentrated HNO_3 and by means of a pipet add 25 ml. of 0.1 *N* $AgNO_3$. Make to 100 ml. volume with 80% alcohol. Transfer to a centrifuge bottle and centrifuge at 1800 r.p.m. for 5 minutes. Pipet 50 ml. of the supernatant liquid into a 300 ml. Erlenmeyer flask, add 2 ml. of a saturated solution of ferric ammonium sulfate, and titrate to a permanent light brown color with 0.1 *N* ammonium thiocyanate solution. Multiply the number of ml. of NH_4CNS used by 2 and subtract from 25. Multiply the difference by 0.005843 to obtain the weight of chlorides present, expressed as grams of NaCl. Divide by 5 and multiply by 100 to calculate the percentage of salt present.

COLLABORATIVE WORK

Samples were submitted to 10 laboratories of the Food and Drug Administration and to three commercial laboratories for collaborative analyses. It was requested that chlorides be determined by the present official method (*Methods of Analysis, A.O.A.C.*, 1935, 500, 22) as well as by the suggested rapid method. In addition, collaborators were requested to make determinations in duplicate of the total solids in each sample, and to use the tentative A.O.A.C. method (*Ibid.*, **16**, p. 499). With the exception of the sample submitted to C. A. Greenleaf of The National Canners Association, who received two cans of paste, each sample consisted of one can of paste and one can of purée. These cans of paste and purée were

purchased from a local grocery. Each of the cans of paste bore the same code mark. The same was true of the purée. It was expected that this would insure that all of the cans of each product had come from the same factory batch. However, the results of the analyses indicate that at least two separate batches were represented in the sample of paste. This is confirmed by a statistical comparison of the average salt contents of the two apparent groups of cans. The difference between the two averages clearly shows that two or more batches or universes of cans were sampled. The uniformity from can to can was not so good as could be desired, but the agreement between duplicate analyses or between the present official method and the suggested method on the same can was satisfactory.

Results of analyses for chlorides comparing the two methods are given in Table 2. They indicate that the suggested method gives results slightly higher than those obtained by the official method. This difference appears to be constant and is not proportional to the amount of chlorides present. Greenleaf makes the following comment: "The rapid method for salt works smoothly and is obviously much quicker and more convenient than the official method. The difference in results is somewhat greater than would be expected as a result of the insoluble solids error alone. It would be of interest to determine whether this is due to a positive error in the rapid method or a negative one in the official." Accordingly an authentic tomato paste was prepared. Analysis for chlorides naturally present was made, both by the official and by the rapid method. A weighed quantity of salt was added, and total chlorides were again determined by both methods. Results, which are given in Table 1, are inconclusive.

TABLE 1.—*Results on salt present and added*

	A.O.A.C. METHOD		RAPID METHOD	
	per cent		*per cent*	
Chlorides naturally present	.13	.12	.15	.15
Salt added	*2.80*	*2.80*	*2.80*	*2.80*
Salt, by calculation	2.93	2.92	2.95	2.95
Salt, by analysis	2.95	2.96	2.97	2.97

Several laboratories pointed out that a sharper end point was obtained when 1 or 2 ml. of concentrated nitric acid was added just before titrating with ammonium thiocyanate. Since this is an established and recognized means for securing a sharper end point when using ferric ammonium sulfate as an indicator, it is not believed that incorporating this change in the method would affect the validity of the collaborative results.

The results of the collaborative work on total solids are given in Table 3. Duplicate determinations on the same can gave very good results.

TABLE 2.—*Collaborative results on chlorides (expressed as per cent NaCl)*

COLLABORATOR	CAN NO.	PASTE		PURÉE	
		A.O.A.C.	RAPID	A.O.A.C.	RAPID
		per cent	*per cent*	*per cent*	*per cent*
Doris H. Tilden					
Food and Drug Adm.	1	0.84	0.89	0.10	0.11
San Francisco		0.84	0.86	0.10	0.13
N. J. Linde					
Crown Can Company	2	0.82	0.87	0.10	0.10
Philadelphia		0.82	0.84	0.10	0.10
S. B. Falck					
Food and Drug Adm.	3		0.86		0.13
Cincinnati			0.84		0.13
A. H. Wells					
Food and Drug Adm.	4	0.85	0.90	0.10	0.12
Los Angeles		0.83	0.90	0.10	0.14
F. L. Hart					
Food and Drug Adm.	4		0.92		0.16
Los Angeles			0.91		0.15
C. R. Joiner					
Food and Drug Adm.	5	0.85	0.87	0.11	0.11
New Orleans		0.87	0.88	0.12	0.10
H. D. Grigsby					
Food and Drug Adm.	6	0.86	0.86	0.11	0.14
Philadelphia		0.85	0.88	0.11	0.13
S. M. Berman					
Food and Drug Adm.	7	0.84	0.86	0.09	0.12
Buffalo		0.84	0.86	0.10	0.13
Maurice Siegel					
Strasburger & Siegel	8				
Baltimore		0.81	0.87	0.09	0.12
C. A. Greenleaf					
Nat. Canners Assoc.	9	0.83	0.89		
Washington		0.83	0.87		
C. A. Greenleaf					
Nat. Canners Assoc.	10	1.08	1.13		
Washington		1.08	1.12		
T. C. Dunn					
Food and Drug Adm.	11	1.11	1.13	0.13	0.13
Denver		1.11	1.12	0.12	0.14

TABLE 2.—*Continued*

COLLABORATOR	CAN NO.	PASTE		PURÉE	
		A.O.A.C.	RAPID	A.O.A.C.	RAPID
		per cent	*per cent*	*per cent*	*per cent*
H. M. Boggs		1.06	1.10	0.08	0.09
Food and Drug Adm.	12	1.07	1.07	0.08	0.08
New York		1.06	1.07	0.09	0.06
F. M. Garfield					
Food and Drug Adm.	13	1.07	1.12	0.09	0.13
St. Louis		1.05	1.12	0.10	0.12
L. M. Beacham	14	1.05	1.09	0.07	0.06
Food and Drug Adm.	15	1.04	1.09	0.08	0.06
Washington	16	1.19	1.20	0.08	0.08

There appears to have been considerable variation from can to can. In view of this fact the Associate Referee does not believe conclusions as to the accuracy of this method are warranted. It was noted that care must be taken in using the method to insure that apparent dryness has been reached before the 4 hour drying period begins.

The suggestion has been made that the quantity of sample taken for solids determination be more definitely defined. S. M. Berman of the Buffalo Station of the U. S. Food and Drug Administration suggests that the size of sample for solids determination be stated in terms of grams of paste or purée per centimeter of dish diameter. Greenleaf suggests a minimum of 9 mg. (dried solids) per sq. cm. of drying area and the present maximum of 12 mg.

There are now two official methods for the determination of chlorides in tomato products, one applicable to all tomato products, in which the product is ashed and the chlorides determined in the ash, and one applicable only to tomato juice. The rapid method, on which collaborative work is herein reported, is equally applicable to tomato juice and other tomato products. The special method for chlorides in tomato juice seems to have no advantage over it, either in rapidity or in accuracy.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method presented for determining chlorides in tomato products, amended to require the addition of 2 ml. of nitric acid before titrating with ammonium thiocyanate, be adopted as tentative.

(2) That the present official method for determination of total chlorides

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 23, 61 (1940).

TABLE 3.—*Collaborators results on total solids*

COLLABORATOR	CAN NO.	PASTE	PURÉE
		per cent	*per cent*
Doris H. Tilden	1	26.28	8.26
		26.28	8.28
N. J. Linde	2	26.5	8.31
		26.8	8.29
C. R. Joiner	5	26.28 25.94	8.33
		25.98 25.97	8.32
H. D. Grigsby	6	25.96	8.20
		25.95	8.21
S. M. Berman	7	25.97	8.16
		26.00	8.18
Maurice Siegel	8	26.02	8.14
		25.91	
C. A. Greenleaf	9	25.83	
		25.79	
	10	24.83	
		24.76	
		24.75	
T. C. Dunn	11	24.55	8.10
		24.47	8.11
H. M. Boggs	12	25.87	8.32
		25.87	8.37
		25.87	8.38
F. M. Garfield	13	25.07	8.23
		25.14	8.20
L. M. Beacham	14	25.12	8.27
	15	25.14	8.21
	16	25.32	8.18

in tomato juice, be dropped, first action, since the method provided above is equally applicable to tomato juice and to other tomato products.

(3) That the official method for determining total solids be amended as suggested by the Referee and Associate Referee.

(4) That the above-amended method be subjected to further collaborative study.

ASSOCIATE REFEREE ON ARSENIC AND ANTIMONY

Owing to changes in work and location, C. C. Cassil, Associate Referee on Arsenic and Antimony, has resigned. C. W. Murray of the U. S. Food and Drug Administration has been appointed to fill this vacancy.

ASSOCIATE REFEREE ON DETECTION OF ADULTERATION OF DISTILLED SPIRITS

Owing to a change of work, S. T. Schicktanz has resigned as Associate Referee on Detection of Adulteration of Distilled Spirits. A. D. Etienne, of the Bureau of Internal Revenue, Washington, D. C., has been appointed to fill this vacancy.

CONTRIBUTED PAPERS

DETERMINATION OF THE TOTAL, VOLATILE, AND FIXED ACIDS OF DISTILLED SPIRITS FROM POTENTIOMETRIC DATA

By S. T. SCHICKTANZ* and A. C. BLAISDELL (Alcohol Tax Unit Laboratory, Treasury Department, Washington, D. C.)

Considerable difficulty is encountered in determining the total, volatile, and fixed acids of a distilled spirit by the official method.[1] The value for total acidity may be in error because of the obscure end point normally obtained when colored solutions are titrated and phenolphthalein used as the indicator. Then, too, most distilled spirits contain substances that change color (darken) near the end point and the colors produced are often confused with the true indicator end point color change.

The values for the volatile acid portion may be in error if careful technic is not used during the steam distillation procedure. The titration is accurate, however, since the solutions obtained from the distillation are water white and the phenolphthalein color change is easily recognized.

The accuracy of the value for fixed acids, which is determined as the difference between the experimental value for total and volatile acids, depends totally on the accuracy of these determinations, and therefore any error in either will produce similar errors in the value for the fixed acids.

Normal titration procedure gives results and values that can be interpreted only as total moles of acids that have dissociation values large enough to be titrated when phenolphthalein is used as an indicator. The results do not indicate the presence of acids differing greatly in dissociation value or differentiate them. Thus, the presence of small quantities of strong inorganic acids or of acids weaker than acetic can not be detected.

These adverse conditions can be overcome to a considerable extent by determining the acids potentiometrically. The data of an electrometric titration plotted as *p*H versus ml. of alkali added produce a curve that clearly indicates the types of acids present. The presence of a minute trace of inorganic acid is indicated by an abnormally low initial *p*H, because the dissociation constant of practically all inorganic acids is much greater than that of acetic acid, which is one of the strongest organic acids normally present in distilled spirits. Similarly, the presence of acids having dissociation constants lower than that of acetic acid is indicated by variations in the shape of the titration curve above *p*H 8.5. An added advantage of titrating acids potentiometrically is that colored solutions that normally decrease the accuracy of titration methods that utilize

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[1] *Methods of Analysis, A.O.A.C.*, 1935, 170.

color indicators for determining the end points do not interfere with the analysis.

In Table 1 are given the dissociation constants of acids and types of acids normally found in distilled spirits.

TABLE 1.—*Dissociation constants of acids normally found in distilled spirits*

ACID	K
Acetic	1.86×10^{-5}
Propionic	1.4×10^{-5}
Butyric	1.48×10^{-5}
Isobutyric	1.5×10^{-5}
Isovaleric	1.7×10^{-5}
Lactic	1.38×10^{-4}
Benzoic	6.6×10^{-5}
Vanillic	3.0×10^{-5}
Gallic	4.0×10^{-5}
Phenol	1.3×10^{-10}

All the acids except phenol contain a carboxyl group (COOH). Except for lactic acid, all the dissociation values are of the same magnitude, which indicates that the strength of a carboxyl radical is independent of the type of molecule attached to it. Thus a methyl, an ethyl, a propyl, an isopropyl, and isobutyl, a benzene, a methoxy-hydroxy benzene, or a trihydroxy benzene molecule attached to the carboxyl radical changes the dissociation value very little. The introduction of a hydroxyl group into an aliphatic acid molecule (lactic acid) produces a ten-fold increase in the dissociation value of the carboxyl group, whereas the introduction of a methoxy and a hydroxy radical into the benzene nucleus of benzoic acid produces only a small change. Similarly, the introduction of three hydroxy groups produces little change as illustrated by the data for gallic acid.

Any acidic type molecule present in a distilled spirit that contains a carboxyl acid radical can be titrated when phenolphthalein is used as an indicator. Likewise, the data for an electrometric titration would include all these acids. If the titration is carried out only to a *p*H value of 9.0, no distinction between the types of acids present could be made, since all the acids listed, except lactic acid and phenol, have dissociation values of the same magnitude and consequently would yield titration curves of the same type and shape. By continuing the titration beyond *p*H 9.0, which is possible in an electrometric titration, the presence of the benzene nucleus and its attached weakly acidic hydroxyl radicals, gallic etc. will affect the shape and slope of the curve in the higher *p*H range. Consequently, it would be impossible to assume the presence of an individual acid in a mixture containing only aliphatic acids by the analyses of a complete titration curve, but the presence of an aromatic acid, such as a derivative of benozic, would be easy to detect because of its effect in the higher *p*H range.

Freshly distilled spirits normally contain only small quantities of acids, which are produced during the fermentation process. When the spirit is aged in charred containers, the acid concentration increases owing to the extraction of the acidic type molecules from the charred portions. By analogy of conditions that exist during the charring of a barrel and the destructive distillation of wood, it may be assumed that the products of decomposition in the two cases will be similar. Thus, in the barrel there are available for extraction many of the homologs of acetic acid, phenol, and benzoic acid, respectively.

Since distilled spirits normally contain acids of the aliphatic series, lactic acid and aromatic acids similar to gallic and tannic, it should be possible to obtain empirically total, volatile, and fixed acids from the analysis of an electrometric titration curve. The initial *p*H should also indicate the presence of minute traces of relatively strong acids (inorganic), owing to their high dissociation values.

EXPERIMENTAL

All the potentiometric data were obtained with a glass electrode and a Leeds and Northrup type *p*H meter. Because of the large size of the electrodes, the data were obtained on 100 ml. samples. All titrations were made at room temperature that varied from 28° to 30° C. Because of the empirical nature of the data obtained, no temperature corrections were made.

In the potentiometric analysis, a measured volume of sample sufficient to cover the electrodes (glass and calomel) is introduced into a beaker and the electrodes are submerged. The sample is stirred, preferably mechanically, and the initial *p*H obtained. The titer 0.05 *N* sodium hydroxide is now run slowly from a buret into the beaker until the *p*H of the solution is 6–6.5. The total volume of titer used is noted and the titration continued, small increments (0.5–1.0 ml.) being introduced and the *p*H being noted after each introduction. The titration is continued in this manner until the *p*H of the solution reaches at least 11.5. The values for *p*H and volume of titer used between *p*H 6 and 11.5 are now plotted in the usual manner for calculating the values for total, fixed, and volatile acids. The time required for a potentiometric analysis is thereby shortened, since only a few *p*H readings are required and it is not necessary to plot the complete titration curve.

In a previous investigation Schicktanz and Etienne[2] show that phenolphthalein changes color in the vicinity of *p*H 9.8 when present in 100 proof alcoholic solutions. However, in the tiration of samples of whisky, the writers obtained the best agreement between actual titration and potentiometric measurements when the end point of the latter determinations was taken at *p*H 10.0. This is shown in Table 3, wherein the results

[2] *Ind. Eng. Chem.*, **29**, 157 (1937).

calculated from the titer curves are compared with the values obtained by the modified official methods suggested by G. F. Beyer.[3] In the latter procedure for the determinatlon of total acid, the sample of whisky (in a large evaporating dish) is diluted with 10 volumes of neutralized, boiled distilled water prior to the titration. It may be concluded that this dilution so reduces the color that it does not interfere with the end point color change.

The value for fixed acids, as determined empirically from the titer curves, is taken as the volume of titer required to raise the *p*H of the solution from 8.5 to 11.0. The values obtained are in good agreement with those obtained by the modified official method as suggested by Beyer.[3] In the fixed acids determination, the sample of whisky in a platinum dish is evaporated to dryness on the steam bath and then further dried for 30 minutes in an oven maintained at 100° C. The residue is dissolved in 25 ml. of 50 per cent ethyl alcohol and transferred to a large evaporating dish, where it is diluted with 10 volumes of neutralized, boiled, distilled water prior to the titration.

The value for the volatile acids, in both procedures, is determined as the numerical difference between the experimentally determined values for total and fixed acids.

DISCUSSION

In Figure 1 are shown titration curves for acetic acid, benzoic acid,

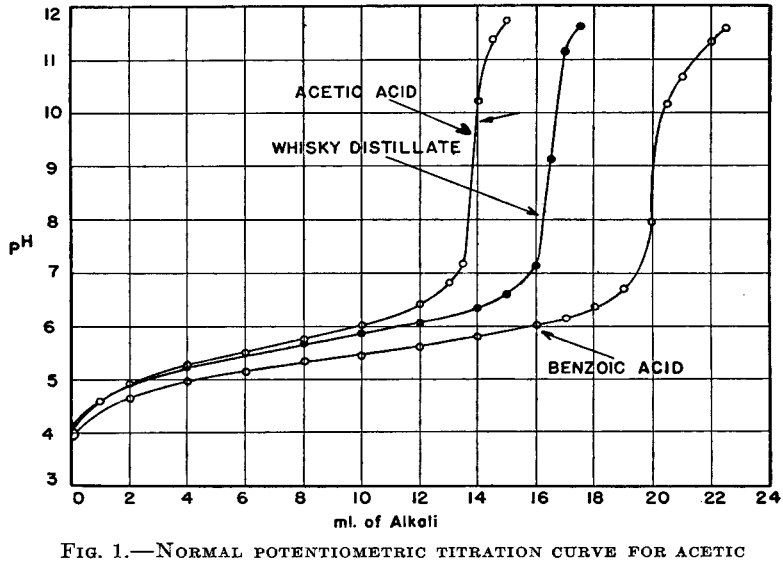

FIG. 1.—NORMAL POTENTIOMETRIC TITRATION CURVE FOR ACETIC AND BENZOIC ACIDS AND A DISTILLATE OBTAINED FROM WHISKY.

[3] *This Journal*, 23, 151 (1940).

and a distillate obtained from whisky in 100 proof alcoholic solution when 0.050 *N* sodium hydroxide was used as titer. The abrupt perpendicular nature of the curves at the end point is indicative of the presence of relatively strong organic acids. The curve for benzoic acid has a greater tendency to slope in the higher *p*H ranges than has either of the other two curves. This probably is due to a buffering action produced by the presence of the benzene nucleus. However, from the values for initial *p*H and the shape of the lower portion of the curve, it may be assumed that benzoic acid has a dissociation constant very near to that of acetic acid (see Table 1). The curve for the whisky distillate is identical to that for acetic acid. From this it may be assumed that the distillate contains only aliphatic type acids.

In Figure 2 are shown the curves for gallic and tannic acids in 100 proof alcoholic solution when titrated with 0.050 *N* sodium hydroxide.

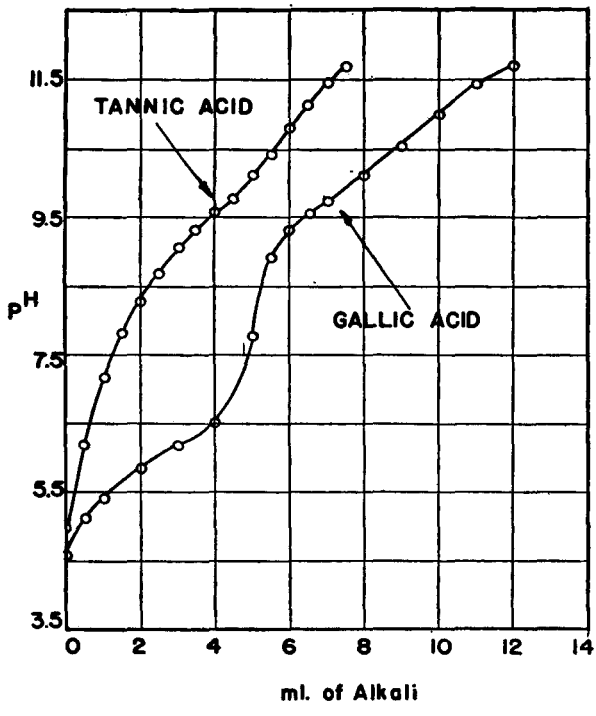

FIG. 2.—NORMAL POTENTIOMETRIC TITRATION CURVES FOR TANNIC AND GALLIC ACIDS IN 100 PROOF ALCOHOL SOLUTION.

The general shape of the curve for gallic acid indicates that the carboxylic acid radical has a relatively high dissociation value and appar-

ently is not affected during the titration by the presence of the phenolic groups. After the carboxylic radical is neutralized, the additional alkali added reacts with the phenolic groups to produce that portion of the curve above *p*H 9.4. The shape of this portion of the curve may be attributed to the weakly acidic power of the phenolic groups and to their buffering action. Since only one carboxylic acid radical is present in a molecule, the molal concentration of gallic acid may be determined by normal titration procedure, with phenolphthalein as the indicator. The same approximate value may be obtained from an analysis of the upper range of the titration curve, since the volume of alkali used to produce the slope between *p*H 9.0 and 11.5 is almost equivalent to the volume required to neutralize the carboxyl radical of the molecule (lower portion of curve). Exclusive of the buffering action, it appears that one of the hydroxyl radicals present in gallic acid has a dissociation value greater than that of the other two, and consequently is the only acid radical titrated between the *p*H range 9.0 and 11.5.

The low value for the initial *p*H for tannic acid indicates the presence of a highly dissociated acidic radical. However, this fact is not corroborated by the peculiar shape of the curve below *p*H 9.5. It appears that because of the complexity of the tannic acid molecule and the presence of numerous phenolic groups, a typical internal buffering action takes place immediately upon the addition of alkali to the acid solution. The portion of the curve above *p*H 9.5 indicates the presence of a neutralizing reaction similar to that which takes place during the titration of gallic acid.

A normal titration of a solution containing small amounts of acetic acid and its homologs and gallic acid and its homologs, with phenolphthalein as the indicator, will give a relatively true value for the carboxylic acid content of the mixture. Potentiometrically, by continuing the titration beyond the color change of the phenolphthalein, the presence of the gallic homologs becomes evident. Thus, from the analyses of the potentiometric data, it is possible to detect the presence of the gallic acid type molecules, which is not the case with the data from an indicator titration measurement.

In Figure 3 are shown the curves obtained by titrating potentiometrically consecutive samples of whisky removed from the same barrel at 6 month intervals. As the aging proceeds the titration curves shift to the right, indicating an increase in concentration of total acids. Simultaneously with the increase in concentration of the acids, the slope of the upper portion of the curves decreases, indicating a gradual increase in concentration of acidic groups having a dissociation value lower than that of acetic acid. It is these acid molecules that are determined as "fixed acids."

The values for fixed acids by potentiometric analysis (Table 2) show

an almost gradual and uniform increase with increase in age, whereas the values obtained by Valaer and Frazier[4] by the official method are considerably higher and show wide irregularities. It may be assumed that the irregularities in the values for fixed acids determined by the official method are due to errors inherent in the method for the determination of volatile acids, rather than to actual variations in concentration.

In Table 3 is given a comparison of the results for total, volatile, and fixed acids as determined by the modified official procedures and the

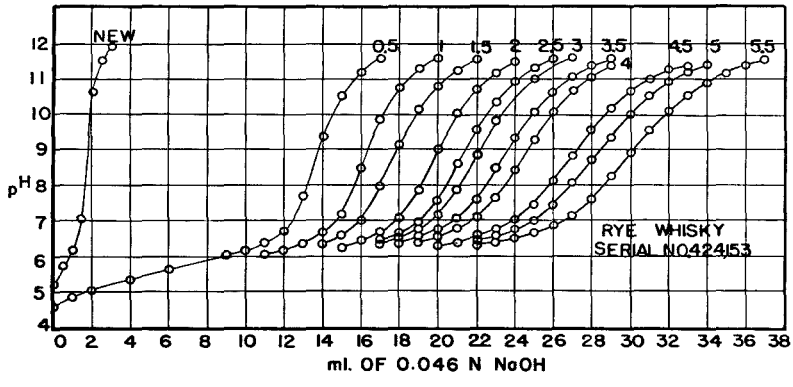

FIG. 3.—TITRATION CURVE OF SUCCESSIVE 6-MONTH OLD SAMPLES OF THE SAME RYE WHISKY.

analysis of potentiometric titration curves. Very little difference is shown in the values obtained by the two methods. Comparatively good checks are obtained for total, fixed, and volatile acids independent of the age of the sample being analyzed. Thus, the method for estimating total, fixed, and volatile acids from potentiometric data, although empirical, gives results that are in good agreement with those determined by the modified official procedures.

In the last column in Tables 2 and 3 are given the initial *p*H readings for the different samples of whiskies. All the values fall within a narrow *p*H range (4.09–4.76) except those for the new whisky sample in Table 3, which has an initial *p*H of 5.77 caused by the very low concentration of acid present.

In Table 4 are given the data for various samples of whiskies, some of which contained traces of sulfurous or sulfuric acid. All of the samples were of approximately the same age.

Each sample that gave a positive test for sulfate gave an initial *p*H considerably lower than that normally obtained on whisky containing the same concentration of total acids.

[4] *Ind. Eng. Chem.*, **28**, 92 (1936).

TABLE 2.—*Values obtained for total, volatile, and fixed acids by the potentiometric and modified official methods*

AGE	TOTAL ACIDS		FIXED ACIDS		VOLATILE ACIDS		INITIAL *p*H
	POTENTIOMETRIC METHOD	OFFICIAL METHOD	POTENTIOMETRIC METHOD	OFFICIAL METHOD	POTENTIOMETRIC METHOD	OFFICIAL METHOD	
years							
0	5.7	4.8	1.3	0	4.4	4.8	5.18
0.5	43.5	42.0	7.2	10.2	36.3	31.8	4.59
1.0	51.6	51.6	8.1	13.2	43.5	38.4	4.54
1.5	56.7	52.8	9.7	10.8	47.0	42.0	4.49
2.0	63.3	60.0	9.5	12.6	53.8	47.4	4.51
2.5	67.8	68.4	10.5	18.6	57.3	49.8	4.48
3.0	70.2	67.2	11.1	16.8	59.1	50.4	4.50
3.5	74.7	72.0	12.4	18.0	62.3	54.0	4.56
4.0	77.7	74.4	12.4	17.4	65.3	57.0	4.63
4.5	86.1	—	14.3	—	71.8		4.33
5.0	90.0	—	15.0	—	75.0		4.31
5.5	95.7	—	16.3	—	79.4		4.27

TABLE 3.—*Values obtained for total, volatile, and fixed acids by the potentiometric and modified official methods*

AGE	TOTAL ACIDS		FIXED ACIDS		VOLATILE ACIDS		INITIAL *p*H
	POTENTIOMETRIC METHOD	OFFICIAL METHOD	POTENTIOMETRIC METHOD	OFFICIAL METHOD	POTENTIOMETRIC METHOD	OFFICIAL METHOD	
years							
2	64.5	66.6	12.3	9.9	52.2	56.7	4.52
4	72.3	75.3	12.3	12.6	60.0	62.7	4.76
6	76.8	74.4	15.3	15.6	61.5	58.8	4.39
4	72.3	73.2	12.9	12.9	60.1	60.3	4.69
2	74.1	75.0	12.6	13.5	61.5	62.5	4.43
2	63.3	65.4	9.9	9.9	53.4	55.5	4.44
3.5	76.5	76.8	12.9	12.6	63.6	64.2	4.38
5	69.0	69.9	10.2	12.3	58.8	57.6	4.36
5.5	96.6	96.0	16.2	18.0	80.4	78.0	4.27
5.5	86.7	86.4	16.8	15.6	69.9	70.8	4.19
7.0	103.5	100.8	18.3	25.2	85.2	75.6	4.20
—	87.6	85.2	18.3	18.0	69.3	67.2	4.18
—	80.4	79.2	16.8	16.8	63.6	52.4	4.16
—	93.3	93.6	19.2	16.8	74.1	76.8	4.09
—	104.7	103.2	20.1	19.2	84.6	84.0	4.12

In Figure 4 are plotted the values of initial *p*H against total acid concentration as grams per 100 liters for the results given in Tables 2, 3, and 4. All the samples containing sulfate ions are indicated by shaded circles. From their position they are easily distinguished from all samples that do not contain any inorganic acid.

TABLE 4.—*Results obtained on samples of whisky*

H_2SO_4	I.*p*H	TOTAL ACID	FIXED ACID	VOLATILE ACID
SO_4	3.90	51.8	7.8	44.0
	4.95	24.9	7.2	17.7
	4.95	17.1	4.8	12.3
SO_4	3.90	50.7	7.8	42.9
SO_4	4.45	27.6	4.8	12.8
	5.10	22.5	4.5	18.0
	5.35	13.2	3.6	9.6
SO_4	3.75	57.6	10.5	47.1
	4.92	18.9	4.8	14.1
SO_4	4.40	18.9	4.2	14.7
SO_4	4.22	54.0	9.6	44.4

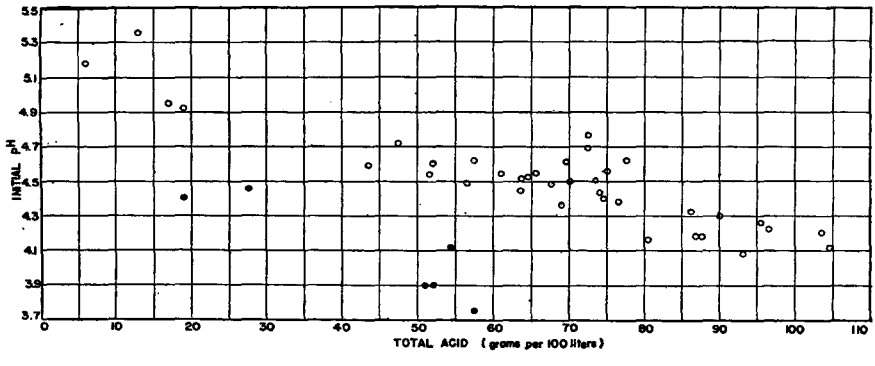

FIG. 4.—RELATION BETWEEN INITIAL *p*H AND TOTAL ACIDS FOR SAMPLES OF WHISKY.

SUMMARY

The results obtained by the analysis of electrometric titration curves for distilled spirits are in good agreement with those obtained by the modified official procedures.

Since the presence of colored and indicator type substances does not interfere with the determination, the results are easily reproduced.

Traces of highly dissociated acidic substances are easily detected.

The total, volatile, and fixed acid values, and the presence or absence of traces of inorganic acids are obtained from a single titration curve.

The complete analysis requires about 30 minutes.

CORRELATION OF FUSEL OIL VALUES BY THE ALLEN-MARQUARDT AND ACETYL CHLORIDE METHODS

By S. T. SCHICKTANZ,* A. D. ETIENNE, and J. L. YOUNG
(Alcohol Tax Unit Laboratory, Treasury Department, Washington, D. C.)

In the Allen-Marquardt procedure,[1] which has been adopted as the official method by the A.O.A.C., the higher alcohols (fusel oil) are oxidized and subsequently estimated by titration with 0.100 *N* standard alkali. In the Schicktanz-Etienne[2] acetyl chloride procedure, the higher alcohols are esterified and the degree of esterification is estimated by a titration procedure in which 0.100 *N* standard alkali and 0.100 *N* sulfuric acid, respectively, are used.

The acetyl chloride method has been modified to give more consistent results when applied as a routine procedure.

The details of the modified method follow:

FUSEL OIL

REAGENTS

Eastman's reagent grade acetyl chloride is used in preparing a 0.23 *M* solution in dry toluene. The pyridine solution is made approximately 0.50 *M* in toluene. Both solutions may be kept safely in regular well-ground glass-stoppered bottles.

APPARATUS

The distillation unit recommended in the official Allen-Marquardt method is used to separate the fusel oil fraction and other volatile constituents from the solids of the whisky.

The reaction flask, Figure 1, is made from a 125 ml. Erlenmeyer flask to which is attached standard-taper ground joint No. 15. The neck of the flask is elongated as shown, in order to assure no loss of reagents during pipetting.

In Figure 2 is shown the dehydrating and dealcoholizing still used to remove the ethyl alcohol and water from the composite CCl_4 extracts. The still is packed with small glass helixes 0.64 cm. (0.125 inch) in diameter.

PROCEDURE

It was found that the percentage of fusel oil removed by extraction with carbon tetrachloride depends on both the alcoholic concentration of the aqueous layer saturated with sodium chloride and its volume.

Thus, instead of using arbitrarily a 50 ml. volume of sample, independent of the proof, as previously suggested, it appeared necessary to use a standard volume, made up to a predetermined proof. The proof was taken as 90° to conform to most of the samples of distilled spirits in commerce. To simplify the analysis, the volume of sample taken is such that when made up to 50 ml. with water, the proof will be approximately 90°. For example, if the sample in question is 100° proof, then only 45 ml. is used. The volume of sample required is calculated by the following equation:

$$\frac{4500}{\text{proof}} = \text{x, where}$$

x is the ml. required to produce 50 ml. of 90 proof material.

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[1] *Methods of Analysis, A.O.A.C.*, 1935, 172.

[2] *Ind. Eng. Chem., Anal. Ed.*, 11, 390 (1939).

The required quantity of sample is pipetted into the 500 ml. Erlenmeyer flask. Enough distilled water is added to make the volume 50 ml., after which 30 ml. of

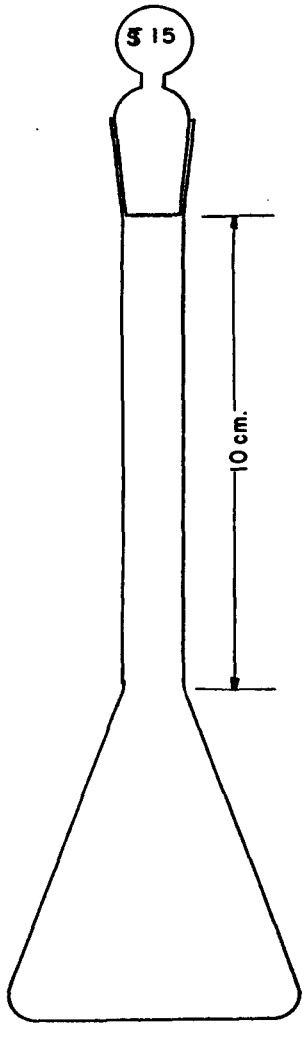

FIG. 1. REACTION FLASK.

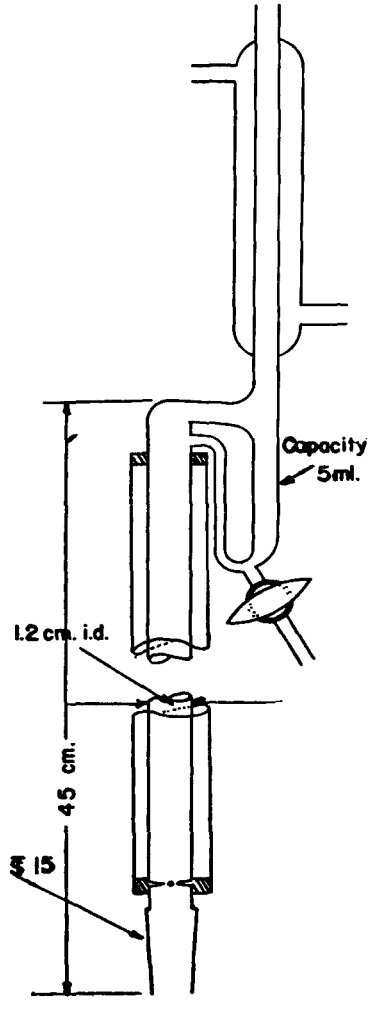

FIG. 2. DISTILLATION UNIT.

0.1 *N* alkali is added and also a few boiling stones. The flask is attached to the regular Allen-Marquardt distillation unit and 50 ml. is distilled into a small separatory funnel (125 ml. capacity). The distillation is stopped at this point, 25 ml. of distilled water is added to the Erlenmeyer flask, and the distillation is continued until

the total volume of distillate is 75 ml. By this procedure the alcoholic concentration and the volume of distillate are the same in all instances.

To the distillate, in the separatory funnel, is now added 14 grams of NaCl and the funnel is shaken for 1 minute. The saturated aqueous solution is extracted successively with 40, 30, 20, and 10 ml. portions of CCl_4, and shaken 1 minute on each addition of extractant. The CCl_4 extract is collected in the reaction flask (Figure 1), to which have been added a few pieces of carborundum to ensure even boiling. The reaction flask is attached to the still (Figure 2), and 50 ml. of distillate is collected by allowing the still to reflux for 5 minutes before removing the first fraction and then removing nine more consecutive fractions at 5 minute intervals. The reaction flask is allowed to cool 1 minute, removed while still warm, loosely stoppered, and cooled for 3–5 minutes in an ice bath. To the flask are now added from precision pipets, 10 ml. of pyridine solution and 20 ml. of acetyl chloride solution. During the pipetting procedure the respective reagents are introduced well down in the flask.

Immediately following the addition of the acetyl chloride solution the flask should be tightly stoppered. (It is advisable to put a very small quantity of lubricant on the stopper, not enough, however, to allow the stopper to blow out of the flask during the following heating period.) The stoppered flask is then shaken and placed in a water bath[3] kept at 60° C. The flask is allowed to remain in the bath for 30 minutes, with shaking every 5 minutes. It is then placed in an ice bath for 5 minutes, after which 25 ml. of water is added, the neck of the flask being washed down during the addition. The contents of the flask are transferred with washing to a 500 ml. Erlenmeyer and 100 ml. of 0.100 *N* alkali added from a standard 100 ml. pipet. The contents are thoroughly shaken and then carefully backtitrated with standard 0.100 *N* H_2SO_4, phenolphthalein being used as the indicator.

A blank should be run with each set of experiments and the alcoholic hydroxyl groups in the sample estimated as the difference in alkali used in the sample and in the blank. 1 ml. of 0.1 *N* NaOH is equivalent to 0.0001 mole (0.0088 gram) of fusel oil, or 17.6 grams of fusel oil per 100,000, calculated as amyl alcohol.

In Table 1 is given a comparison of the results obtained by the two methods on samples of whisky, Scotch, and brandy.

TABLE 1.—*Results of analysis of whisky, Scotch, and brandy*

LAB. NO.	PROOF	FUSEL OIL CALCULATED TO PROOF		DIFFERENCE (ACETYL-OFFICIAL)
		OFFICIAL METHOD	ACETYL CHLORIDE METHOD	
		I. WHISKY		
95748	117.4	284	246	−38
81125	101.5	145	145	0
96372	102.6	115	96	−19
98022	107.5	142	136	−6
99715	109.5	166	166	0
99716	109.3	165	150	−15
90881	113.7	262	243	−19
59106	102.8	120	122	+2
89706	103.6	116	113	−3
94313	112.0	203	218	+15

[3] Smith and Bryant, *J. Am. Chem. Soc.*, 57, 61 (1935).

TABLE 1—*Continued*

LAB. NO.	PROOF	FUSEL OIL CALCULATED TO PROOF		DIFFERENCE (ACETYL-OFFICIAL)
		OFFICIAL METHOD	ACETYL CHLORIDE METHOD	
		I. WHISKY—*Continued*		
52904	102.0	184	177	−7
55726	103.8	170	171	+1
91353	109.6	132	122	−10
87297	113.4	169	170	+1
70580	100.2	181	188	+7
98020	104.4	174	171	−3
96990	100.9	130	138	+8
96991	100.9	135	138	+3
96992	101.3	147	149	+2
96993	101.3	141	130	−11
98251	106.2	169	167	−2
84687	112.5	185	157	−28
90881	113.7	262	250	−12
96043	110.1	162	141	−21
96044	110.3	161	141	−20
		II. SCOTCH		
98698	125.5	37	19	−22
98118	87.6	100	78	−22
134	87.3	58	36	−22
86788	86.5	47	35	−12
95674	87.0	63	46	−17
128	86.2	59	40	−19
113	86.2	98	71	−27
114	84.7	71	50	−21
116	86.7	134	102	−32
119	87.3	77	55	−22
139	88.3	130	82	−48
		III. BRANDY		
83930	101.4	85	95	+10
83523	151.6	127	147	+20
83921	173.6	127	125	−2
83938	107.0	184	196	+12
84030	109.2	229	89	−140
85244	172.0	174	152	−22
83929	101.6	64	69	+5
84014	102.4	111	81	−30
84026	107.4	59	54	−5
84047	103.4	92	89	−3
85979	102.6	84	85	+1
89517	103.2	136	128	−8

In the majority of instances, the results obtained by the two methods on whisky are in good agreement. Usually when check results were not obtained, the values for fusel oil by the official method were high. A possible explanation is that the fusel oil in these samples contained a large portion of n-propyl alcohol or isobutyl alcohols, which contribute to the value by the official method, but are removed during the dealcoholization in the acetyl chloride procedure. Likewise, the excellent agreement obtaining in the majority of cases can be attributed to the fact that the fusel oil consists mainly of the higher boiling amyl alcohols and only a small percentage of the lower boiling alcohols such as n-propyl and isobutyl.

In every case the values obtained by the acetyl chloride method on Scotch are much lower than those obtained by the official method. The constancy of the variation between the results obtained by the two methods makes it possible to correlate the evaluation by means of a correction factor. The differences between the values are attributed again to the presence of large percentages of either n-propyl and isobutyl alcohol, or of both.

Again, as with the samples of whiskies, the differences between the results by the two methods on brandy are not constant and can not be correlated by means of a correction factor.

From the results obtained by the acetyl chloride procedure it may be assumed that the values are dependent mainly on alcohols having boiling points above that of isobutyl. Since the concentrations of alcohols boiling above n-amyl are negligible, it may be postulated further that the acetyl chloride procedure gives a relatively true quantitative measure of the concentration of the n-butyl and the isomeric amyl alcohols occurring in the so-called fusel oil fraction.

If it is true that the taste intensity factor of an alcohol increases with increase in molecular weight and also with branching in the molecule, it may also be true that the taste characteristics of a distilled spirit due to the fusel oil is entirely dependent on the concentration of the amyl alcohols. The low-taste-intensity factor of alcohols, such as n-propyl, iso, and n-butyl, although present in appreciable concentrations, would add very little to the taste characteristics of the spirit. Likewise, although the isomeric hexanols and heptanols might have a high taste intensity factor, their low concentrations would prohibit them from adding greatly to the fusel-oil taste characteristics.

Thus, the fusel oil, or more correctly the amyl alcohol value, obtained by the acetyl chloride procedure, probably gives a true picture of the concentration of those alcohols that are responsible for the characteristic and essential taste and bouquet of distilled spirits. However, it is necessary to investigate further to be sure that the above assumptions and postulations are true.

From the results obtained, it may be assumed that in the majority of

instances, especially in the analysis of whiskies and brandies, either method could be used to obtain results that would indicate a true value for fusel oil content. Although the time required to complete an analysis by the acetyl chloride procedure is only 3 hours as compared to 12 to 14 for the official method, the actual man hours of work is approximately the same for both methods. The time requirement of the official method could be shortened somewhat by elimination of the saponification step. This was verified by a set of experiments with samples of whisky, brandy, and neutral spirits. In all the determinations no esters were recovered in the distillate by the regular Allen-Marquardt procedure when a mixture of 50 ml. of sample plus 30 ml. of 0.100 *N* alkali was distilled immediately.

Although the actual time required for a determination by the acetyl chloride procedure is less than that of the official method, a higher degree of technic in manipulation is required by the former method to produce constant and check results.

DECOMPOSITION OF DOLOMITIC LIMESTONE IN FERTILIZERS

By E. R. COLLINS and F. R. SPEER (Agronomy Department, North Carolina Experiment Station, Raleigh, N. C.)

The recent trend in the manufacture of mixed fertilizers has been toward sources of nitrogen that leave an acid residue in the soil. At the same time the more concentrated and purer materials that have been used permit only relatively small amounts of the secondary nutrients as impurities. During the transition period, fertilizers were generally sold on the basis of their content of nitrogen, phosphoric acid, and potash, and with little regard to the need for other essential elements or secondary effects on the soil. For the last few years manufacturers have neutralized the potential acidity of fertilizers with lime. The inclusion of such neutralizing materials as dolomitic limestone, which contains considerable magnesium, raised a question as to the availability of this magnesium as a nutrient to the plant.

The experiments reported in this paper constitute part of a coordinated program of study to determine the availability of magnesium in dolomitic limestone of different degrees of fineness when used in amounts calculated to make the fertilizer non-acid forming.

LITERATURE CITATIONS

Morgan and Salter (10), Ames and Schollenberger (1), White (16), MacIntire and Shaw (9), Steward and Wyatt (11), and Hartwell and Damon (6), working under a wide range of conditions, all came to the

general conclusion that the finer limestone particles are more rapidly decomposed in the soil than are the coarser particles.

Taylor and Pierre (13), working with a physiologically neutral 6–8–4 fertilizer, concluded (1) that the two important factors that determine the rate of decomposition are fineness of division of the limestone and acidity of the soil; (2) that a very good relationship exists between decomposition and the *p*H of the soil in the fertilized zone; and (3) that the coarser grades of dolomitic limestone are not very effective in neutralizing the acidity due to an acid-forming fertilizer. Taylor and Pierre (14) substantiated these conclusions by water-soluble magnesium tests.

Carolus and Brown (2) found that potatoes obtained an adequate supply of magnesium from dolomitic limestone included in a physiologically neutral fertilizer.

Cook and Connor (4) compared relative values of different agents used to neutralize acid fertilizers and found that the finely ground dolomite was superior to the coarsely ground material. Hester and Zimmerly (8) report that dolomitic material should be of at least 100-mesh fineness to be effective in maintaining soil reaction.

Dawson, Snyder, Leighty, and Reid (5) and Collins and Speer (3), in preliminary reports of work with a physiologically neutral fertilizer, found that the change in *p*H resulting from the inclusion of the dolomitic separates varied with the fineness of division. The residual carbonate data were generally consistent with the treatments, soil characteristics, and *p*H data in that greater decomposition took place with the finer particles.

Although considerable work has been done on decomposition of limestone, the value of dolomitic supplements to mixed fertilizers as an adequate source of magnesium for the immediate crop has not been fully established.

EXPERIMENTAL METHODS

A. General Procedure.—The experiments reported in this paper comprise greenhouse pot experiments in which the dolomitic materials are applied to the soil as the neutralizing material of a complete fertilizer and allowed to react with the soil in the presence of a growing crop under conditions simulating those in the field.

Nineteen treatments, as presented in Table 1, were used in duplicate on five soils comprising four soil types. All of the fertilizer used, with the exception of treatment 2, carried acid-forming sources of nitrogen and differed only in regard to the amount and kind of dolomitic limestones or of magnesium sulfate included. The soils selected were a Dunbar very fine sandy loam, Ruston sandy loam, Norfolk fine sandy loam, and two Portsmouth fine sandy loams.

The general procedure was essentially the same as that used by Taylor and Pierre (13), a description of which follows.

TABLE 1.—*Treatment for each soil type studied*

TREATMENT	FORMULA	DESCRIPTION	SUPERPHOSPHATE, 20.65% P_2O_5	AMMONIA, 3% OF SUPERPHOSPHATE	AMMONIUM SULFATE C.P., 21.2% N	POTASSIUM CHLORIDE C.P., 63.1% K_2O	POWDERED QUARTZ	MAGNESIUM MATERIAL TO BE ADDED WHEN MgO CONTENT= 19.30% DOLOMITE-A	WEIGHT OF SPECIFIED MATERIALS TO ADD PER 100 GRAMS OF OTHER COMPONENTS OF MIXTURE[1]
			lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	
1	0-0-0	No treatment							
2	0-8-6	Without Mg or N	775	—	—	190	1035	—	
3	6-8-6	Without Mg	775	24	472	190	539	—	
4	6-8-6	Hydrated dolomite ⅓ full rate	775	24	472	190	—	180	7.83
5	6-8-6	Hydrated dolomite ⅔ full rate	775	24	472	190	—	360	15.66
6	6-8-6	Hydrated dolomite full rate	775	24	472	190	—	539	23.49
7	6-8-6	Dolomite A, 20–40-mesh	775	24	472	190	—	539	36.74
8	6-8-6	Dolomite A, 40–60-mesh	775	24	472	190	—	539	36.93
9	6-8-6	Dolomite A, 60–80-mesh	775	24	472	190	—	539	36.95
10	6-8-6	Dolomite A, 80–100-mesh	775	24	472	190	—	539	36.93
11	6-8-6	Dolomite A, 100–200-mesh	775	24	472	190	—	539	36.66
12	6-8-6	Dolomite A, through 200-mesh	775	24	472	190	—	539	37.16
13	6-8-6	Selectively calcined dolomite	775	24	472	190	—	539	27.54
14	6-8-6	$MgSO_4$ in No. 4 to Mg	775	24	472	190	—	180	13.94
15	6-8-6	$MgSO_4$ to Mg in No. 5	775	24	472	190	—	360	27.88
16	6-8-6	Composite Dolomite A	775	24	472	190	—	539	36.76
17	6-8-6	Composite Dolomite B	775	24	472	190	—	539	42.17
18	6-8-6	Composite Dolomite C	775	24	472	190	—	539	33.58
19	6-8-6	$MgSO_4$ to Mg in No. 6	775	24	472	190	—	539	41.82

[1] The magnesium components of the 6-8-6 mixture were kept separate from the rest of the mixture. The quantities of the different magnesium material required to supplement the mixture to form a physiologically neutral 6-8-6 mixed fertiliser, or its equivalent, are given in this column.

After thoroughly mixing the bulk of the soil, equal quantities of air-dry soil from each soil type were potted in 2 gallon pots in the usual manner. One-tenth of the soil in each pot was removed and mixed with a quantity of fertilizer calculated to be equivalent to 1333 pounds per acre (on the basis of all the soil in the pot). A metal cylinder, which enclosed exactly one-tenth of the area of the inside of the pot, was placed in the center of the empty pot, and the fertilized soil was placed inside and gently packed by jolting the pot up and down. The untreated soil was then placed in the pot on the outside of the metal cylinder and settled in a similar manner. A circular metal collar protected with asphaltum paint, 2 inches in height and about 3 inches in diameter, was placed over the end of the cylinder and the cylinder was removed, leaving the circular collar to mark the fertilized zone.

Cotton was planted in the area outside the fertilized zone, water was added on alternate days, and the soil was kept at optimum moisture content by bringing to constant weight at 15 day intervals. The plants were removed at the end of 65–75 days and dried for analysis. Soil samples were removed from the center of the fertilized zone by means of a circular cylinder that was about one-half of the diameter of the zone of fertilized soil.

A portion of each sample was leached for the determination of soil reaction, and the remainder was air-dried for the residual carbonate determination by the Schollenberger method (12).

The quinhydrone electrode was used to determine the soil reaction values of the leached samples according to the method of Volk and Truog (15). The soil was leached in order to simulate the equilibrium *p*H values that would result in the field under leaching conditions.

Analyses of plants for calcium and magnesium were by conventional methods (17).

Supplementary tests were made at shorter intervals after application of the fertilizer mixture to the soil to indicate the rate at which magnesium was being liberated to meet the immediate needs of the growing crop. These tests were carried out in duplicate on a Dunbar fine sandy loam of *p*H 5.5. The soil, fertilized at the rate used in the preceding experiments, was placed in water-proof quart containers and maintained at an optimum moisture with tap water. Samples were taken at 10 and 23 day intervals for *p*H, residual carbonate, and calcium and magnesium determinations.

Soil reaction values were obtained on the unleached samples with a glass electrode, and the residual carbonate data were obtained according to the simplified method of Schollenberger.

Changes in quantities of magnesium in the soil were determined by Hester's (7) rapid test method, which is used as a measure of the available forms.

B. Testing of Methods.—A review of the literature failed to reveal a method for determining residual carbonates that combined accuracy and sufficient speed for routine determinations. The method of Schollenberger (12) appeared to be the most satisfactory of those available and, therefore,

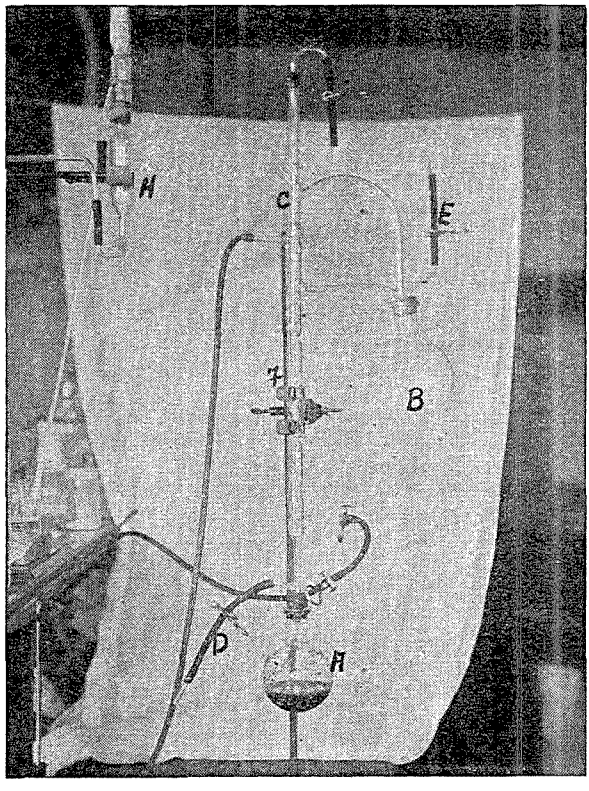

FIG. 1.—APPARATUS USED FOR RESIDUAL CARBONATE DETERMINATIONS.
A. Flask for decomposition of carbonates.
B. Flask for barium hydroxide solution.
C. Manometer.
D. Opening for exhausting air and introducing HCl–$FeCl_2$ mixture
F. Condenser.
H. Automatic buret.

was adapted to this work. The good features were retained and the ease of manipulation appreciably increased by constructing the apparatus as a single compact unit with rubber connections largely eliminated. The picture and description of the apparatus used (Figure 1) illustrate the compactness and simplicity of operation of the unit.

The analytical procedure used in these determinations is essentially the same as that followed by Schollenberger and the general description is as follows: 20 grams of soil is placed in A and the system exhausted with a vacuum pump until the manometer contained in the upper part of the condenser registers approximately 2 cm. of mercury. D is closed with a pinch clamp, the entire apparatus is moved so that the delivery tip of automatic pipet H may be inserted in E, and 50 ml. of barium hydroxide is introduced. The system is again exhausted and the intro-

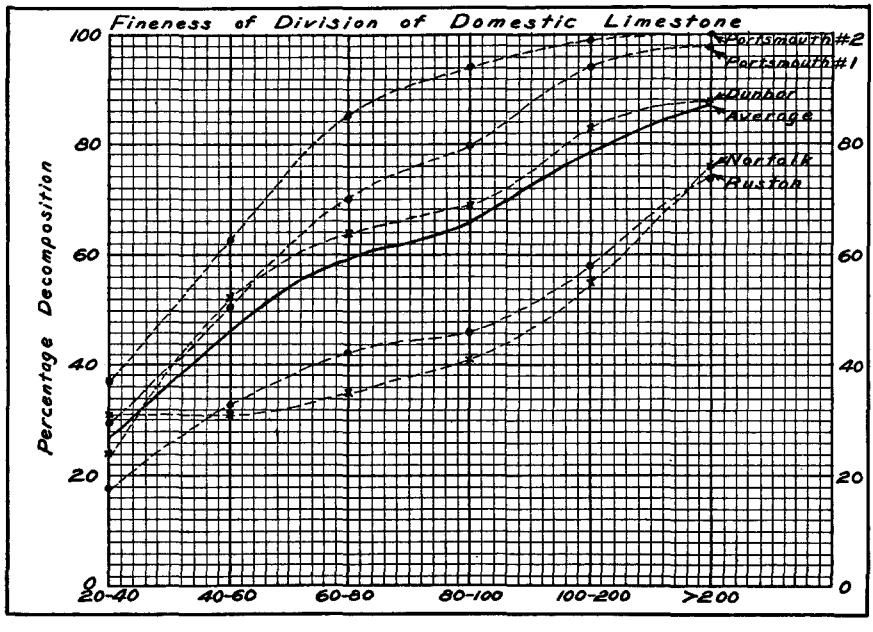

FIG. 2.—PERCENTAGE DECOMPOSITION OF DOLOMITIC LIMESTONE OF DIFFERENT DEGREES OF FINENESS.

duction of 60 ml. of hydrochloric acid (1+9) and 5 ml. of ferrous chloride solution is effected through D. A piece of glass tubing is inserted in D and the HCl–$FeCl_2$ mixture is easily drawn in because of the reduced pressure. The suspension is heated with a micro-burner (boiling occurs readily below 30° C.), and recovery of all carbon dioxide from soil containing dolomitic limestone can usually be effected in 30–50 minutes. As long as decomposition of the carbonate continues, the mixture boils quietly, but when evolution of the gas has ceased, the mixture bumps violently, which indicates that digestion is complete. The vacuum is released by drawing carbon dioxide-free air in through D. B is removed,

TABLE 2.—*Percentage decomposition of dolomitic limestone when used as a neutralizing agent in an acid-forming fertilizer*

		DUNBAR VERY FINE SANDY LOAM	RUSTON SANDY LOAM	NORFOLK FINE SANDY LOAM	PORTSMOUTH NO. 1 FINE SANDY LOAM	PORTSMOUTH NO. 2 FINE SANDY LOAM	AVERAGE
Original *p*H of the respective samples		5.45	5.60	5.42	5.18	4.50	
Time of contact with soil (days)		75	75	65	72	78	
PLAT NO.	TREATMENT	PER CENT DECOMPOSITION					
7	6-8-6+Dolomite A, 20–40-mesh	22.4	17.8	30.4	29.3	37.4	27.5
8	6-8-6+Dolomite A, 40–60-mesh	52.2	33.1	30.4	51.5	63.3	46.1
9	6-8-6+Dolomite A, 60–80-mesh	64.0	42.7	35.4	70.7	87.4	60.0
10	6-8-6+Dolomite A, 80–100-mesh	67.7	45.9	41.8	79.9	94.0	65.9
11	6-8-6+Dolomite A, 100–200-mesh	83.2	58.0	55.7	94.5	99.4	78.2
12	6-8-6+Dolomite A, through 200-mesh	87.6	75.2	76.0	97.6	100.0	87.3
16	6-8-6+Composite A	59.0	45.9	36.7	70.7	80.1	58.5
17	6-8-6+Composite B	70.1	53.3	44.3	78.1	83.9	65.9
18	6-8-6+Composite C	55.9	38.9	37.3	62.8	79.5	54.9
	Per cent $CaCO_3$ in check soil	0.0	0.006	0.008	0.0015	0.0007	

TABLE 3.—*The pH of leached soil after contact with treatments shown for the period indicated*

PLAT NO.	TREATMENT		DUNBAR VERY FINE SANDY LOAM		RUSTON SANDY LOAM		NORFOLK FINE SANDY LOAM		PORTSMOUTH NO. 1 FINE SANDY LOAM		PORTSMOUTH NO. 2 FINE SANDY LOAM	
	FERTILIZER	MAGNESIUM CARRIER	1	2	1	2	1	2	1	2	1	2
1	None	None	5.45	5.33	5.77	5.67	5.37	5.37	4.86	4.92	4.57	4.57
2	0-8-6	None	5.29	5.23	5.43	5.43	5.24	5.20	4.93	4.87	4.43	4.43
3	6-8-6	None	4.84	4.77	5.29	5.26	4.99	5.03	4.84	4.86	4.29	4.29
4	6-8-6	⅓ rate hydrated dolomite	4.92	4.99	5.29	5.45	5.13	5.03	4.99	5.07	4.35	4.27
5	6-8-6	⅔ rate hydrated dolomite	5.14	5.07	5.60	5.63	5.17	5.29	5.03	5.04	4.33	4.29
6	6-8-6	Full rate hydrated dolomite	5.21	5.28	5.80	5.87	5.65	5.72	5.27	5.20	4.46	4.46
7	6-8-6	Dolomite A, 20–40-mesh	4.87	4.92	5.51	5.51	5.13	5.13	4.88	4.82	4.57	4.61
8	6-8-6	Dolomite A, 40–60-mesh	5.02	4.97	5.63	5.63	5.15	5.14	5.01	4.98	4.58	4.60
9	6-8-6	Dolomite A, 60–80-mesh	5.06	5.12	5.82	5.82	5.25	5.23	5.04	5.06	4.62	4.67
10	6-8-6	Dolomite A, 80–100-mesh	5.16	5.16	5.80	5.80	5.22	5.33	5.14	5.23	4.65	4.64
11	6-8-6	Dolomite A, 100–200-mesh	5.26	5.23	5.99	6.07	5.33	5.31	5.25	5.26	4.62	4.68
12	6-8-6	Dolomite through 200-mesh	5.29	5.41	6.03	6.17	5.53	5.57	5.29	5.22	4.75	4.68
13	6-8-6	Selectively calcined dolomite	5.50	5.51	6.51	6.38	5.73	6.00	5.24	5.18	4.57	4.57
14	6-8-6	$MgSO_4$=to Mg in No. 4	5.02	5.02	5.63	5.33	5.47	5.28	4.69	4.65	4.29	4.29
15	6-8-6	$MgSO_4$=to Mg in No. 5	4.84	4.84	5.33	5.43	5.11	5.20	4.55	4.56	4.33	4.25
16	6-8-6	Composite dolomite A*	5.12	5.12	5.43	5.39	5.38	5.32	4.91	4.95	4.43	4.43
17	6-8-6	Composite dolomite B	5.23	5.23	5.56	5.70	5.38	5.51	5.01	5.00	4.45	4.45
18	6-8-6	Composite dolomite C	5.02	5.06	5.53	5.53	5.21	5.25	5.17	4.87	4.40	4.30
19	6-8-6	$MgSO_4$=to Mg in No. 6	4.79	4.79	5.14	5.14	5.15	5.13	4.63	4.58	4.23	4.25
	Original *p*H of soils		5.45		5.60		5.42		5.18		4.50	
	Time of contact with soil (days)		75		75		65		72		78	

* Composite dolomites A, B, and C represent material from different quarries, and each one was composed of equal parts of 20–40, 40–60, 60–80, 80–100, 100–200, and through 200-mesh dolomite.

a one-hole stopper is inserted, and the delivery tip of a buret is inserted in the hole. The excess barium hydroxide is titrated with 0.1 *N* hydrochloric acid, phenolphthalein being used as the indicator. The buret reading is deducted from that of a blank determination conducted in the same way except that no sample is included, and the quantity of carbon dioxide absorbed is calculated from the following formula: (blank − buret reading) × normality of HCl × 0.022 = wt. CO_2 absorbed.

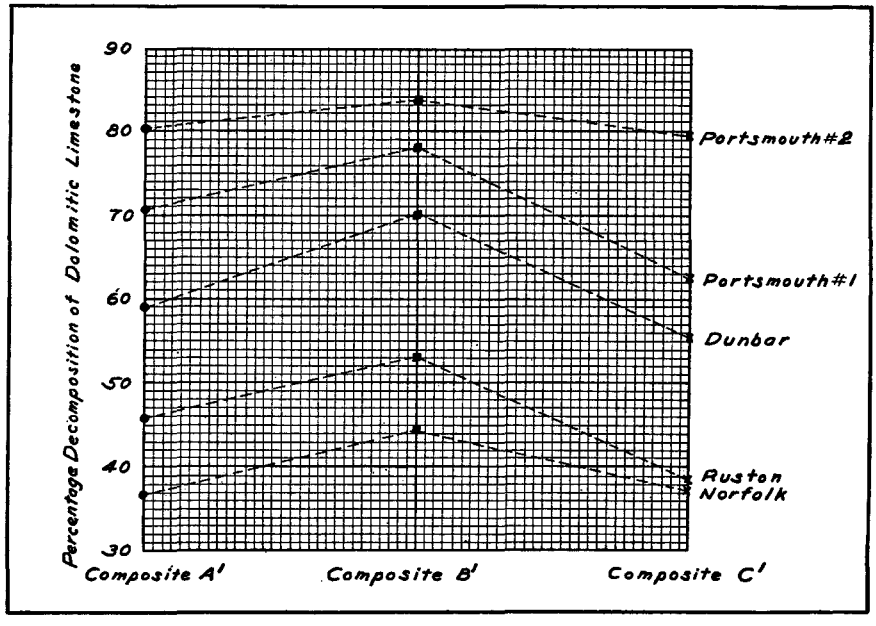

FIG. 3.—PERCENTAGE DECOMPOSITION OF COMPOSITE DOLOMITES A, B, AND C.

RESULTS

The percentage decomposition of the dolomitic limestone in contact with the five soils of the acidity given and for the periods of time indicated, as calculated from the residual carbonate determinations, is shown in Table 2. These results for the particle sizes are depicted graphically in Figure 2 and for the composite dolomitic limestones in Figure 3. These values take into account the quantity of residual carbonates found in treatment 3 (Table 1), which received the same 6–8–6 fertilizer but no neutralizing supplement.

The *p*H of the leached soil after contact with the fertilizer treatments shown for the period of time indicated is given in Table 3. The relative

TABLE 4.—*Magnesium content of cotton crop*

TREATMENT NO.	TREATMENT		DUNBAR		NOROFOLK		PORTSMOUTH NO. 1		RUSTON		PORTSMOUTH NO. 2	
	FERTILIZER	MAGNESIUM CARRIER	MgO	CaO/MgO RATIO	MgO	CaO/MgO RATIO	MgO	CaO/MgO RATIO	MgO	CaO/MgO RATIO	MgO	CaO/MgO RATIO
			per cent		*per cent*		*per cent*		*per cent*		*per cent*	
1	None	None	1.39	1.89	0.78	3.45	0.89	2.15	0.52	3.12	0.74	2.99
2	0-8-6	None	0.94	2.18	0.62	3.69	0.93	2.51	0.48	3.52	0.71	3.06
3	6-8-6	None	0.97	2.49	0.63	3.81	1.07	2.09	0.44	4.14	0.82	3.02
4	6-8-6	⅓ rate hydrated dolomite	1.14	2.12	0.76	3.05	1.09	2.08	0.55	2.71	0.84	2.56
5	6-8-6	⅔ rate hydrated dolomite	1.18	1.96	0.80	2.80	1.16	1.90	0.67	2.27	0.82	2.49
6	6-8-6	Full rate hydrated dolomite	1.18	1.94	0.85	2.71	1.14	2.20	0.66	2.08	0.88	2.44
7	6-8-6	20–40-mesh dolomite	1.16	2.12	0.67	3.40	1.08	2.40	0.42	3.86	0.82	2.66
8	6-8-6	40–60-mesh dolomite	1.12	2.33	0.70	3.20	1.00	2.16	0.46	3.15	0.97	2.65
9	6-8-6	60–80-mesh dolomite	1.06	2.38	0.80	3.26	1.07	2.21	0.42	4.10	0.86	2.70
10	6-8-6	80–100-mesh dolomite	1.20	2.00	0.79	2.99	1.22	1.99	0.57	2.63	0.90	2.33
11	6-8-6	100–200-mesh dolomite	1.25	1.83	0.80	2.40	1.30	2.22	0.53	2.85	0.83	2.37
12	6-8-6	Through 200-mesh dolomite	1.30	1.85	0.76	2.58	1.28	1.87	0.69	2.25	0.76	2.37
13	6-8-6	Selectively calcined dolomite	1.30	1.81	0.91	2.43	1.34	2.06	0.64	2.30	0.64	2.89
14	6-8-6	$MgSO_4$ = to Mg in No. 4	1.08	2.17	0.75	2.65	1.06	2.55	0.56	2.73	0.77	2.71
15	6-8-6	$MgSO_4$ = to Mg in No. 5	1.20	1.83	0.83	2.77	1.08	2.16	0.51	3.71	0.90	2.57
16	6-8-6	Composite dolomite A	1.30	2.01	0.74	2.91	1.19	2.03	0.67	2.69	0.84	2.45
17	6-8-6	Composite dolomite B	1.33	1.79	0.78	3.45	1.35	2.03	0.72	2.25	0.78	2.51
18	6-8-6	Composite dolomite C	1.25	2.22	0.64	3.56	1.14	2.09	0.66	2.83	0.84	2.50
19	6-8-6	$MgSO_4$ = to Mg in No. 6	1.56	1.79	0.94	2.94	1.18	1.93	0.86	1.81	0.98	2.08

effect of the dolomitic separates and composite dolomitic limestone A, B, and C on the soil reaction is shown in Figures 4 and 5, respectively.

The concentration of magnesium and the CaO/MgO ratios found existing in the cotton plants are presented in Table 4. Dolomitic decomposition as reflected in magnesium uptake by the plant is depicted graphically in Figure 6.

The foregoing results deal with the reaction and residual carbonates at the end of the normal growing season. The question as to whether de-

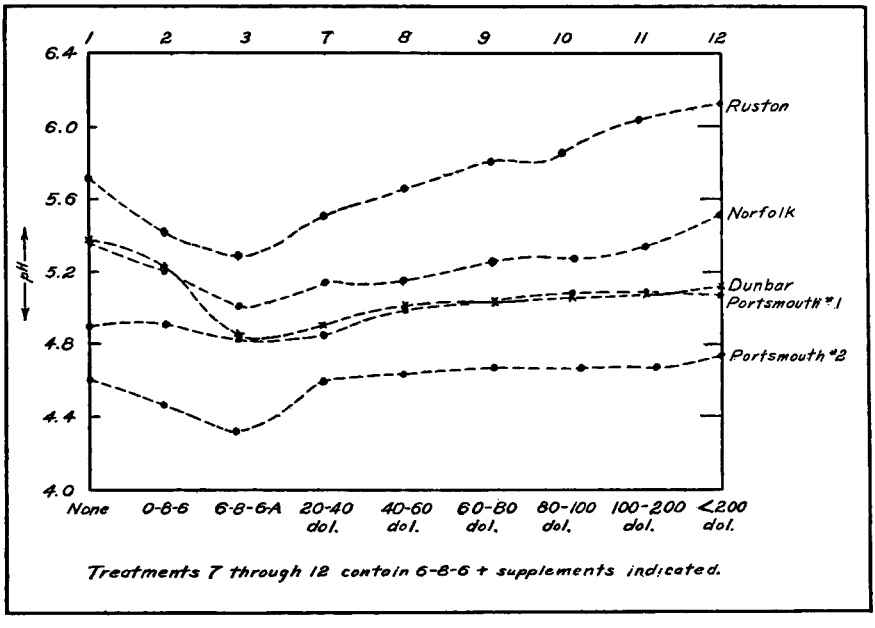

FIG. 4.—RELATIVE EFFECT ON *p*H OF DOLOMITIC LIMESTONE OF DIFFERENT DEGREES OF FINENESS

composition takes place gradually or rapidly at first and then at a slower rate would make considerable difference in the quantity of magnesium available during the early growing season. With this in mind, the writers set up a series of small jars and applied the different fertilizers to the entire soil at the rate used in the center core of soil in the previous experiments. One set of samples was taken for determination of soil reaction, available magnesium, and percentage decomposition at the end of 10 days, and the other set after a period of 23 days. The results are presented in Table 5.

TABLE 5.—*Available magnesium, pH and decomposition of carbonate after the reaction periods indicated for the materials shown*

PLAT NO.	FERTILIZER	MAGNESIUM CARRIER	MAGNESIUM IN FERTILIZER ZONE		pH		DECOMPOSITION IN CARBONATES (%)		
			10 DAYS	23 DAYS	10 DAYS	23 DAYS	10 DAYS	23 DAYS	75 DAYS
			p.p.m.	*p.p.m.*					
3	6-8-6	None	0	0	4.78	4.81	—	—	—
6	6-8-6	Full rate hydrated dolomite	100	100	6.26	6.26	—	—	—
7	6-8-6	Dolomite A, 20–40-mesh	15	15	5.32	5.25	4.1	8.1	22.4
8	6-8-6	Dolomite A, 40–60-mesh	15	45	5.33	5.36	7.5	15.5	52.2
9	6-8-6	Dolomite A, 60–80-mesh	45	75	5.73	5.60	10.0	21.1	64.0
10	6-8-6	Dolomite A, 80–100-mesh	75	100	5.81	5.83	12.7	24.9	67.7
11	6-8-6	Dolomite A, 100–200-mesh	75	100	5.84	6.01	14.6	28.6	83.2
12	6-8-6	Dolomite A, through 200-mesh	100	100	6.27	6.29	16.1	31.6	87.6
13	6-8-6	Selectively calcined dolomite	100	100	6.61	6.50	—	—	—
16	6-8-6	Composite dolomite A	100	100	5.78	5.76	11.5	23.2	59.0
17	6-8-6	Composite dolomite B	100	100	5.93	6.08	13.2	26.1	70.1
18	6-8-6	Composite dolomite C	45	75	5.62	5.62	10.8	21.8	55.9

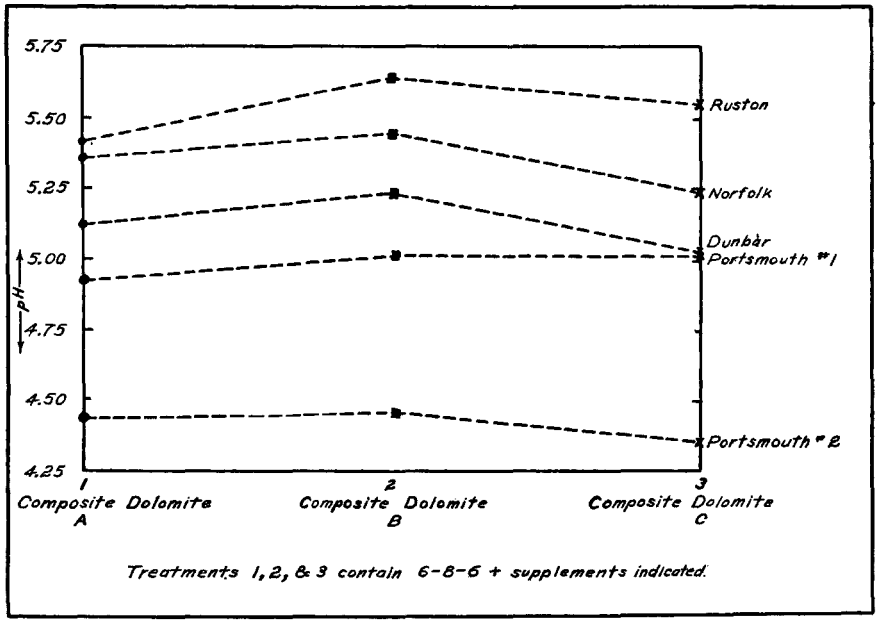

FIG. 5.—RELATIVE EFFECT ON *p*H OF COMPOSITE DOLOMITES A, B, AND C.

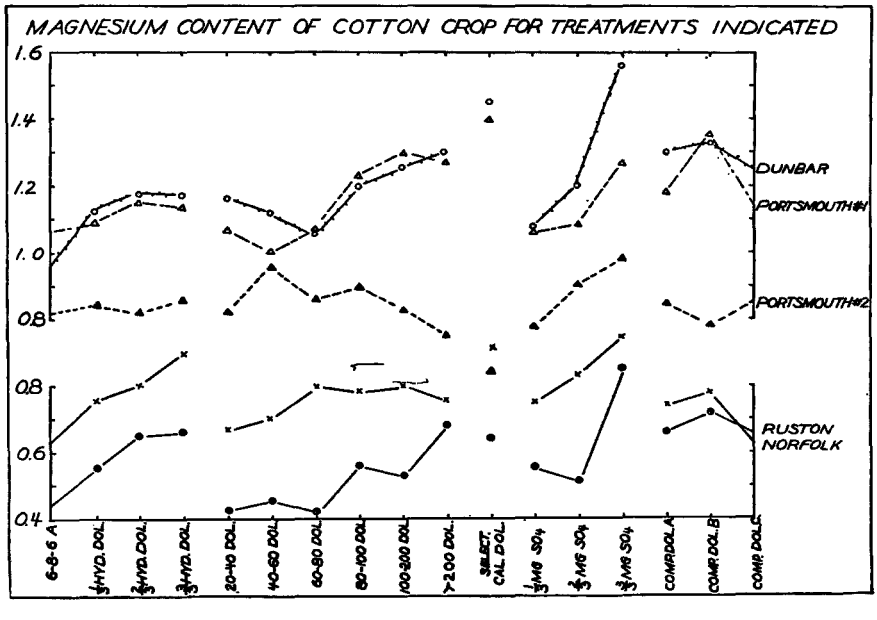

FIG. 6.—CONCENTRATION OF MAGNESIUM IN COTTON PLANTS.

The percentage decomposition is shown graphically in Figure 7 for the 10, 23, and 75 day periods of contact with the soil.

Available magnesium was determined by Hester's rapid test method (7), and the figures presented (Table 5) are for p.p.m. of magnesium in the fertilized zone.

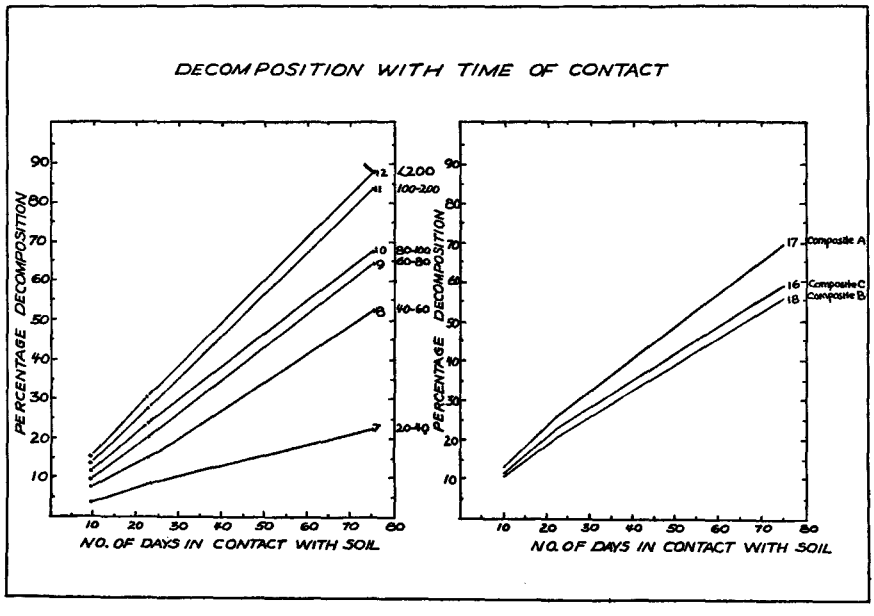

FIG. 7

DISCUSSION

The good correlation between particle size and percentage decomposition is illustrated in Figure 2. On all five soils studied, an increase in the fineness of division of the dolomitic supplements is accompanied by a greater degree of decomposition. The Portsmouth soils of higher organic matter content, greater buffer capacity, and lower *p*H values show a marked increase in decomposition of all the dolomitic fractions over that of the more sandy and less acid Norfolk and Ruston soils. Differences in the relative decomposition rates found are apparently due to differences in soil characteristics.

On the whole, the decomposition of the dolomitic separates is appreciable and should be accompanied by the liberation of considerable magnesium during the growing season.

Differences in rates of decomposition of the composite dolomitic limestones from different quarries are obvious from Figure 3. Composite B

shows the greatest decomposition, with composite A intermediate between B and C. This would indicate that dolomitic limestones, of equal fineness, from different quarries, differ in their rate of reaction with the same soils under these conditions.

The results in Figure 4 show that in general the change in soil reaction produced by the dolomitic separates increases with the degree of fineness of grinding. The differences in the effect of the composite dolomitic limestones from different quarries on the *p*H of the soil are shown in Figure 5.

As shown in Table 4 and Figure 6, the magnesium content of the plant is higher in all cases where $\frac{1}{3}$, $\frac{2}{3}$, and full rates of hydrated dolomitic supplements were added, with the possible exception of the No. 2 Portsmouth soil. The general tendency was for the magnesium content of the plant to increase as the particle sizes of the dolomitic supplements decreased, except with the No. 2 Portsmouth soil. Plants growing in the pots receiving fertilizer supplemented with selectively calcined dolomite contained quantities of magnesium that compared favorably with the finer particle dolomitic limestone supplements, except on the No. 2 Portsmouth soil. Hydrated, calcined, composite, and 80-mesh and finer dolomitic limestones are about equally effective in supplying magnesium, as indicated by uptake data. They are not so effective as magnesium sulfate but compare very favorably with the results obtained for magnesium concentration in the plant receiving the acid-forming fertilizer.

The results in Table 5 and Figure 7 show that there is no one period in which the rate of decomposition is materially greater than any other period as the decomposition-time curve approaches a straight line. The data on the water-soluble magnesium (in fertilizer zone, Table 5) show that no magnesium was found in the soil receiving the acid-forming fertilizer. However, the soil receiving the fertilizer with dolomitic supplements showed increasing quantities of magnesium as the particle size decreased and as the reaction period progressed.

SUMMARY AND CONCLUSIONS

Studies were made in the greenhouse to determine the extent of decomposition of dolomitic limestone of different degrees of fineness when used in quantities calculated to form a neutral fertilizer. These experiments comprised pot trials with Dunbar, Norfolk, Ruston, and Portsmouth soils under conditions simulating those in the field. The extent of decomposition during the course of a growing season of 65–75 days was evaluated by change in soil reaction, determination of residual carbonates, and magnesium uptake by the plant. Additional studies were conducted in which soil samples were removed at 10 and 23 day intervals and carbonate decomposition measured by change in soil reaction, determination of residual carbonates, and increase in magnesium extracted by Hester's method. The data presented indicate the following conclusions:

1. That *p*H and buffer capacity of the soil are the major soil factors involved in dolomitic limestone decomposition.
2. That determination of residual carbonates constitutes the most satisfactory index of carbonate decomposition.
3. That calcined, 80-mesh and finer, and composite dolomitic limestones of the quality used in these experiments should supply at least a large part of the magnesium needs of plants.

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ABSENCE OF REVERSION IN AMMONIATED AND LIMED SUPERPHOSPHATES OF LOW FLUORINE CONTENT*

By W. H. MACINTIRE and L. J. HARDIN (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

Loss of P_2O_5 availability in ammoniated and limed superphosphates is of academic interest and practical importance. The range of economic ammoniation and the role of calcium sulfate in reversion reactions have been dealt with in several contributions (6, 4, 3, 5). Differential behavior of limestone and dolomite in phosphatic mixtures has been reported in contributions from the Tennessee Experiment Station (8, 10, 12, 16), and by Beeson and Ross (2). Divergent behavior of additions of calcium

* A cooperative study conducted at The University of Tennessee Agricultural Experiment Station under the auspices of the Tennessee Valley Authority, Department of Chemical Engineering.

silicates and calcium silicate slag of high fluorine content also was determined (15, 17).

Ross, Jacob, and Beeson (19) stated, "The principal phosphatic component of heavily ammoniated superphosphates is hydrated tricalcium phosphate." From a study of the joint usage of dolomite and ammonia, Keenen and Morgan (7) concluded that resultant formation of phosphatic compounds less soluble than ordinary tricalcium phosphate is due solely to the ammonia. The present writers postulated that development of citrate insolubility in processed superphosphates is due to a true reversion of engendered tricalcium phosphate into fluorphosphate (15, 17, 14), rather than calcium hydroxyphosphate.

The objective of this study was to determine whether standard and triple superphosphates derived from defluorinated rock can be ammoniated or supplemented by ordinary liming materials without loss of P_2O_5 availability.

EXPERIMENTAL APPROACH

Experiments related to the immediate objective brought an explanation for P_2O_5 retrogradation in mixtures of calcined rock phosphate and commercial superphosphates (14). The experimental control was a special superphosphate of meager "I and A" content and almost devoid of fluorides (9). The standard and the concentrated superphosphates of the present study were made, however, by acidulation of raw and ignited rock phosphates, partly defluorinated and completely defluorinated fused brown rocks, and a commercial rock phosphate calcine of low fluorine content. These experimental superphosphates were comparable, therefore, to conventional superphosphates of widely variant fluorine content.

The P_2O_5 and fluorine contents of the starting phosphatic solids are given in Table 1. Acidulations were by either 65 per cent sulfuric or 78 per cent phosphoric acid, on basis of total calcium content of the starting phosphates. The P_2O_5 analyses of the superphosphates and their mixtures were by A.O.A.C. methods (1). Fluorine determinations were made by igniting analytical charges with admixed calcium peroxide (13) and then distilling with perchloric acid at 135° C., according to the method of Willard and Winter (20).

TABLE 1.—*Partial analyses of rock phosphates used in making the triple superphosphates*

MATERIAL	P_2O_5	FLUORINE[a]
	per cent	*per cent*
Tenn. brown rock phosphate	34.90	3.88
Tenn. brown rock phosphate, heated 1 hr. at 600° C.	35.20	3.90
Calcined rock phosphate	36.80	0.15
Fused Tenn. brown rock phosphate	29.80	0.67
Special fused brown rock phosphate	27.50	0.003
Florida rock phosphate	32.25	3.75

[a] Fluorine determinations of this table and of subsequent tables were made by J. W. Hammond.

TABLE 2.—*Influence of component fluorides of superphosphates upon development of citrate-insolubility following ammoniation and curing at 45° C. for one month*

(Results expressed in percentage.)

SUPERPHOSPHATES[a]			ANALYSES OF SUPERPHOSPHATES AND THEIR AMMONIATED PRODUCTS											
			INITIAL SUPERPHOSPHATES[a]					AMMONIATED SUPERPHOSPHATES[g]						
					P_2O_5					P_2O_5				
											WATER-SOLUBLE		CITRATE-INSOLUBLE	
DERIVED FROM—	BEHAVIOR OF PHOSPHATE WHEN ACIDULATED	CODE	MOISTURE LOST AT 100° C.	FLUORINE	TOTAL	WATER-SOLUBLE	CITRATE INSOLUBLE	FLUORINE	AMMONIA	TOTAL	FOUND	CORRECTED[h]	FOUND	CORRECTED[h]
Tenn. brown rock phosphate	Normal	663 669	11.52 4.47	1.53 1.33	19.20 51.20	18.00 49.50	*0.00* *0.00*	1.54 1.31	6.77 10.14	19.34 48.98	5.00 27.50	4.96 28.76	4.10 2.20	*4.07* *2.30*
Tenn. brown rock phosphate, heated 1 hr. at 600° C.	More reactive than unheated rock	664 670	12.24 2.36	1.49 1.35	20.00 52.00	17.50 48.75	*0.84* *0.82*	1.52 1.31	7.12 8.81	20.47 50.83	3.25 27.25	3.17 27.87	5.60 2.40	*5.47* *2.45*
Calcined rock phosphate[b]	Vigorously reactive. Both products dry after 5 min.	665 671	6.22 2.70	0.09 0.13	21.20 53.20	17.25 48.75	*0.64* *0.52*	0.08 0.12	5.93 8.06	20.40 51.66	2.50 24.25	2.59 25.00	0.65 0.40	*0.67* *0.41*
Fused Tenn. brown rock phosphate	Vigorously reactive with H_3PO_4. Product dry overnight	666 672	slurry[f] 6.02	— 0.24	— 45.20	— 41.25	— *0.92*	— 0.30	— 9.39	— 44.25	— 21.00	— 21.26	— 1.00	— *1.02*
Fused Tenn. brown rock phosphates[c]	Vigorously reactive. Product dry overnight[d]	667 673	6.79 1.80	0.02 0.10	18.10 44.80	17.88 42.75	*0.00* *0.00*	0.03 0.14	4.95 5.33	18.10 44.53	4.75 25.00	4.75 25.15	0.00 trace	*0.00* *trace*
Florida rock phosphate	Rapidly reactive with H_3PO_4. Product nearly dry overnight[e]	668 674	10.34 3.86	1.45 1.27	18.80 50.00	16.25 49.50	*1.68* *0.28*	1.45 1.20	5.34 9.73	18.66 48.57	6.75 26.75	6.80 27.53	2.10 2.00	*2.11* *2.06*

[a] Acidulation with 65% H_2SO_4 and 78% H_3PO_4, on basis of total calcium content; analysis after curing 18 days.
[b] Obtained from Darling and Company.
[c] A special material containing only 0.003% of F_2.
[d] Vigor of reaction was immediate with H_3PO_4, product 673, but slow in starting with H_2SO_4, product 667.
[e] Less rapidly reactive than Tennessee rock in H_2SO_4 acidulation; product 668 required several days to become dry.
[f] Mixture was still a slurry 2 months after the H_2SO_4 acidulation. Incomplete acidulation also with 78% H_2SO_4.
[g] Ammoniated to constant weight by exposure of agitated 12-mesh superphosphate.
[h] Corrected to original basis to compensate for change in weight during curing.

The ammoniated products (Table 2) were made by bringing agitated 200 gram charges of 12-mesh superphosphates to constant weight in an atmosphere of ammonia. Fixations of ammonia and diminutions in water-soluble P_2O_5 are reported, but these results will be considered only as they serve to facilitate interpretations, the primary objective being to determine the effect of fluoride components upon formation of citrate-insoluble P_2O_5. The mixtures (Tables 3, 4, and 5) were made of 20-mesh superphosphates and 100-mesh liming materials and then aged at 45° C. for one month. The aged mixtures were sized to 30-mesh for analysis, and analytical values found for each final product were computed to initial basis to compensate for changes in weight.

DEVELOPMENT OF CITRATE-INSOLUBILITY[1]

In ammoniated superphosphates.—The eleven superphosphates (Table 2) were cured 18 days before ammoniation. A standard superphosphate from the regular fused rock was not included, because only a slurry resulted when this material was mixed with either 65 per cent or 78 per cent sulfuric acid. The regular fused rock responded readily, however, to acidulation by 78 per cent phosphoric acid.

The analyses (Table 2) show that ammoniation induced substantial reversion in the standard and concentrated superphosphates derived from both raw and ignited Tennessee brown rock. In contrast, ammoniation induced no reversion in the superphosphates derived from either the calcined rock or the fused rocks of low fluorine content. Ignition of the brown rock was intended to inactivate iron and aluminum compounds and minimize their influence upon reversion. The preliminary heating had no retardative effect, however, upon reversion in the subsequently ammoniated products.

Reversion in the ammoniated standard superphosphates from the brown Tennessee rock, both raw and ignited, was greater than in the corresponding concentrated superphosphates. This is in agreement with practical experience and was attributed by Keenen (6) to formation of tertiary calcium phosphates through secondary reactions between calcium sulfate and dibasic phosphates.

In ammoniated superphosphate-dolomite mixtures.—The eleven products of Table 3 were made by allowing the superphosphates to react with 100-mesh dolomite for 24 hours and then ammoniating with ammonia to constant weight. Each mixture was representative of a rate of 250 pounds of dolomite per 1750 pounds of superphosphate. The neutralizing effect of the incorporated dolomite during the preliminary 24-hour period was reflected by decreases in ammonia fixations and by less reversion in the systems that contained fluorides. Although substantial reversion still developed in the mixtures carrying normal fluoride content, practically no

[1] In the present paper such development will be connoted by "reversion."

TABLE 3.—*Influence of component fluorides of superphosphates upon development of citrate-insolubility following ammoniation of superphosphate-dolomite mixture*[a] *and curing at 45° C. for one month*
(Results expressed in percentage).

SUPERPHOSPHATES			ANALYSES OF SUPERPHOSPHATES AND THEIR DOLOMITE-AMMONIATED MIXTURES									
			INITIAL SUPERPHOSPHATE-DOLOMITE MIXTURES[b]				CURED SUPERPHOSPHATE-DOLOMITE AMMONIATED MIXTURES					
				P_2O_5			AMMONIA		P_2O_5			
									WATER-SOLUBLE		CITRATE-INSOLUBLE	
DERIVED FROM—	CODE	MOISTURE LOST AT 100° C.	FLUORINE	TOTAL	WATER-SOLUBLE	CITRATE-INSOLUBLE	FOUND	CORRECTED[c]	FOUND	CORRECTED[c]	FOUND	CORRECTED[c]
Tenn. brown rock phosphate	663	11.52	1.34	16.80	15.75	*0.00*	5.11	5.24	3.75	3.66	1.05	*1.02*
	669	4.47	1.16	44.80	43.31	*0.00*	5.54	5.54	25.50	25.50	2.19	*2.19*
Tenn. brown rock phosphate, heated 1 hr. at 600° C.	664	12.24	1.30	17.50	15.32	*0.18*	5.41	5.73	2.00	1.88	2.00	*1.88*
	670	2.36	1.18	45.50	42.65	*0.28*	5.44	5.55	25.50	25.12	2.60	*2.56*
Calcined rock phosphate	665	6.22	0.08	18.55	15.09	*0.56*	5.17	5.25	3.25	3.10	0.50	*0.49*
	671	2.70	0.11	46.55	42.65	*0.46*	5.29	5.29	23.50	23.50	0.50	*0.50*
Tenn. brown fused rock phosphates[d]	667	6.79	0.02	15.84	15.66	*0.00*	4.26	4.32	4.75	4.68	0.02	*0.02*
	673	1.80	0.08	39.20	37.40	*0.00*	5.32	5.14	21.50	20.77	0.10	*0.09*
	672	6.02	0.21	39.55	36.09	*0.81*	5.69	5.60	18.25	17.98	1.15	*1.03*
Florida rock phosphate	668	10.34	1.27	16.45	15.31	*0.18*	4.38	4.56	4.50	4.36	0.70	*0.67*
	674	3.86	1.11	43.75	43.31	*0.20*	5.80	5.65	27.50	26.82	2.20	*2.14*

[a] Containing 250 lbs. of 100-mesh dolomite and 1750 lbs. of superphosphate; mixtures aged 24 hours and then exposed to NH_3 gas for 3 days.
[b] Same as those of Table 2, which carries the initial analyses and describes the behavior of each starting phosphate upon its acidulation with either H_2SO_4 or commercial H_3PO_4.
[c] Computed from analyses made immediately before mixing dolomite.
[d] A special material containing only 0.003% of F_2 was used to make products 667 and 673; that used in making Product 672 contained 0.67% of F_2.
[e] Corrected to original basis to compensate for change in weight during curing.

reversion occurred in the mixtures that contained meager quantities of fluorides.

In superphosphate-limestone and superphosphate-dolomite mixtures.—The eleven superphosphate-limestone and the eleven superphosphate-dolomite mixtures (Table 4) were not ammoniated. Otherwise, they were respectively identical to those of Table 3.

Limestone caused considerable reversion in the mixtures that contained the superphosphates derived from starting materials of normal fluorine content. Reversion in the dolomite mixtures was decidedly less than in the corresponding limestone mixtures, as explained in previous contributions (10, 11, 7). Practically no reversion was induced, however, by additions of either limestone or dolomite to superphosphates derived from any of the three fused rock phosphates or from the calcined rock.

Reversions in the unammoniated dolomite mixtures of Table 4 were decidedly less than in the corresponding ammoniated dolomite mixtures of Table 3. Formation of basic phosphates in the dolomite mixtures of Table 4 was localized to grains of dolomite, in contrast to the extensive formation of basic phosphates induced solely by diffused ammonia in the mixtures of Table 3. Ammoniation therefore resulted in greater surface contact between component fluorides and engendered tertiary phosphates, with resultant increase in formation of fluorphosphate.

In superphosphate-lime and superphosphate-cyanamid mixtures.—The results of Table 5 show P_2O_5 transitions induced by admixtures of calcium hydroxide, and of cyanamid, at the rate of 125 pounds per 1875 pounds of superphosphate. The two high-calcic materials caused considerable reversion in all mixtures that contained superphosphates derived from phosphatic materials of normal fluorine content. Again, however, reversion did not occur in those mixtures that contained superphosphates derived from the phosphatic materials of low fluorine content.

DISCUSSION

In previous studies, addition of wetted calcium silicate slag of high fluoride content to triple superphosphates resulted in reversion far beyond that induced by corresponding addition of limestone (15, 16). It was postulated that rapid formation of dicalcium phosphate induced by the slag was followed by gradual transition into tertiary forms, which then reacted with fluorides of the superphosphate and those of the slag to form fluorphosphate (15, 17). Further work demonstrated that the tricalcium phosphate of admixed defluorinated calcined rock phosphate reacted with the component fluorides of both standard and triple superphosphates and caused substantial reversion, which was accelerated by enhanced humidity and elevated temperature (14). No such reversion occurred, however, in corresponding mixtures of the calcined rock phosphate and an experimental superphosphate of meager fluorine content,

and almost devoid of the extraneous components that occur in commercial superphosphates.

To assure such components, the standard and triple superphosphates of the present study were derived from rock phosphates, either raw or processed. The several heat treatments imparted different physical properties to the starting materials and caused variable losses of fluorine, and undoubtedly affected the nature of "I and A" content. Losses of organic matter, carbon dioxide, and sulfur from the moderately ignited rock are deemed to have no effect upon the composition of phosphatic components. Variation in fluorine content, therefore, was the chief difference in the percentage composition of the several experimental superphosphates.

Substantial reversion resulted from the direct ammoniation of those superphosphates that contained fluorides, whereas no reversion occurred in the systems that were practically fluoride-free. Maximal, intermediate, and minimal reversions in the ammoniated superphosphates were in direct relation to the fluorine content of the respective starting materials—raw and ignited rocks, partly defluorinated fused rock, and substantially defluorinated rock products. Formation of tricalcium phosphate through secondary reactions between engendered dibasic phosphates and the calcium sulfate content of the standard superphosphates from brown rock was reflected by more extensive ultimate transitions to fluorphosphate. This effect was not extensive, however, in the ammoniated superphosphates derived from Florida rock.

When the superphosphate-dolomite mixtures of normal fluoride content were aged 24 hours and then ammoniated, reversion in the triple superphosphate mixtures exceeded that in the corresponding standard superphosphate mixtures. The dolomite supplements were identical, and the triple superphosphate mixtures therefore contained larger residues of water-soluble P_2O_5 for reaction with the subsequently introduced ammonia. Development of the ultimate fluorphosphate in the triple superphosphate-dolomite mixtures that contained fluorides was greater, therefore, than in the standard superphosphate-dolomite parallels. The results of Table 3 indicate that an interval between incorporation of dolomite and injection of ammonia would minimize reversion, when standard superphosphates are processed by joint usage of these materials.

Reversion by straight ammoniation of fluoride-bearing superphosphates exceeded that induced by additions of either dolomite, limestone, hydrated lime, or calcium cyanamid. Diminutions in water-soluble P_2O_5 reflected the localized activities of the solid particles, in contrast to the greater neutralizing effect of the diffused gas. Avidities of the high calcic solids also were evidenced by reversion beyond the slight effect induced by the less active dolomite in the mixtures characterized by normal fluoride content.

TABLE 4.—*Influence of component fluorides of superphosphates upon development of citrate-insolubility in their mixtures*[a] *with limestone and with dolomite during curing at 45° C. for one month*

(Results expressed as percentage.)

SUPERPHOSPHATES[b]			ANALYSES OF SUPERPHOSPHATES AND THEIR MIXTURES WITH LIMESTONE AND WITH DOLOMITE											
			INITIAL SUPERPHOSPHATE-LIMESTONE AND SUPERPHOS.-DOLOMITE MIXTURES[c]				CURED SUPERPHOSPHATE-LIMESTONE MIXTURES				CURED SUPERPHOSPHATE-DOLOMITE MIXTURES			
				P_2O_5			P_2O_5				P_2O_5			
							WATER-SOLUBLE		CITRATE-INSOLUBLE		WATER-SOLUBLE		CITRATE-INSOLUBLE	
DERIVED FROM—	CODE	LOST AT 100° C.	FLUORINE	TOTAL	WATER-SOLUBLE	CITRATE-INSOLUBLE	FOUND	CORRECTED[e]	FOUND	CORRECTED[e]	FOUND	CORRECTED[e]	FOUND	CORRECTED[e]
Tenn. brown rock phosphate	663	11.52	1.34	16.80	15.75	*0.00*	11.50	10.65	0.55	*0.51*	14.25	12.78	0.04	*0.03*
	669	4.47	1.16	44.80	43.31	*0.00*	39.75	37.68	0.62	*0.59*	42.50	38.64	0.12	*0.10*
Tenn. brown rock phosphate, heated 1 hr. at 600° C.	664	12.24	1.30	17.50	15.32	*0.18*	7.50	6.70	2.20	*1.96*	12.50	11.37	0.55	*0.50*
	670	2.36	1.18	45.50	42.65	*0.28*	38.50	36.50	1.25	*1.18*	42.50	41.26	0.45	*0.44*
Calcined rock phosphate	665	6.22	0.08	18.55	15.09	*0.56*	12.50	11.96	0.65	*0.62*	12.50	11.57	0.55	*0.51*
	671	2.70	0.11	46.55	42.65	*0.46*	39.75	38.78	0.60	*0.58*	41.25	39.28	0.50	*0.47*
Tenn. brown fused rock phosphates[d]	667	6.79	0.02	15.84	15.66	*0.00*	6.25	5.72	0.05	*0.04*	11.00	10.00	0.02	*0.02*
	673	1.80	0.08	39.20	37.40	*0.00*	22.25	20.22	0.15	*0.15*	36.25	35.02	0.10	*0.09*
	672	6.02	0.21	39.55	36.09	*0.81*	23.50	21.36	1.20	*1.09*	29.00	26.37	0.95	*0.86*
Florida rock phosphate	668	10.34	1.27	16.45	15.31	*0.18*	12.25	11.50	0.30	*0.27*	14.75	11.41	0.45	*0.41*
	674	3.86	1.11	43.75	43.31	*0.20*	40.25	39.30	0.50	*0.46*	43.00	40.56	0.05	*0.04*

[a] Containing 250 lbs. of 100-mesh limestone or dolomite and 1750 lbs. of superphosphate.
[b] Same as those of Table 2, which carries initial analyses and describes the behavior of each starting phosphate upon its acidulation with either H_2SO_4 or commercial H_3PO_4.
[c] Computed from analyses made immediately before mixing limestone or dolomite.
[d] A special material containing only 0.003% of F_2 was used to make products 667 and 673. Product 672 was made from a fused product that contained 0.67% of F_2.
[e] Values corrected to original basis to compensate for change in weight during curing.

TABLE 5.—*Influence of component fluorides of superphosphates upon the development of citrate-insolubility in their mixtures*[a] *with hydrated lime and with calcium cyanamid during curing at 45° C. for one month*

(Results expressed in percentage.)

SUPERPHOSPHATES[b]			ANALYSES OF SUPERPHOSPHATES AND THEIR MIXTURES WITH HYDRATED LIME AND WITH CALCIUM CYANAMID											
			INITIAL SUPERPHOSPHATE-LIME AND SUPERPHOS.-CYANAMID MIXTURES[c]				CURED SUPERPHOSPHATE-LIME MIXTURES				CURED SUPERPHOSPHATE-CYANAMID MIXTURES			
				P_2O_5			P_2O_5				P_2O_5			
							WATER-SOLUBLE		CITRATE-INSOLUBLE		WATER-SOLUBLE		CITRATE-INSOLUBLE	
DERIVED FROM—	CODE	MOISTURE LOST AT 100° C.	FLUORINE	TOTAL	WATER-SOLUBLE	CITRATE-INSOLUBLE	FOUND	CORRECTED[e]	FOUND	CORRECTED[e]	FOUND	CORRECTED[e]	FOUND	CORRECTED[e]
Tenn. brown rock phosphate	663	11.52	1.43	17.55	16.88	*0.00*	9.50	8.75	0.55	*0.50*	9.75	9.11	0.73	*0.69*
	669	4.47	1.25	48.00	46.40	*0.00*	38.25	36.42	0.59	*0.56*	40.50	40.00	0.37	*0.36*
Tenn. brown rock phosphate, heated 1 hr. at 600° C.	664	12.24	1.40	18.75	16.46	*0.79*	7.50	6.94	1.15	*1.06*	7.50	6.98	1.25	*1.16*
	670	2.36	1.27	48.75	45.70	*0.30*	35.50	33.80	1.35	*1.28*	39.50	38.44	0.85	*0.82*
Calcined rock phosphate	665	6.22	0.08	19.88	16.17	*0.60*	8.25	7.86	0.75	*0.71*	13.00	12.50	0.65	*0.62*
	671	2.70	0.12	49.88	45.70	*0.49*	38.25	38.25	0.65	*0.65*	41.25	40.64	0.55	*0.54*
Tenn. brown fused rock phosphates[d]	667	6.79	0.02	16.97	16.75	*0.00*	8.00	7.48	0.03	*0.03*	8.50	8.01	0.03	*0.03*
	673	1.80	0.09	42.00	40.08	*0.00*	29.50	28.00	0.07	*0.06*	31.75	31.75	0.17	*0.17*
	672	6.02	0.22	42.38	38.67	*0.86*	28.75	26.50	1.20	*1.10*	27.00	25.71	1.10	*1.04*
Florida rock phosphate	668	10.34	1.35	17.63	16.46	*0.19*	12.50	11.57	0.85	*0.79*	14.75	14.11	0.75	*0.74*
	674	3.86	1.19	46.88	46.40	*0.22*	38.75	36.47	0.55	*0.51*	38.25	37.20	0.70	*0.68*

[a] Containing 125 lbs. of either hydrated lime or calcium cyanamid and 1875 lbs. of superphosphate.
[b] Same as those of Table 2, which carries the initial analyses and describes the behavior of each starting phosphate upon its acidulation with either H_2SO_4 or commercial H_3PO_4.
[c] Computed from analyses made immediately before mixing hydrated lime or cyanamid.
[d] A special material containing only 0.003% of F_2 was used to make products 667 and 673. Product 672 was made from a fused rock that contained 0.67% of F_2.
[e] Values corrected to original basis to compensate for change in weight during curing.

Table 1 shows that ignition of brown rock for one hour at 600° C. had caused no loss of fluorine. The moderately ignited rock was more responsive than the raw rock to acidulation, but the ignition of the rock did not diminish reversion when the superphosphates derived from it were subsequently processed. Additions of high-calcic liming materials caused more reversion in superphosphates derived from the ignited brown rock phosphate than in superphosphates derived from the raw rock.

Considered on the whole, the 66 mixtures of superphosphates and liming materials of Tables 4 and 5 demonstrate that added liming materials will not develop reversion in the absence of component fluorides.

The term "component" is used to connote natural incidence of fluorides. Regardless of whether the fluorine in the superphosphate is present as calcium fluoride or as calcium silico-fluoride, the effect is the same when the superphosphate is either limed or ammoniated. It has been found that reversion by fluorides, dispersed during acidulation, greatly exceeds reversion induced by additive solid fluorides, in limed and also in ammoniated superphosphates. Unpublished data demonstrated that marked reversion occurred in experimental ammoniated superphosphates produced by acidulation of a fluoride-free marble with phosphoric acid that had been saturated with calcium fluoride. In contrast, no reversion occurred upon ammoniation of a similar superphosphate made from the same solid by acidulation with the same acid without the dissolved calcium fluoride.

Although it has been shown (17, 18) that additions of precipitated calcium fluoride caused no measureable reversion during conventional analysis of processed superphosphates, Rader and Ross (18) found that the addition of soluble sodium fluoride caused a definite increase in citrate-insoluble P_2O_5. Such an addition would react with the calcium compounds of the superphosphate, and the resultant finely divided and thoroughly disseminated calcium fluoride would be expected to show an effect similar to that induced by component fluorides of either limed or ammoniated superphosphates.

Considered in connection with the several previous observations—(a) extensive reversion in mixtures of commercial superphosphates with calcium silicates and the same effect in mixtures of fluoride-free special superphosphates with calcium silicate slag of high-fluoride content, (b) disappearance of pulverulent forms of calcium fluoride from its moistened and aged 1–12 mixtures with precipitated tricalcium phosphate and concomitant decrease in P_2O_5 solubility, and (c) the incompatibility of calcined rock phosphate with commercial superphosphates (14)—the present findings substantiate the conclusion that formation of calcium fluorphosphate is the cause of development of citrate-insolubility, or true reversion.

The results shown in this paper demonstrate that superphosphates

derived from starting materials of low fluorine content can be ammoniated fully or supplemented by liming materials without detrimental effect upon P_2O_5 availability. It is obvious that superphosphates of meager fluorine content would fill a need in hydroponics, in nutrient-sand media in greenhouse culture. Mixtures of such superphosphates with the proper amounts of pulverized limestone would give dicalcium phosphate for use as an animal-feed supplement. The possibilities of fluoride-free starting phosphates for such usages should stimulate development of economic processes for the removal of fluorine from raw rock phosphate.

SUMMARY AND CONCLUSIONS

Economic ammoniation of superphosphates and incorporation of high-calcic liming materials are restricted by resultant loss of P_2O_5 availability. The result is accelerated by the presence of calcium sulfate and by elevation of humidity and temperature. The writers have endeavored to show that this is due to the development of calcium fluorphosphate through reaction between engendered basic phosphates and component fluorides of the superphosphates.

This paper deals with the effect of ammoniation and of four liming materials upon P_2O_5 availability in standard and triple superphosphates made from starting phosphates of variant fluoride content. Marked reversion occurred when superphosphates derived from starting materials of normal fluoride content were processed and aged one month at 45° C., whereas no reversion developed in the processed superphosphates derived from phosphates of low fluoride content. Superphosphates of meager fluoride content can be ammoniated fully or mixed with ordinary high calcic liming materials, without loss of P_2O_5 availability. Utility of fluoride-free superphosphates for special purposes is pointed out.

THORIUM NITRATE TITRATION OF MICRO QUANTITIES OF FLUORINE IN AQUEOUS AND ALCOHOLIC SYSTEMS*

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Quantitative determination of fluorine has been recognized as a difficult and tedious procedure. Fahey (4) recently pointed out that 21 analytical technics—gravimetric, volumetric, colorimetric, and nephelometric—were proposed between the appearance of the original Berzelius method in 1816 and the review by Stevens in 1936 (16). Fahey observed that Merwin's modification (11) of Steiger's method (15) is used widely in

* Contribution from cooperative studies conducted at The University of Tennessee Agricultural Experiment Station under auspices of the Tennessee Valley Authority, Department of Chemical Engineering.

rock analysis and that the lead chlorofluoride technic developed by Hoffman and Lundell (5) is adapted to analysis of materials of relatively high fluorine content. These methods are not applicable, however, to minute quantities of fluorine.

The thorium nitrate volumetric method proposed by Willard and Winter (17) was a distinct contribution and has been adapted to analysis of foods, wines, waters, plants, and soils. Armstrong (1) compared thorium nitrate titrations in aqueous and alcoholic systems and concluded that as little as 0.5 micrograms of fluorine can be determined in an aqueous system devoid of chlorides. He also concluded that the thorium nitrate solution registers a constant fluorine equivalence in aqueous solutions of variant fluoride concentrations, and a variable equivalence for a fluorine range of 1–10 micrograms in alcoholic systems. In a study of aqueous systems, Dahle, Bonnar, and Wichmann (3) considered (a) quantity of indicator, (b) variation in *p*H, and (c) influence of different neutral solutes. They concluded that the fluorine equivalent of the thorium nitrate solution is uniformly 66 per cent of theory in titration range of 2–50 micrograms. Upon this assumption, they also concluded that fluorine could be determined with an accuracy of 0.5 microgram in the range of 0.5–50 micrograms. They did not stipulate, however, that the thorium nitrate solution intended for a titration of microgram range should be standardized against a corresponding range supplied by a material of known fluorine content. In his subsequent report as Referee, Dahle (2) recommended that, "The procedure for titrating small quantities of fluorine be studied collaboratively."

Rowley and Churchill (14) assigned stoichiometrical value to their thorium nitrate solution and prescribed titration in aqueous systems for a fluorine range of 1–50 micrograms in 100 ml., with *p*H 3.0–3.1 buffered with monochloroacetic acid, as recommended by Hoskins and Ferris (6). The normality of the thorium nitrate was determined, however, by titration against a fluoride of known assay in 48 per cent alcoholic solution. Reynolds and Hill (13) studied the interference of PO_4 and SO_4 and other ions and concluded that such interference was less in aqueous than in alcoholic systems. They recommended 0.04 *N* thorium nitrate solution for titration of a 50 ml. aliquot, with *p*H adjustment by monochloroacetic acid buffer. They observed that titrations of aqueous and alcoholic systems give concordant results for fluorine incidence between 0.1 and 60 mg., but not for systems containing less than 0.1 mg. Mason and Ashcraft (8) reported concordant results for titrations with 0.01008 *N* thorium nitrate solution in alcoholic and aqueous systems containing more than 1 mg. of fluorine per aliquot.

McClure (9) summarized the procedures advocated for analysis of ash of bone and teeth and concluded that thorium nitrate titrations in aqueous systems are not adapted to precise determination of less than 10

micrograms of fluorine per 2 ml. aliquot buffered with monochloroacetic acid, or for less than 1.0 p.p.m. in milk powder. MacIntire and Hammond (7) reported complete and expedited recovery of fluorine from soils and organics fortified with fluorides and ignited with calcium peroxide and magnesium peroxide, respectively, before steam current distillation with perchloric acid and titration of distillate with 0.001 *N* thorium nitrate in *p*H-adjusted alcoholic systems. These preliminary ignitions obviate explosions during the perchloric acid distillations. Collaborative work by McHargue and Hodgkiss (10) demonstrated the effectiveness of this treatment. In an adaptation of the Willard and Winter method to determination of fluorine content of wine, Rempel (12) used a 0.01 *N* thorium nitrate solution, standardized against sodium fluoride in alcoholic solution.

The objectives of this study were to determine (a) the influence of range of fluorine content upon relative accuracy of thorium nitrate titrations in buffered aqueous and 48 per cent alcoholic systems, and in corresponding systems with *p*H adjusted by 0.05 *N* hydrochloric acid; and (b) the relation between normality and empirical values of thorium nitrate for titration of milligram and microgram occurrences of fluorine.

EXPERIMENTAL CONDITIONS

Essential precautions were taken to obviate coatings of colloidal silica in the distillation flasks, to maintain close proximation to 135° C. during distillation, to assure reagent purity, and to provide a constant of indicator. Two drops of 0.05 per cent aqueous solution of sodium alizarin sulfonate per 10 ml. aliquot were used in each titration. The aqueous and alcoholic systems were all brought to *p*H of 3.0 ± 0.2. One series of each type was neutralized and then adjusted to that *p*H by 1 ml. of 0.05 *N* hydrochloric acid per 20 ml. aliquot. Another series of each type was likewise adjusted by means of 1 ml. of the buffer solution containing 9.448 grams of monochloroacetic acid and 2 grams of sodium hydroxide per 100 ml. The thorium nitrate solutions were 0.0175 *N* and 0.00175 *N* for respective titrations of milligram range and microgram range. The two solutions were standardized against ammonium oxalate and against the fluorine content of rock phosphate No. 56 of the Bureau of Standards.

RESULTS

The results in Table 1 show the fluorine values obtained for aqueous and alcoholic solutions having fluorine occurrences of 2–50 micrograms and 0.2–5 mg. per aliquot of 10 ml. and 10–20 ml., respectively. When normality values obtained as above indicated were applied, the buffered and hydrochloric acid-adjusted alcoholic systems were in fair accord with the known quantities of fluorine for both ranges of concentration. Adjustment with hydrochloric acid and use of the buffer solution gave comparable milligram values for the alcoholic and aqueous systems. In

general, quantities of fluorine registered by both the alcoholic and the aqueous systems were in accord with known milligram occurrences of fluorine. Agreement between quantities of fluorine indicated by titrations and the known occurrences in both aqueous and alcoholic systems was, however, much closer in the milligram series than in the corresponding microgram series.

TABLE 1.—*Fluorine values for microgram and milligram ranges by thorium nitrate titrations in alcoholic and in aqueous systems*

FLUORINE CONTENT	VOLUME TITRATED	THORIUM NITRATE USED[a]				FLUORINE FOUND[c]			
		IN 48% ALCOHOLIC SYSTEM[b]		IN AQUEOUS SYSTEM[b]		IN 48% ALCOHOLIC SYSTEM[b]		IN AQUEOUS SYSTEM[b]	
		WITH HCl	WITH BUFFER	WITH HCl	WITH BUFFER	WITH HCl	WITH BUFFER	WITH HCl	WITH BUFFER
microgram	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*microgram*	*microgram*	*microgram*	*microgram*
none	10–12	0.080	0.12	0.10	0.36	—	—	—	—
2.0	10	0.135	0.18	0.19	0.58	1.84	2.0	2.84	7.3
4.0	10	0.200	—	0.27	—	4.00	—	5.68	—
5.0	10	0.225	0.30	0.31	0.74	4.84	5.9	7.02	12.6
8.0	10	0.305	—	0.47	—	8.02	—	12.02	—
10.0	10	0.380	0.48	0.61	0.96	10.02	11.9	17.04	19.9
20.0	10	0.670	0.70	1.12	1.65	19.71	19.3	35.09	42.8
40.0	10	1.270	—	—	—	40.00	—	—	—
50.0	12	1.540	1.78	2.08	3.25	49.61	54.2	66.20	96.0
mg.						mg.	mg.	mg.	mg.
none	10–20	0.008	0.05	0.01	0.05	—	—	—	—
0.10	10	0.325	—	0.40	—	0.105	—	0.130	—
0.20	10	0.610	0.70	0.71	0.95	0.200	0.216	0.234	0.299
0.50	10	1.525	1.50	1.49	1.57	0.505	0.482	0.498	0.505
1.00	10	2.950	3.00	2.95	3.15	0.983	0.980	0.983	1.030
2.00	10	5.950	5.95	5.93	5.90	1.985	1.960	1.978	1.950
3.00	20	8.950	—	8.93	—	2.987	—	2.980	—
5.00	20	14.620	14.40	14.75	14.38	4.861	4.770	4.880	4.770

[a] The thorium nitrate solutions were 0.0175 *N* and 0.00175 *N* for respective titrations of milligram range and microgram range.
[b] All systems neutralized with NaOH and then adjusted to *p*H 3.0, ±0.2, by either 1 ml. of 0.05 *N* HCl or 1 ml. of monochloroacetic acid buffer solution.
[c] Corrected for respective "blanks."

In the microgram range, the buffered alcoholic solutions gave results somewhat higher than the results for the corresponding systems adjusted by hydrochloric acid, and results slightly higher also than the true values. In the aqueous systems of the 2–50 microgram range, however, results for the five occurrences were greatly in excess of the true values. This was true of the systems adjusted with hydrochloric acid and also those buffered. Each microgram result in the series of buffered aqueous systems

was much greater than the result obtained for the same incidence of fluorine in the corresponding system adjusted by hydrochloric acid. Blank titrations in the buffered microgram series were also in excess of blanks for the systems adjusted with hydrochloric acid, particularly in the aqueous systems. Inclusion of the buffer, therefore, tended to magnify the

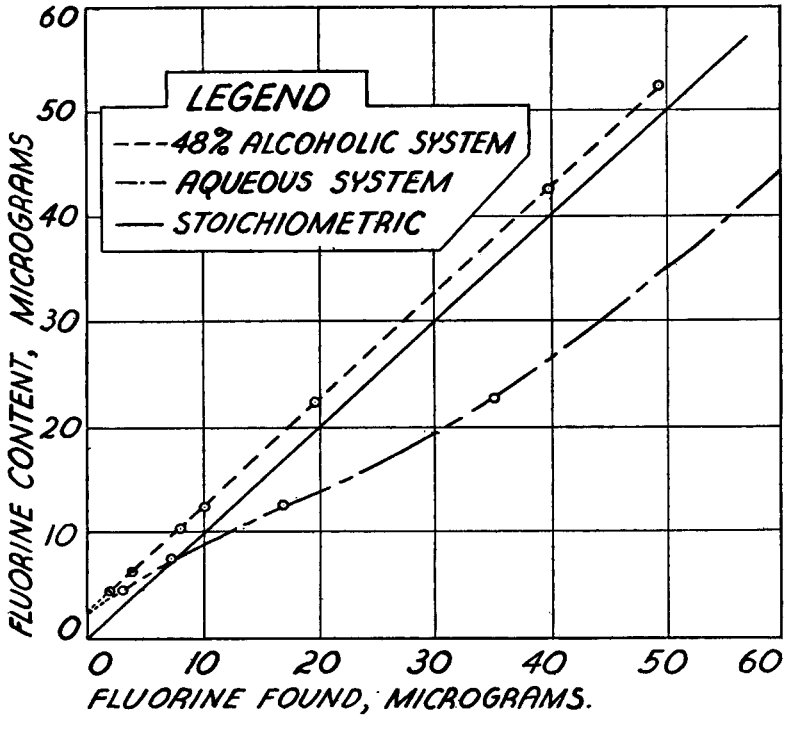

FIG. 1.—THORIUM NITRATE TITRATION VALUES FOR FLUORINE CONTENT OF MICROGRAM RANGE, IN AQUEOUS AND ALCOHOLIC SYSTEMS AT *p*H 3, ±0.2.

error in quantities of fluorine indicated by titration in the 2–50 microgram series.

The foregoing results demonstrate that *micro* quantities of fluorine can not be determined accurately in aqueous systems by application of the normality value of a thorium nitrate solution. Were normality value applied for the 2–50 microgram range in the aqueous systems adjusted with hydrochloric acid, the mean apparent incidence would be 1.5 times the true value. Similar application of normality value for microgram range in the buffered aqueous systems would give a medial apparent indication 2.05 times the true medial. For such microgram range, titration

value of a thorium nitrate solution must be determined empirically against corresponding quantities of fluorine from a material of known assay and for the specific solvent, definite volume, and identical quantity of indicator. When titrations are made in alcoholic solution, however, application of the stoichiometrical value of the standard thorium nitrate

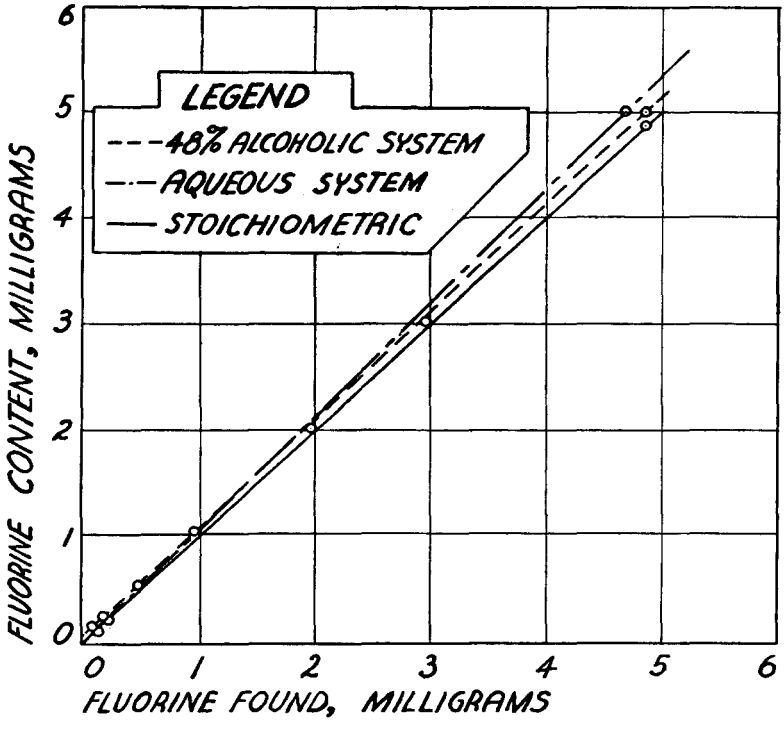

FIG. 2.—THORIUM NITRATE TITRATION VALUES FOR FLUORINE CONTENT OF MILLIGRAM RANGE, IN AQUEOUS AND ALCOHOLIC SYSTEMS AT *p*H 3, ±0.2.

will give accurate values in a solution of adjusted *p*H and without inclusion of a buffer solution.

Figures 1 and 2 show the relationship of the curves obtained by titrations of solutions of known fluorine content and the lineal arrangement of stoichiometrical values for the stipulated milligram and microgram ranges.

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COMPARISON OF CHEMICAL METHODS FOR ESTIMATING THE AVAILABILITY OF MAGNESIUM*

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The principal carriers of magnesium from domestic sources now offered for sale in this country are dolomite or dolomitic limestone and magnesium oxide. Active magnesium oxide is now available for fertilizer use, and it has been increasingly used in mixed fertilizers during the past few months as a source of magnesium. As is always the case with a material new to the fertilizer trade, the question of solubility and availability has been raised both by agronomists and by manufacturers. A preliminary estimate of the availability of the magnesium in this new material can be gained from its behavior with respect to reagents.

The choice of a suitable solvent and technic for estimating the availability of dolomitic limestone to plants has been given considerable attention in recent years, especially by Smith and Deszyck (11, 12, 13, 14, 15). The rate of decomposition in ammonium chloride under standard conditions has been suggested as a measure of the reactivity of dolomite by Shaw (9) while the rate of decomposition in hydrochloric acid was used by Siems and Batton (10). Kuzmeski (3) studied the solubilities of nine different dolomitic limestones in a citrate solution of *p*H 4 and Whittaker, Rader, and Zahn (17) studied the solubility of dolomite in various solvents alone, and after incorporation in fertilizer mixtures. The final choice of a solvent and technic has, however, not been made. The present paper presents (a) the results of a study of the behavior toward water and various citrate solutions of active magnesium oxide both before and after in-

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corporation in mixed fertilizers, and (b) a comparison of the solubilities of various mesh sizes of dolomite in several solvents with their availability as indicated by actual crop tests.

ACTIVE MAGNESIUM OXIDE

Since active magnesium oxide is used mainly in mixed fertilizers it is important to know its solubility when incorporated in mixtures under the conditions ordinarily obtaining in practice.

Whittaker, Rader, and Zahn (17), in their study of the reactions of magnesium oxide in mixed fertilizers, made up a series of mixtures containing varying quantities of magnesium oxide and stored one-third of each mixture at 30°, 60°, and 90° C. for 20 days. These same mixtures were used in the present study, much of the work having been done concurrently with the work on the reactions of this material. The composition of these mixtures is given in Table 1.

TABLE 1.—*Composition of mixtures containing active magnesium oxide*

NO.	BASE	ACTIVE MAGNESIUM OXIDE
		lbs./ton
1	Superphosphate	40
2	Superphosphate	80
3	Superphosphate	160
4	Ammoniated Superphosphate (3%)	40
5	Ammoniated Superphosphate (3%)	80
6	Ammoniated Superphosphate (3%)	160
7	6–8–6 Fertilizer[1]	20
8	6–8–6 Fertilizer[1]	40
9	6–8–6 Fertilizer[1]	80

[1] This 6-8-6 fertilizer had the following formula:

Superphosphate, 20.56% P_2O_5	775
Ammonia, 3% of superphosphate	24
Ammonium sulfate, 21.2% N,	472
Potassium chloride, 63.1% K_2O	190
Magnesium oxide and quartz flour as required to fill formula	539
	2000

In the study of these mixtures water solubility was determined by washing a 1 gram sample on the filter with 250 ml. of water at room temperature and determining magnesium oxide in the filtrate. For purposes of comparison water solubility was also determined in some of the mixtures by boiling a 1 gram sample for 30 minutes in 200 ml. of water. In the later discussion, unless otherwise specified, the expression "water-soluble magnesium" means the fraction that was dissolved by leaching with water at room temperature. Solubility in neutral ammonium citrate was determined by the technic prescribed for citrate-soluble phosphorus in *Methods of Analysis, A.O.A.C.* Magnesium was determined in the insoluble residue and the citrate-soluble portion obtained by difference

between the total and citrate-insoluble magnesium oxide. Solubility was also determined by the same technic in the special solvent suggested by Smith and Deszyck (15). This solvent, which will be referred to as "special citrate," is composed of 6 per cent citric acid brought to *p*H 4 with ammonium hydroxide. All magnesium determinations were made by the tentative method given in the 1935 edition of *Methods of Analysis, A.O.A.C.*

Table 2 shows the fraction of the total magnesium in the stored mixtures that was dissolved in each of the citrate solutions and in water by leaching (17) and by boiling. Large fractions of the magnesium oxide in the stored mixtures were water soluble, although only about 0.1 per cent of the magnesium oxide alone was water soluble. Therefore this increased solubility must be due to reactions that occurred during storage or during the course of the analysis, or both. The third column of Table 2 shows the fraction of the magnesium that dissolved on leaching mixtures of the same composition, freshly prepared from dry materials to prevent reaction prior to the leaching (17). Since the magnesium oxide itself is practically insoluble in water, these figures should represent the extent of the reactions during the extraction with water. In the stored mixtures most of these reactions should have gone to completion, since sufficient moisture was present to permit the reactions to occur. At present no means are available for determining what part of the reaction occurred during storage and what part occurred during the analysis.

When the sample is boiled in determining water solubility considerably higher values are obtained, and it would appear, therefore, that the boiling initiates reactions that would not ordinarily occur during storage. No data are available at present to indicate which of these methods gives the best appraisal of the water solubility of the magnesium oxide after it enters the soil.

The magnesium oxide alone was found to be 99.74 per cent soluble in neutral ammonium citrate, but in some cases it was appreciably less soluble in the same solvent when incorporated in the mixtures and stored. This reduced solubility is perhaps due to exhaustion of the reagent by other components of the mixture. It is much too large in many cases to be due to analytical error. The effect of increased storage temperatures on the solubility of magnesium oxide when incorporated in mixed fertilizers has been discussed elsewhere (17). Much less reduction in solubility was noted when the special citrate was used, a significant decrease being found in only 7 of the 27 mixtures studied. If it is later shown by plant tests that all the active magnesium oxide is available, then it would appear that the special citrate is more suitable for determining the availability than is neutral ammonium citrate. As will be shown later, the special citrate gives the best estimate of the availability of dolomitic magnesium of any reagent yet tried, and it would be a fortunate circum-

TABLE 2.—*Solubility of active magnesium oxide after incorporation in fertilizer mixtures*

BASE	MgO ADDED	FRACTION BECOMING WATER SOLUBLE[1]				FRACTION SOLUBLE IN BOILING H_2O		FRACTION SOLUBLE IN—					
		DURING ANALYSIS	AFTER 20 DAYS' STORAGE AT—			AFTER 20 DAYS' STORAGE AT—		NEUTRAL AMMONIUM CITRATE[2] AFTER 20 DAYS' STORAGE AT—			SPECIAL CITRATE[2,3] AFTER 20 DAYS' STORAGE AT—		
			30°	60°	90°	30°	90°	30°	60°	90°	30°	60°	90°
	lbs/ton	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Superphosphate	40	60	90	84	47			98	95	69	99	100	83
Superphosphate	80	52	69	38	31	96	67	98	97	97	99	99	93
Superphosphate	160	36	37	36	32			99	99	96	100	99	98
Ammoniated Superphosphate	40	64	64	47	45			85	91	87	95	99	97
Ammoniated Superphosphate	80	49	52	27	34	87	70	96	93	79	99	99	93
Ammoniated Superphosphate	160	30	46	41	28			98	97	94	100	99	99
6-8-6 fertilizer	20	69	68	39	40			85	86	68	97	97	95
6-8-6 fertilizer	40	52	55	25	32			91	93	83	99	99	98
6-8-6 fertilizer	80	36	51	27	25	96	87	96	96	95	99	99	99

[1] Washed on filter with 250 ml. of water at room temperature.
[2] Used as in the official method for P_2O_5.
[3] 6% citric acid brought to *p*H 4 with ammonia.

stance if the same reagent could be used for both forms of magnesium.

Water-soluble magnesium is assumed to be available, and by analogy with the accepted standards for the availability of P_2O_5 the citrate-soluble fraction should also be available. Selectively calcined dolomite is a material that also contains magnesium oxide, and except for minor differences caused by its content of calcium carbonate it reacts very similarly to active magnesium oxide from other sources. After detailed chemical tests MacIntire, Hardin, and Oldham (4) concluded that the magnesia of selectively calcined dolomite in mixtures with ordinary or ammoniated superphosphate was available to plants. This observation is a further reason for supposing that the magnesia in active magnesium oxide is completely available.

DOLOMITE

Smith and Deszyck (15) considered the agronomic data on the availability of the magnesium in dolomite of varying particle size and reached the following conclusion: "It seems conservative to estimate that ordinary dolomites in the average soil at *p*H 5–5.5 for two or three months will

TABLE 3.—*Analyses of dolomites*

MATERIAL	MESH SIZE	CaO	MgO	$CaCO_3$ EQUIVALENT
		per cent	*per cent*	*per cent*
Dolomite A[3]	20–40	28.02	19.42	98.2
Dolomite A	40–60	28.00	19.32	97.9
Dolomite A	60–80	27.97	19.31	97.9
Dolomite A	80–100	28.02	19.32	98.0
Dolomite A	100–200	28.14	19.46	98.4
Dolomite A	<200	27.68	19.20	97.0
Dolomite A	Composite[1]	28.06	19.41	98.3
Dolomite B[4]	Composite[1]	28.36	16.92	92.6
Dolomite C[2]	Composite[1]	30.38	21.25	107.0
Selectively calcined dolomite[5]		37.86	25.91	132.0

[1] Composed of equal amounts of the six mesh fractions.
[2] Considered a "hard" or unreactive dolomite.
[3] Considered a "medium" or moderately reactive dolomite.
[4] Considered a "soft" or very reactive dolomite.
[5] Mostly 200-mesh or finer.

decompose at about the following rates: 20 to 40 mesh, 15 per cent; 40 to 60 mesh, 25 per cent; 60 to 100 mesh, 50 per cent; 100 to 200 mesh, 60 per cent; finer than 200 mesh, 75 per cent. More data are needed for less acid soils, heavier soils, and very light soils." This conclusion was based largely on the work of Morgan and Salter (8), Taylor and Pierre (16), Dawson *et al.* (2), Collins and Speer (1), and on unpublished data of Smith and Deszyck. The work of MacIntire and Shaw (5) also seems to be in fair agreement.

The experiments of Dawson *et al.*, and of Collins and Speer, referred to above, were conducted with dolomites furnished by this laboratory, and

therefore an opportunity was afforded the writers to test chemical methods of determining availability of dolomitic magnesium with the same dolomites that were used in the plant tests. The work described here, therefore, is more nearly comparable with that of these workers than it is with that of some of the others. Dawson *et al.*, and Collins and Speer determined the residual carbonates in the soil after cropping and from those results calculated the fraction of the dolomite that decomposed. For present purposes it is assumed that all dolomite that decomposed liberated its magnesium in an available form. "Percentage decomposition," therefore, is also "per cent available." The figures in the last column of Table 4, headed "Fraction of Dolomite Decomposed in the

TABLE 4.—*Comparison of chemical methods and plant tests for determining availability of dolomitic magnesium*

MATERIAL	MAGNESIUM CARRIER ONLY FRACTION OF MgO SOLUBLE IN—				MAGNESIUM CARRIER IN 6–8–6 FERTILIZER	FRACTION OF DOLOMITE DECOMPOSED IN SOIL[5]
	NEUTRAL NH_4 CITRATE[1]	CITRIC ACID (2%)[2]	CITRATED NH_4NO_3[3]	SPECIAL CITRATE[1]	FRACTION OF MgO SOLUBLE IN SPECIAL CITRATE[1]	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Dolomite A 20–40 m.	2.1	9.4	17	31	27	23
Dolomite A 40–60 m.	4.3	15	24	47	43	41
Dolomite A 60–80 m.	5.3	18	26	49	51	53
Dolomite A 80–100 m.	5.9	20	26	52	56	60
Dolomite A 100–200 m.	7.4	19	31	54	62	74
Dolomite A < 200 m.	16	36	41	68	79	85
Composite Dolomite A	8.9	22	26	46	52	54
Composite Dolomite B	11	29	28	61	65	61
Composite Dolomite C	4.0	12	20	29	28	52
$Ca(OH)_2+Mg(OH)_2$[4]	100	93	99	81	100	
Selectively Calcined Dol.	99	98	97	73	100	
$MgSO_4 \cdot 7H_2O$	100	100	100	100	97	
Active Magnesium Oxide	100			100		

[1] See footnote 2 to Table 2.
[2] Used as in the official method for available P_2O_5 in Thomas slag.
[3] Solvent and method as suggested by MacIntire, Hardin, and Shaw for available P_2O_5. MgO determined in residue.
[4] Mixture of $Ca(OH)_2$ and $Mg(OH)_2$ in molecular proportions.
[5] Estimated from residual carbonates in soil. Average of combined results of Dawson and coworkers and of Collins & Speer.

Soil," were obtained by averaging all the results found by these workers for the fraction of dolomite decomposed during 75–85 days in a soil on which a crop was growing. The initial *p*H of the eight soils ranged from 4.6 to 5.6. Part of the data used in obtaining the averages was kindly supplied by Dawson and by Collins in private communications.

In the present study the solubility of 1 gram samples of each dolomite alone was determined in each of the four solvents, neutral ammonium citrate, the special citrate solution, 2 per cent citric acid, and the citrated

ammonium nitrate suggested by MacIntire, Shaw, and Hardin (6) for the determination of available P_2O_5. The first two citrate solutions were used in the same manner that neutral ammonium citrate is used in the official method for available P_2O_5 in acidulated samples (7), but the 2 per cent citric acid was used as directed in the method for available P_2O_5 in Thomas slag (7), except that a 1 gram sample in 100 ml. was used instead of 5 grams in 500 ml. The citrated ammonium nitrate was used as specified by MacIntire, Shaw, and Hardin, except that magnesium was determined in the insoluble residue rather than in the solution, as is recommended for P_2O_5. In addition to the work on the dolomites alone, their solubility in the special citrate solution was also determined when freshly incorporated in the 6–8–6 base used by Dawson *et al.* and by Collins and Speer. This is the base described in Table 1. Freshly made mixtures were used to avoid changes in the solubility of the dolomitic magnesium due to reactions with other components of the fertilizer and to make the work strictly comparable with the plant tests, in which the dolomite was kept separate from the fertilizer until both were incorporated in the soil. The mixtures were adjusted to give the same amount of total MgO in each case. For purposes of comparison a synthetic mixture of the hydrated oxides of calcium and magnesium in equimolecular proportions,[1] selectively calcined dolomite, and magnesium sulfate were included in the tests. The results are collected in Table 4. When used on the 6–8–6 mixtures, the special citrate gave results that were in fair agreement with the averaged pot tests and nearly as good agreement was obtained when this solvent was used on 1 gram samples of the dolomite alone. Composite dolomite C is completely out of line in both cases. If this dolomite be excepted, then the agreement is good except for the two finest fractions. The other three solvents gave results that were consistently low. In a previous study of these three composite dolomites B had been rated as reactive, A as of medium reactivity, and C as relatively inactive. This rating was based on their solubility in neutral ammonium citrate when ground to pass 100-mesh. It is interesting to note that all the pot and chemical tests here reported place them in the same order. The difference between A and C in the pot tests is hardly significant.

The selectively calcined dolomite alone was about equally soluble in three of the solvents, but was relatively less soluble in the special citrate. The digestion of a 1 gram sample of this material in the special citrate results in the formation, during the digestion, of a copious white precipitate, and it is suggested that sufficient of the citrate ion was removed by this precipitate to reduce the activity of the solvent and so give low results by dissolving less magnesium. This precipitate was not observed in the other solvents. However, when selectively calcined dolomite was

[1] This mixture was designated "hydrated dolomite" in the papers by Dawson *et al.* (2) and Collins and Speer (1).

included in the mixture it was practically 100 per cent soluble in the special citrate. The magnesium sulfate by itself was completely soluble in all four solvents but was very slightly less soluble after incorporation in the mixtures.

SUMMARY

A comparison of chemical methods for determining the availability of magnesium from various carriers is presented. Tests with several solvents indicate that active magnesium oxide is completely available, although confirmation by actual plant tests is lacking. A special citrate solution recommended by Smith gave results for the availability of dolomitic magnesium that more closely approximated the results of plant tests than did any of the other solvents tried.

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IMPROVED METHOD FOR RAPID ESTIMATION OF LEAD IN MAPLE PRODUCTS*

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An accurate and rapid method for the estimation of minute quantities of lead is necessary for the work that is being conducted here to find the source of the lead contamination of maple sap and maple sirup and of foods made from the sirup. The adoption of reasonable means to prevent

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contamination and to remove the lead from the foods already contaminated are also objectives. The electrolytic and sulfide methods are not suitable. A study of the colorimetric determination of lead, with dithizone as the reagent, which was first suggested by Fisher and Leopoldi (6), together with the many modifications suggested by others (1, 2, 7, 8, 9, 10, 11, 12, 13, 14), has resulted in a method that is based on the "mixed color" method of Clifford and Wichmann (3), but uses a photoelectric photometer instead of the neutral wedge photometer as well as a modification of the sampling and details of the lead extraction. The procedure of the modified method is as follows:

LEAD IN MAPLE PRODUCTS

REAGENTS

High grade reagents are used throughout, but only the dithizone is purified.

(a) *Hydrochloric acid.*—Approximately 35%.

(b) *Ammonia-cyanide-citrate solution.*—40 grams of KCN, 20 grams of citric acid, and 500 ml. of ammonia (sp. gr. 1.18) diluted to 1000 ml. with water.

(c) *Ammonia-cyanide.*—20 grams of KCN and 150 ml. of ammonia diluted to 1 liter.

(d) *Chloroform.*—Must be free of oxidizing material so that a very dilute solution of dithizone will retain the green tint and not change to yellow on standing.

(e) *Indicator.*—1% o-cresolphthalein in alcohol.

(f) *Cotton.*—Absorbent cotton made lead free by washing with 1% HNO_3 and water, then dried.

(g) *Dithizone (diphenylthiocarbazone).*—The Eastman reagent has always been purified by the A.O.A.C. method. Three dithizone solutions are used:

Solution A.—A stock solution of high concentration (0.2 gram per 100 ml. of $CHCl_3$) from which the standard solutions B and C are prepared. For convenience this stock solution is kept in a separatory funnel. To prevent oxidation it is kept under a strong aqueous reducing solution of either SO_2 or a 10% solution of hydroxylamine hydrochloride. Before any of this dithizone-chloroform stock solution is used, it is shaken vigorously with the overlying reducing agent and the two layers are allowed to separate.

Solution B.—Used in the final extraction of the lead. A portion of solution A is diluted with $CHCl_3$ so that when 25 ml. of the resulting solution reacts with 60 gamma of lead to form lead dithizonate there will be a slight excess of dithizone, which is shown by a faint purple color. Greater accuracy in the photoelectric measurements of the quantities of red lead dithizonate formed is obtained by using as dilute solutions of dithizone as possible. The tintorial power of the dithizone in $CHCl_3$ is so great that very slight differences in concentrations would cause serious error. The strength of this solution is therefore not expressed in the usual manner of per cent but instead in terms of light absorption as measured by the photoelectric photometer and galvanometer. Fifteen ml. of the dithizone solution is filtered through cotton into a test tube, which is placed in the photometer, and the strength of the solution is recorded in terms of galvanometer deflection. All subsequent solutions B of the standard dithizone are made so that this same galvanometer reading is obtained. This is the only dithizone solution that need be made with great accuracy.

Solution C.—Prepared from the strong stock solution A by dilution with $CHCl_3$ until the concentration of dithizone is approximately twice that of B as measured by the color photometer.

APPARATUS

(1) *Separatory funnels.*—250 ml. Squibb type for first extraction and 150 ml. Scientific Glass Apparatus No. J1880 for second or final extraction. A 15 ml. calibration mark is made on the narrow portion of each.

(2) *Sirup pipet.*—26 cm. × 1.5 cm. with large open tip calibrated to transfer 16.67 grams of standard sirup at room temperature.

(3) *Photoelectric color photometer and galvanometer.*—The Evelyn (13, 14) type colorimeter was chosen because it permits the measurement of absorption of light by any solution from zero to 100% without resorting to dilution methods, and of even greater importance because it utilizes test tubes for the absorption cells, which are cheap and more easily used than the rectangular type. The galvanometer should be sufficiently sensitive to give a full scale deflection when the necessary filters are used.

(4) *Eastman Kodak Wratten Light Filter No. 65A.*—This is a blue-green filter with a maximum of light transmission near that of the unchanged dithizone but a high absorption in the region of the light transmitted by the lead dithizonate. Accordingly the amount of light absorbed is almost completely dependent upon the quantity of lead present.

STANDARDIZATION OF INSTRUMENT

Since the photoelectric cell measures by means of the galvanometer the amount of light absorbed by the red, lead dithizonate formed, a relationship exists between galvanometer reading and the amount of lead present in the test solution. A curve can be constructed calibrating the galvanometer in terms of amount of lead present in the test solution. The lead standards are prepared by dilution with 1% HNO_3 portions of a stock solution that contains 0.75 gram of lead as recrystallized $Pb(NO_3)_2$ in 100 ml. of 1% HNO_3. The concentration of lead in the series of standards was 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 60 gamma, respectively, per 50 ml. of acid solution. Fifty ml. of each of the standard lead solutions is transferred to the 150 ml. separatory funnels. Two drops of the indicator are added, and the solutions are neutralized (*p*H 9) with the ammonia-cyanide solution. Twenty-five ml. of standard dithizone solution B is added, and the mixture is shaken well to extract all the lead from the aqueous solution. The two layers are allowed to separate and the $CHCl_3$ solution is drawn off and filtered through cotton into the test tube (absorption cell). The instrument is adjusted so that the galvanometer shows maximum deflection with no tube in the instrument. This setting of the instrument is to be followed in all subsequent analyses. Each test tube is in turn placed in the instrument, and the galvanometer deflection for each one is observed. The plotting of the galvanometer values vs. that of the gamma of lead gives the standardization curve.

The relationship between the amount of transmitted light and the quantity of lead is not linear. However, the curve has the greatest slope for the lowest lead values; thus this portion of the curve can be read with greatest accuracy. It is this portion of the curve most frequently used in maple sirup work. The curve has been repeatedly verified and is thoroughly reliable and reproducible.

PREPARATION OF SAMPLE

By means of a pipet 16.67 grams of a sirup is transferred to a 250 ml. separatory funnel containing 25 ml. of the dilute HCl. If sugar is used, 16.67 grams is dissolved in water and then transferred to the separatory funnel containing the acid, and the mixture is shaken well to insure complete solution of the lead; 25 ml. of dithizone solution C and two drops of indicator are added, and the mixture is neutralized by addition of the ammonia-cyanide-citrate solution. The same quantity is used in all the sirup samples of a given series. The mixture is shaken vigorously for 1 minute,

the layers are allowed to separate, and 15 ml. of the $CHCl_3$-dithizone layer is run into the calibrated 150 ml. separatory funnels. Some maple sirup samples produce an emulsion during this first lead extraction. In such case the emulsion is filtered into an intermediate 150 ml. separatory funnel equipped with a 2-holed stopper containing a short-stemmed funnel with cotton filter and a tube through which the suction is applied. Sufficient cotton should be used to absorb most of the water of the emulsion and produce a clear layer of the $CHCl_3$-dithizone, 15 ml. of which is then transferred to the calibrated separatory funnel. Fifty ml. of 1% HNO_3 is added, and the solution is shaken 2 or 3 minutes, or until the dithizone layer appears blue-green, indicating complete removal of the lead. After the layers have separated, the $CHCl_3$ is drawn off and discarded, and 25 ml. of solution B and two drops of indicator are added, and the mixture is neutralized with the ammonia-cyanide. After all the lead from the aqueous layer has been extracted by shaking, a portion of the $CHCl_3$-lead dithizonate solution is transferred by filtering through cotton into the colorimeter test tubes. The reading is made as in the standardization, and this value is translated into gamma of lead by means of the calibration curve. Simultaneously a blank determination is run under the same specifications used in the analysis of the sample. The amount of lead found is deducted from that observed for the sample.

The weight chosen for the amount of sample to be analyzed is 16.67 grams since the final analysis is made on a 15/25 or 10 gram aliquot of the original sample. As the standardization curve gives the lead concentration in terms of gamma of lead, the value automatically expresses the lead analysis in tenths of a part per million.

RESULTS

The method was checked on sirup to which known quantities of lead were added and found to give complete recovery. The results were also compared with results obtained by other chemists using different methods for the analysis of a given sample of sirup.

Aliquots of a sample of maple sirup known to contain lead were sent to 11 chemists interested in this subject. No method of analysis was recommended or suggested, nor did the chemists report the method used. The samples analyzed in these experiments were sent out from two different sources, three from the New York State Agricultural Experiment Station and three from the Central Experimental Farms, Ottawa, Canada. The results of the analyses are given in the table.

Since the true lead content of these sirups is not known, it must be assumed that the average of all the submitted results is the true value. The values obtained by this method are consistently in closer agreement with the mean average than are the values obtained by the methods used by other chemists.

DISCUSSION

With the photoelectric cell the lead can be determined with an accuracy of less than 0.2 p.p.m. Since a small sample is used, the quantity of zinc that often accompanies lead as an impurity in maple sirup will be low, and therefore the amount of reagents necessary to prevent interference of the zinc can also be kept low. The adjustment of the *p*H of the solution

TABLE 1.—*Analysis of check samples*

ANALYST	NEW YORK STATE SAMPLES						CANADIAN SAMPLES					
	NO. 1		NO. 2		NO. 3		NO. 1		NO. 2		NO. 3	
	p.p.m.	*g./lb.*	*p.p.m.*	*g./lb.*	*p.p.m.*	*g./lb.*	*p.p.m.*	*g./lb.*	*p.p.m.*	*g./lb.*	*p.p.m.*	*g./lb.*
A			1.2	.009	1.3	.009	1.2	.009	2.6	.018	5.6	.039
B	4.7	.033	1.2	.008			1.0	.007	2.4	.016		
C	4.4	.031	1.2	.008	1.1	.008	0.9	.006	2.4	.017	4.6	.032
D	5.0	.035	1.3	.009	1.3	.009	1.1	.008	2.4	.017	4.6	.032
E			0.9	.006	(1.3)	.009	0.9	.006				
F			1.2	.009			0.8	.006	2.8	.020	5.1	.035
G	4.6	.032	1.0	.007	1.0	.007	1.0	.007	2.4	.017	4.3	.030
H	4.7	.033	0.9	.006			0.9	.006	(2.7)	.019		
J	4.7	.033	1.3	.009	1.4	.009	1.0	.007	2.6	.018	5.0	.035
K			1.0	.007	1.2	.008	1.1	.008	2.8	.019	4.7	.033
C. O. Willits	4.5	.032	1.2	.008	1.2	.008	1.0	.007	2.7	.019	4.7	.033
Average	4.7	.033	1.1	.0087	1.1	.008	1.0	.007	2.6	.018	4.8	.034
Deviation of this method from the average	0.2	.001	0.0	.001	0.1	.000	0.0	.000	0.1	.001	0.1	.001
Maximum deviation from the average	0.3	.002	0.2	.003	0.3	.001	0.2	.002	0.2	.002	0.8	.005

with ammonia is carefully controlled by the use of an indicator since an excess of ammonia will extract some of the color of the green dithizone from the chloroform, which would cause serious error in the photoelectric measurement. The chloroform solutions must be filtered before being tested since the presence of droplets of water will cause incorrect reading of the instrument. In the analysis of maple sirup the emulsions that often occur during the first extraction may be difficult to break by centrifuging, but the method of filtering the emulsion through a pledget of cotton has always completely separated the aqueous and chloroform layers. The chloroform aliquot of the first extraction is easily drawn off from a separatory funnel and does not require the introduction of a pipet through the upper aqueous layer. The number of transfers of the sample has been kept to a minimum, which partially eliminates a source of error.

As this method does not require the running of a set of standards each time an analysis is made, there is a consequent saving of time and reagents, especially if only a few samples are to be run. It is advisable, however, to recheck the standardization curve once every two or three months. The adjustment of the concentration of the dithizone-chloroform solution by means of the photometer instead of by the weight dilution method permits making duplicate standard solutions, which must be obtainable when a standardization curve is used. The accuracy of the determination of lead by the dithizone method is greatly improved by adjusting the strength of the standard dithizone solution colorimetrically.

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ESTIMATION OF UNITS OF VITAMIN D AND VITAMIN A IN FISH LIVER OILS AND THEIR CONCENTRATES

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VITAMIN D

The A.O.A.C. method for the determination of vitamin D in fish oils[1] has been decidedly improved since work on it was begun. The next logical step is the expression of the results in units of vitamin D. This form of expression is more desirable than the mere statement that the oil is as guaranteed, or especially, that it is below the guaranteed standard. One object of the present paper is to aid in developing a procedure for expression of the results as units of vitamin D.

For this work there were used the A.O.A.C. method, with slight modifications; 5 units of the U.S.P. reference cod liver oil per 100 grams of feed as a negative control; and 15 units per 100 grams of feed as a positive control. The U.S.P. reference cod liver oil for vitamin D was therefore fed at levels of 5, 10, and 15 units per 100 grams. The oil to be tested was fed at 10 units (calculated from guaranteed potency) and was thus bracketed with three comparisons of the standard oil. This method of feeding is desirable, because the spread in bone ash between the levels of feeding of the U.S.P. oil gives some idea as to the adequacy of the test.

Six separate tests were run by this procedure on a total of 17 oils. The percentages of bone ash are given in Table 1. In four of the six sets, the spread in the bone ash between the three levels of 5, 10, and 15 units of U.S.P. reference oil seems to be satisfactory. In set C, however, the 15 units of vitamin D from the reference oil produced only 37.98 per cent bone ash, compared with 37.46 per cent produced by 10 units. In set E, the ash produced by 10 units of vitamin D from the reference oil was only 34.43 per cent compared with 33.78 per cent produced by 5 units and 41.02 per cent produced by 15 units. Obviously the ash with 15 units is too low in set C, and the ash with 10 units is too low in set E.

METHOD OF CALCULATION

The following method of calculating the units of vitamin D was devised:

For oils giving less ash than the 10 units of U.S.P. reference oil, subtract the ash for 5 units of the reference oil from the ash for 10 units of the reference oil. Divide the difference by 5, which gives the value of 1 unit (Factor A). Then subtract the ash obtained from 10 units of the oil tested from the ash for 10 units of the reference oil. Divide this difference by Factor A above, to 0.1 unit, and subtract the result from 10. This gives the value of the oil tested as related to 10 units. To obtain the

[1] *Methods of Analysis, A.O.A.C.*, 1935, 351.

TABLE 1.—*Vitamin D in fish oils*

OIL TESTED	UNITS FED	ASH IN BONES (PER CENT)					
		TEST A	TEST B	TEST C	TEST D	TEST E	TEST F
Reference oil	5	36.20	32.92	33.73	35.26	33.78	36.60
Reference oil	10	38.61	36.25	37.46	41.09	34.43	39.98
Reference oil	15	43.42	40.84	37.98	43.65	41.02	41.82
1	10	37.74			37.76		
2	10	37.26			36.82		
3	10	36.51		37.03			39.17
4	10	35.49		39.16			37.48
5	10		35.23		37.00		
6	10		35.39		39.09		
7	10		35.39		39.19		
8	10		33.21		39.16		38.58
9	10		35.30		40.54		39.73
10	10			35.88		36.00	
11	10			35.15		35.81	38.95
12	10			36.54		33.34	40.40
13	10			35.41		35.38	
14	10			36.58		34.51	
15	10			34.47		34.36	36.65
16	10			33.67		39.74	43.09
17	10				40.41	36.56	

number of vitamin D units per gram of oil multiply this last figure by the guaranteed units of vitamin D in the tested oil and divide by 10.

For the oils giving more ash than the 10 units of reference oil, subtract the ash obtained from 10 units of reference oil from that obtained by 15 units of reference oil, and divide by 5 (Factor B). Subtract the ash obtained from 10 units of reference oil from the ash obtained from the test oil. Divide the difference by Factor B, to 0.1 unit, and add the results to 10 units. This gives the relative quantity. Then as above, multiply by the guaranteed potency and divide by 10 to get units. Factors A and B are given in Table 2.

TABLE 2.—*Bone ash equivalent to 1 unit of vitamin D*
(10% of the 10 units fed)

	FACTOR A BELOW 10 UNITS	FACTOR B ABOVE 10 UNITS
Set A	.48	.96
Set B	.67	.92
Set C	.75	.10
Set C (corrected)	.75	.50
Set D	1.17	.51
Set E	.13	1.32
Set E (corrected)	.72	.72
Set F	.68	.37

The results obtained with this method of calculation are given in Table 3. As stated previously, the ash with 15 units of vitamin D from the reference oil in set C is too low compared with that from the 5 and 10 units. Unless correction is made, the units for oil No. 4, which had an ash value above the 10 units of reference oil, would be too high. Such is clearly the case, as shown in Table 3. The units for the remainder of the oils in test C are correct since the percentage of ash obtained for them was below the 10 units of standard oil.

TABLE 3.—*Units of vitamin D in fish oils*

	GUARANTEE UNITS PER GRAM	UNITS FOUND							
		TEST A	TEST B	TEST C	TEST D	TEST E	TEST E CORRECTED	TEST F	AVERAGE
1	85	70			61				66
2	400	288			256				272
3	100	56		94				88	79
4	400	140		(54)				252	196
5	400		340		260				300
6	400		348		332				340
7	400		348		336				342
8	400		(220)		336			316	326
9	400		344		380			384	369
10	400			316		(448)	324		320
11	400			(276)		(440)	312	340	326
12	400			352		(64)	(176)	444	398
13	100			73		(107)	72		73
14	200			176		(212)	120		148
15	85			51		(81)	49	43	48
16	85			(42)		(119)	113	156	135
17	400				376	(464)	352		364

In test E the percentage of bone ash with 10 units of standard oil is too low as compared with the bone ash at 5 units or 15 units. When this low result is used as a basis of comparison, the units of vitamin D for the oils tested will be too high. The results of this test, given in parentheses in Table 3, when compared with the results of other tests on the same oil, are obviously too high for 6 out of 8 oils. The units of vitamin D were also calculated by using the average bone ash of the 15 units and the 5 units as the value for 10 units. These results, as given, are more probable. The average units in the 17 oils tested are also given in Table 3. In making these averages, the usual practice of excluding wide results was followed. The averages with 3 of the oils are too irregular to be satisfactory, but the results with the other 14 oils are quite satisfactory and indicate that the method is workable. It cannot be expected that as close agreement between repetitions can be made with a biological method as with many

other ordinary chemical methods, and this fact must be considered when the results are examined.

The irregular result in ash obtained with the 10 unit level in test E seems to be fairly well corrected for by using the average of the 5 and 15 unit levels as was done in this work. Another way to guard against irregular results would be to have two lots of chickens on the 10 unit level. This procedure was tried with test F; the second lot gave 39.96 per cent ash as compared with 40.02 per cent for the first.

VITAMIN A

Guarantees of vitamin A as well as of vitamin D are made on the fish oils, but so far no tests have been reported. In the tests here reported, a spectrographic and a biological method were used. The spectrographic method is the procedure used by Fraps and Kemmerer for spectro vitamin A in butter,[2] and briefly is as follows:

> Saponify 1 gram of cod liver oil with alcoholic potash and extract therefrom with ethyl ether. After washing free from alkali and drying over anhydrous sodium sulfate, remove the ether in vacuo and take up the residue in 10 ml. of methanol. Chill the methanol solution of vitamin A in an ice-salt mixture, filter, and dilute an aliquot part of the filtrate to the proper volume. Determine the spectro vitamin A in this solution by measuring the absorption at 328 millimicrons.

The U.S.P. reference oil was subjected to the same test, and the value of the spectro vitamin A in terms of U.S.P. units was ascertained by dividing the units of the reference oil (3000 per gram) by the units of spectro vitamin A. The spectro vitamin A of the oils tested was then multiplied by this factor to obtain the U.S.P. units of vitamin A in the oils tested.

The biological potency of the oils was also compared with that of the U.S.P. reference oil by a method similar to the U.S.P. method. Rats that had been depleted of vitamin A were placed on the regular test diet for vitamin A in 10 groups of 10 rats each, the groups being equalized with respect to weight and sex. The biological estimations were made in two tests. In the first test, the U.S.P. reference oil was fed at 4 and 6 International units twice a week and the remaining 8 groups were fed the oils to be tested at 6 International units twice a week. In the second test the U.S.P. reference oil was fed at 3 levels of 2, 4, and 6 International units twice a week, and 7 test oils were fed at 4 International units twice a week. The oil was diluted with refined cottonseed oil. At the end of the 4-week test period, the rats were weighed, and the average weights for those receiving the U.S.P. reference oil were plotted on semi-log paper. By comparison with this curve, the vitamin units of the oils being tested were determined.

Table 4 shows a comparison of the results by both methods. According

[2] Texas Agr. Expt. Sta. Bull., 560 (1938).

to these results, 18 of the samples of oil were below guarantee, while 6 were up to guarantee or past. The ratio of the biological to the spectrographic results in 11 comparisons varies from 0.58 to 1.41. These two,

TABLE 4.—*Vitamin A by spectrographic and biological methods*
(U.S.P. units per gram)

NO.	U.S.P. UNITS			RATIO OF BIOLOGICAL TO SPECTRO UNITS
	GUARANTEE OF VITAMIN A	SPECTROGRAPHIC	BIOLOGICAL	
1	3000	2156	—	
2	3000	2389	2850	1.19
3	600	674	950	1.41
4	3000	2105	—	
5	1300	1478	—	
6	3000	846	—	
7	1000	1007	833	.83
8	3000	2118	—	
9	3000	2415	2100	.87
10	3000	1743	1007	.58
11	3000	2492	—	
12	3000	2944	—	
13	3000	1989	2050	1.03
14	3000	1420	1287	.91
15	3000	2260	—	
16	3000	2401	1900	.79
17	3000	2299	—	
18	3000	2131	2375	1.12
19	3000	1678	1875	1.12
20	800	898	—	
21	3000	2272	—	
22	1500	1850	1642	.89
23	600	833	—	
24	3000	1162	—	

however, are the extremes. Six of the oils deviated 13 per cent, and 3 deviated 13–21 per cent. The spectrographic method gave higher values than the biological method with 6 of the 11 oils compared, while one was practically the same. Two were within 12 per cent, one within 19 per cent, and one was 41 per cent higher by the biological than the spectrographic method. The spectrographic method is satisfactory for rapid preliminary testing of the oils.

SUMMARY

Vitamin D was determined by the A.O.A.C. chick method modified by the feeding of the U.S.P. reference oil at 5, 10, and 15 units per 100 grams of feed, and the oils to be tested at 10 units (as guaranteed) to 100 grams of feed. Six sets were run on 17 oils. The units of vitamin D were cal-

culated from the ash analyses and reasonably concordant results were obtained.

Vitamin A was estimated by spectrographic and by biological methods on 24 samples of fish liver oils or concentrates, and 18 samples were appreciably below guarantee. The spectrographic method is satisfactory for rapid preliminary testing of the oils.

DETERMINATION OF CAROTENE IN PRESENCE OF LYCOPENE

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As has been pointed out by Shinn, Kane, Wiseman and Cary,[1] and by Fraps and Kemmerer,[2] carotene solutions prepared by the tentative A.O.A.C. method contain impurities. Fraps and Kemmerer[2] report that these impurities can be removed from alfalfa hay and other substances, at least to some extent, merely by shaking 50 ml. of the crude carotene solution with 2.5 grams of magnesium carbonate, especially prepared so as not to adsorb carotene but to adsorb crude xanthophyl prepared from alfalfa leaf meal. This magnesium carbonate is called the xanthophyl reagent.

Some foods, such as watermelons, tomatoes, and red peppers, contain lycopene or other reddish pigments that are not completely removed by treatment of the solution with the xanthophyl reagent. The lycopene is present in sufficient amounts to give a reddish tinge to the solution of crude carotene prepared by the tentative A.O.A.C. method. It was adsorbed, however, by one of the adsorbents prepared as reported in previous work.[2]

Experiments were therefore made to ascertain how to prepare a reagent that would adsorb lycopene but would not adsorb carotene. A test solution of crude lycopene was prepared from canned tomato pulp by the following procedure: One hundred grams of canned tomato pulp was refluxed for 30 minutes with 250 ml. of saturated alcoholic potash. After being cooled to room temperature, the carotene fraction, which contained the lycopene also, was extracted with petroleum ether, as in the Hughes-Peterson modification of the Guilbert method.[3] The petroleum ether extract was washed with 90 per cent methanol to remove xanthophyl and then with water to remove any traces of methanol. The solution was then dried over anhydrous sodium sulfate. This solution contains a large quantity of lycopene and a small quantity of carotene and other pigments. As the presence of carotene would interfere in testing the lycopene-adsorb-

[1] *J. Biol. Chem.*, 119, Proc. 31st meeting, 89 (1937).
[2] *This Journal*, 22, 190 (1939).
[3] *Ibid.*, 79.

ing properties of a reagent that should not adsorb carotene, further purification was necessary. The solution was diluted to 200–400 ml. with light petroleum ether and shaken with 50 grams of the xanthophyl reagent. This treatment removed some impurities. The filtrate was next shaken with a preliminary preparation of magnesium carbonate that would adsorb much of the lycopene but would not adsorb carotene. The magnesium carbonate was added gradually with shaking until 50–75 per cent of the color was removed. The adsorbent containing the lycopene was filtered off and washed three times with petroleum ether. The purified lycopene was eluted from the magnesium carbonate with petroleum ether containing 2 per cent ethanol. The eluate was washed free from alcohol, dried over sodium sulfate, and made up to contain 0.5–0.8 p.p.m. of color estimated as carotene.

This lycopene solution is unstable. The quantity of lycopene decreased very noticeably in a week, even though the solution was kept in the dark in cold storage. Therefore it should be freshly prepared every week and tested before use.

To test the reagent, the color of the test solutions of lycopene and of carotene were first read in a KWSZ photoelectric photometer, with color filters adapted for the estimation of carotene. Then 3–5 grams of the reagent was shaken with 50 ml. of each test solution and the color again read. To be satisfactory the reagent must adsorb all the lycopene from a solution containing 0.5–0.8 p.p.m. of lycopene and no carotene from a solution containing the same amount of carotene. Since xanthophyl is more easily adsorbed than lycopene, it will also be adsorbed by this lycopene reagent. After a number of trials, a lycopene reagent was prepared by the following method:

PREPARATION OF LYCOPENE REAGENT

Heat 100 grams of $MgCO_3$ in an electric furnace at 200° C. for 1 hour. Test the reagent for adsorption of carotene. If the reagent does not adsorb carotene, test with the lycopene solution. If 3–5 grams of the reagent extracts all the red pigment from 50 ml. of the lycopene solution, the reagent is ready for use. However, if the reagent adsorbs carotene, small quantities of water (3 ml. at a time) may be added and the reagent again tested. Repeat this treatment until the reagent does not adsorb carotene. Then test with lycopene solution as before. As this lycopene reagent is unstable, it must be kept in a tightly closed container to prevent its moisture content from changing and it must be tested each time it is used.

This method was successful with the magnesium carbonate used. Differences between different lots of the same kind of adsorbents have been pointed out by Fraps, Kemmerer, and Greenberg.[4] Some modifications of the procedure, such as longer heating, may be required to adapt the method to different lots of magnesium carbonate.

A comparison of the lycopene reagent and the xanthophyl reagent in

[4] *Ind. Eng. Chem., Anal. Ed.*, 12, 16 (1940).

the analysis of crude carotene solutions prepared by the tentative A.O.A.C. method is given in Table 1.

TABLE 1.—*Effect of lycopene reagent and xanthophyl reagent on removal of impurities from crude carotene*

KIND OF MATERIAL	CRUDE CAROTENE IN SAMPLE	PURE CAROTENE—		IMPURITIES	
		AFTER XANTHOPHYL REAGENT	AFTER LYCOPENE REAGENT	BY XANTHOPHYL REAGENT	BY LYCOPENE REAGENT
	p.p.m.	*p.p.m.*	*p.p.m.*	*per cent*	*per cent*
Old alfalfa hay	19.2	15.0	14.8	21.9	22.9
Alfalfa leaf meal, dehydrated	135.0	108.0	103.4	20.0	23.4
Watermelon, Cletex	46.4	42.6	10.8	8.3	76.7
Watermelon, Black Diamond	20.2	—	2.4	—	88.1
Watermelon, Black Diamond	23.0	—	5.0	—	78.3
Dried apricots	45.6	41.7	31.5	8.6	31.0
Red peppers	4.7	2.9	2.1	37.7	55.9
Tomatoes	43.9	43.8	18.0	0.2	59.0

The lycopene reagent removed only 2–3 per cent more impurities than did the xanthophyl reagent from crude carotene solutions prepared from alfalfa. Either of the reagents could be used for alfalfa samples. With watermelon the lycopene reagent removed 76.7 per cent impurity and the xanthophyl reagent 8.3 per cent; with dried apricots, 31.0 per cent and 8.6 per cent; and with red peppers and tomatoes, 55.9 per cent and 59.0 per cent and 37.7 per cent and 0.2 per cent impurities, respectively. For these and similar materials a reagent that removes lycopene should be used.

Plants contain a number of different kinds of carotinoids and products derived from them. The application of this very simple method of selective adsorption to the determination of carotene in different feeds and foods will require the development and use of reagents with adsorptive powers suitable to the material being studied. This paper shows how the method can be applied to foods containing lycopene. As the investigation is pursued, other improvements will be reported.

SUMMARY

A simple method was developed for the preparation of a medium suitable for the selective adsorption of lycopene and related reddish pigments from a crude carotene solution, as prepared by the tentative A.O.A.C. method for carotene. The lycopene reagent removed 76.7 per cent impurity from crude carotene solutions derived from watermelons, compared with 8.3 per cent by the xanthophyl reagent. With dried apricots the lycopene reagent removed 31 per cent and the xanthophyl reagent

8.6 per cent impurity. The crude carotene solution from red peppers and tomatoes also lost much more impurity to the lycopene reagent than to the xanthophyl reagent. The lycopene reagent removed 2 or 3 per cent more impurities from crude carotene from alfalfa, than did the xanthophyl reagent.

The results recorded in this paper and those reported previously[5] show that it is possible to prepare at least two adsorbents with different selective powers for purifying carotene solutions. One (xanthophyl reagent) adsorbs xanthophyl but not lycopene or carotene. The second (lycopene reagent) adsorbs both xanthophyl and lycopene but not carotene.

DETERMINATION OF CHLORIDES IN FEEDING STUFFS*

By GEORGE E. GRATTAN and ALFRED POTVIN

The new Canadian Feeding Stuffs Act requires that in certain concentrated feeds and mineral supplement feeds the percentages of the various mineral ingredients be declared in terms of calcium (Ca), phosphorus (P), iron (Fe), iodine (I), and salt (NaCl). Because the official methods (A.O.A.C.) contained no quantitative procedure for estimating salt, it was necessary to devise such a method.

A review of recent literature on quantitative chemical analysis gave little information on the determination of chlorides in the presence of organic matter. Incineration of the organic matter followed by the extraction and determination of the chlorides in the ash tends to cause loss of chlorides by direct volatilization and also the loss of hydrochloric acid by the action of non-volatile acid oxides on the chlorides. Alkali chlorides are volatile at red heat and it is necessary to run blank determinations when alkaline earth oxides are added to fix the chlorides.

Davies[1] proposed a method for determining small quantities of chlorine in organic matter. He added sufficient silver nitrate solution to react with all the chlorides present and destroyed the organic matter with potassium permanganate and nitric acid. In feeding stuffs containing high percentages of salt, this method would tend to increase the cost of analysis unduly because of the large quantity of silver nitrate necessary to precipitate all the chlorides.

As a preliminary investigation, leaching on a filter paper with both hot and cold water was tried, but the organic matter swelled and prevented filtration. Digestion with water in a flask was also tried in an endeavor to obtain sufficient clear supernatant liquid, but the amount of soluble organic material prevented complete coagulation and interfered with the proper end point.

[5] *This Journal*, 22, 190 (1939).

* Contribution from the Chemical Laboratories, Feed, Fertilizer, Insecticide and Fungicide Control, Dept. of Agriculture, Ottawa, Canada..

[1] *Analyst*, 57, 79–84 (1932).

It was therefore found necessary to destroy the interfering organic matter in such a manner that there would be no loss of chlorides. The samples were first analyzed to determine the percentage of chlorides present, then known quantities of pure sodium chloride were added, and the resulting mixture was used for analysis. The samples were ignited at low heat (550° C.) in the presence of sodium carbonate, *Methods of Analysis, A.O.A.C.*, 1935, 131, 34. This method proved to be quite satisfactory, but it was time consuming and tedious and required an efficient fume cupboard for the ignition.

Oxidation of the organic materials present in a wet condition was tried as a substitute for burning. Digestion with nitric acid gave consistently low results. Chromates and bichromates gave colored solutions that interfered with the volumetric determination. Potassium permanganate was then tried, and this study is presented in this paper.

EXPERIMENTAL

A mixed feed containing numerous plant and animal organics and very little inorganic material was selected as the basic sample. It was ground to pass an 80-mesh sieve and thoroughly mixed. The chlorides were then determined by the sodium carbonate ignition method referred to above.

Sodium chloride of the highest purity obtainable was heated for several hours at 500° C., ground to pass a 100-mesh sieve, heated again to 350° C., and cooled in a desiccator.

Samples were then prepared to contain 5, 10, 15, and 20 per cent of added sodium chloride, and one sample had no added chlorides. To ascertain the approximate amount of potassium permanganate required for oxidation, 5 gram portions of each of the samples were treated with 1, 2, 3, 5, and 8 grams of potassium permanganate in a flask with 200–300 ml. of water, boiled for about 10 minutes, and filtered. The results are given in Table 1.

TABLE 1.—*Appearance of the solution obtained after filtration*

SAMPLE NO.	DESCRIPTION OF SAMPLES	$KMnO_4$ USED—				
		1 GRAM	2 GRAMS	3 GRAMS	5 GRAMS	8 GRAMS
1	low in NaCl or mineral matter	viscous, colored, difficult to filter	colored, difficult to filter	colored	very slightly colored	practically colorless
2	5% NaCl	"	"	"	"	"
3	10% NaCl	"	titration possible, although appearance same as above	slightly colored	"	"
4	15% NaCl	"	"	"	"	"
5	20% NaCl	"	"	"	"	"

The quantity of potassium permanganate required for oxidation varies directly with the amount of organic matter present (Table 1). When Samples 3, 4, and 5 were treated with 2 grams of potassium permanganate, the solution was dark colored and the silver chloride flocculated only after the addition of a large quantity of nitric acid. The solution was then somewhat decolorized, which made the titration possible.

Additional 5 gram portions of the same samples were then treated with 5 grams and 8 grams of potassium permanganate and analyzed. As a comparison the samples were also analyzed by the sodium carbonate-ignition method.

The results are shown in Table 2.

TABLE 2.—*Chlorides in feed*

SAMPLE NUMBER	SODIUM CARBONATE-IGNITION METHOD	OXIDATION WITH $KMnO_4$	
		5 GRAMS	8 GRAMS
	per cent	*per cent*	*per cent*
1	0.84	0.88	0.82
2	5.58	5.72	5.80
3	10.50	10.88	10.87
4	15.62	15.58	15.30
5	20.43	20.77	20.83

It will be observed (Table 2) that the potassium permanganate procedure yields slightly higher results than does the ignition method, with practically 100 per cent recovery of the added salt in the four samples that contained known amounts. There is also a saving of one-third of the time required in addition to the cost of the combustions.

The method follows.

SALT (CHLORINE) IN FEEDS

REAGENTS

(a) *Standard sodium chloride solution.*—Heat reagent-quality (A.C.S.) NaCl in a muffle furnace or over a Meker burner until decrepitation is over but do not melt. Cool in a desiccator. Dissolve 1.648 grams in 1 liter of distilled water. This solution contains 0.001 gram of Cl per ml.

(b) *Standard silver nitrate solution.*—Dissolve 5 grams of $AgNO_3$ in 1 liter of distilled water and adjust the solution so that 1 ml. is equal to 1 ml. of the standard sodium chloride solution.

(c) *Standard potassium sulfocyanate solution.*—Dissolve 2.5 grams of KCNS in 1 liter of distilled water and adjust the solution so that 1 ml. is equal to 1 ml. of the $AgNO_3$ solution.

(d) *Potassium permanganate solution.*—Dissolve about 25 grams of $KMnO_4$ in 100 ml. of boiling distilled water.

(e) *Ferric alum indicator.*—To a saturated solution of $FeNH_4(SO_4)_2.12H_2O$ add approximately an equal amount of HNO_3.

DETERMINATION

Weigh 5 grams of the sample and place in a 500 ml. volumetric flask. Add 200–300 ml. of distilled water and 10–25 ml. of the $KMnO_4$ solution (depending on quan-

tity of organic matter present in sample) in 5 ml. portions. Heat the flask to boiling. Cool somewhat before each addition and continue this procedure until most of the organic matter is oxidized and a black precipitate settles, leaving a clear, easily filtered solution. If an excess of $KMnO_4$ has been added, remove it by the addition of a small quantity of perhydrol H_2O_2. (If organic materials are excessive frothing may occur. This may be minimized by boiling the sample slowly, particularly at the beginning.) Finally cool the solution, make up to volume, and thoroughly mix.

Filter a portion of this solution and pipet an aliquot into a 250 ml. Erlenmeyer flask. For samples containing less than 15% salt, use a 50 ml. aliquot; for samples containing 15% or over, use a 25 ml. aliquot. Add 10–15 ml. of HNO_3 and measure from a buret sufficient quantity of the $AgNO_3$ solution to precipitate all the chlorides present. Add 10 ml. of the ferric alum indicator and shake the flask vigorously so that the AgCl coagulates. If difficulty is experienced in this respect, heating or a drop or two of ether may help to gather the precipitate.

Make the back titration with the KCNS solution until a reddish tint appears. (Best results are obtained with artificial light on a white enameled plate.)

Calculate the amount of Cl from the amount of $AgNO_3$ required to precipitate the chlorides.

$$\% \text{ Cl} \times 1.648 = \% \text{ salt.}$$

It was found that samples less than 5 grams did not give close results, probably due to segregation of the particles or the difficulty in mixing substances showing wide variations in specific gravity.

Table 3 gives results obtained in these laboratories with this method and also with the ignition method.

TABLE 3.—*Chlorides in feeds as determined in Associate Referee's laboratory*

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	SODIUM CARBONATE IGNITION METHOD			OXIDATION WITH POTASSIUM PERMANGANATE		
1	Specially prepared, low in Cl	.75	.71	.84	.75	.84	.92
2	No. 1 + 5 grams pure NaCl	5.22	5.58	5.70	5.87	5.80	5.49
3	No. 1 + 10 grams pure NaCl	10.50	10.65	10.73	10.87	10.87	10.90
4	No. 1 + 15 grams pure NaCl	15.42	15.62	15.60	15.30	15.76	15.68
5	No. 1 + 20 grams pure NaCl	20.12	20.43	20.24	20.78	20.83	20.72
A	High in organic matter	1.39	1.39		1.35	1.55	
B	High in organic matter	3.89	3.99		3.99	4.21	
C	Mod. high in organic matter	16.63	16.68		17.07	17.14	
D	Much less organic matter	28.70	28.97		29.15	29.40	
E	Low in organic matter	5.98	6.02		6.14	6.26	

Samples A, B, C, D, and E, were taken from official samples sent in for analysis.

PROPOSED MODIFICATION OF THE OFFICIAL COLORIMETRIC METHOD FOR DETERMINING VANILLIN IN VANILLA EXTRACTS

By H. J. LYNCH and NEULON DEAHL (Analytical Laboratories of Parke, Davis and Co., Detroit, Mich.)

In determining the vanillin content of concentrated vanilla flavoring extracts containing added vanillin by the official colorimetric method[1] it has not been possible to recover a considerable portion of the vanillin actually added. Since preliminary investigations proved that the vanillin was not being lost by precipitation, by volatilization or by other physical means, a study was made of the assay procedure itself in an effort to account for failure to get theoretical results.

The official method, originally developed by Folin and Dennis,[2] directs that a quantity of the sample that contains 8–12 mg. of vanillin (usually 5 ml.) be diluted with 75 ml. of water, precipitated with 4 ml. of a solution of basic and neutral lead acetates, and then made up to a volume of 100 ml. with water. A rather voluminous precipitate of lead resinate is obtained at this point.

Folin and Dennis called attention to the fact that even in fairly dilute solutions vanillin may be precipitated by basic lead acetate, but stated that they had satisfied themselves that in the very dilute concentrations of vanillin used in their method no vanillin was precipitated by the lead solution. However, since previous investigations by the writers had demonstrated that the vanillin was not being lost, it seemed logical to conclude that it was either being precipitated by the lead solution or being occluded by or adsorbed on the precipitated lead resinate.

A modification of the official precipitation procedure, which permitted washing of the lead precipitate on the filter with water to remove occluded or adsorbed vanillin, was tried. The proper quantity of the extract was placed in a 125 ml. Erlenmeyer flask and diluted with sufficient water so that the additon of 4 ml. of the lead solution gave a volume of 50 ml. This solution was then filtered into a 100 ml. volumetric flask, and the precipitate was washed with water until 100 ml. of filtrate was obtained. After being mixed, 5 ml. of this filtrate was taken for assay as directed in the official method. This method, which will hereafter be referred to as the "Filtration Modification," gave results definitely higher than those obtained by the official method, but still failed to account for all the vanillin known to be present.

Subsequent investigation showed that the vanillin could be removed from this lead precipitate practically quantitatively, but such a large volume of water was required and the washing time was so long that the

[1] *Methods of Analysis, A.O.A.C.*, 1935, 307.
[2] *J. Ind. Eng. Chem.*, 4, 670 (1912).

method was considered impracticable. It did, however, prove that a quite appreciable quantity of vanillin was carried down with the lead precipitate.

These results seemed to indicate that the practical solution of the problem depended upon precipitation in a much more dilute solution to preclude, as far as possible, precipitation of the vanillin. It appeared that there could be no objection to precipitating the resins at the same time and in the same flask in which the color is developed by the vanillin reagent. Inasmuch as the sodium phosphate formed on the addition of the sodium carbonate solution must be removed by filtration before a colorimetric comparison can be made, the precipitation of the resins in this same solution eliminates one precipitation and filtration procedure.

The details of the proposed procedure are as follows:

Transfer to a 100 ml. volumetric flask a quantity of the sample that contains 8–12 mg. of vanillin, dilute to 100 ml. with water, and mix. Transfer 5 ml. of this dilution to a 50 ml. volumetric flask, add 0.2 ml. of the lead solution (50 grams each of basic and neutral lead acetate per liter), and mix well. Into another 50 ml. volumetric flask pipet 5 ml. of standard vanillin solution (1 ml. = 0.1 mg. of vanillin). To each of these flasks add from a pipet 5 ml. of the reagent, allowing it to flow down the neck of the flask in such a way as to wash down the vanillin solution that may be on the sides of the flask. Mix the contents of the flasks by rotating and after 5 minutes dilute the contents to 50 ml. with saturated $NaCO_3$ solution. Mix thoroughly by inverting the flask several times and allow to stand at least 10 minutes so that the precipitate that forms may separate completely. Filter the solutions through dry filter papers and compare the blue color of the clear solutions in a colorimeter. Report results as grams of vanillin per 100 ml. of extract.

The special concentrated vanilla flavoring extract on which these original assays were made is prepared by the following steps, which gave an excellent opportunity to check the results under different conditions:

A.—Percolate, which is essentially a double-strength extract of vanilla.

B.—A, to which a known quantity of vanillin, dissolved in alcohol, is added.

C.—B, after the addition of sugar, adjustment of alcohol content, and dilution to a definite volume.

Samples of each of these preparations were assayed by the official colorimetric procedure and by each of the modifications previously mentioned. The results are shown in the following table.

VANILLIN (GRAMS PER 100 ML.)

	A	B			C		
		THEORY*	FOUND	PER CENT RECOVERY	THEORY*	FOUND	PER CENT RECOVERY
Official Method	0.4545	1.4061	1.1173	79.46	1.0899	0.9478	86.96
Filtration Mod.	0.4717	1.4194	1.1765	82.88	1.1028	1.0526	95.44
Precipitation Mod.	0.8583	1.7969	1.7892	99.50	1.3928	1.4544†	104.7

* Calculated on basis of assay under "A," plus vanillin added.

† Colorimeter reading between standard and sample varied 7.5 points, and the readings should vary not more than 5.0.

In addition to the assays on the concentrated vanilla extract with added vanillin, a number of lots of vanilla extract of official strength were assayed by the official and modified procedures. The results follow:

	VANILLIN (GRAMS PER 100 ML.)		
	OFFICIAL METHOD	FILTRATION MOD.	PRECIPITATION MOD.
No. 1	0.1818	0.1923	0.2985
No. 2	0.1843	—	0.3233
No. 3	0.1785	—	0.3189

While there is no official standard for the vanillin content of pure vanilla extracts, it is generally accepted that a good extract should contain about 0.2 gram of vanillin per 100 ml., when determined by the official assay method. If the proposed modification should be adopted, as the experimental data presented seem to justify, it will be necessary to revise current ideas on this point.

THE SHAFFER-SOMOGYI REAGENT FOR THE DETERMINATION OF SUGARS IN PLANT MATERIALS*

By T. A. PICKETT (Agricultural Experiment Station, Experiment, Ga.)

During the past twenty years many sugar methods have been developed, primarily for blood and urine analyses. In the field of plant chemistry these methods have been tried with varying degrees of success. In this laboratory the Shaffer-Somogyi method[1] has been used on plant materials over a period of four years, and it is considered satisfactory for some types of work. The Shaffer-Somogyi reagent is more economical than the Fehling solution used in the official methods of the A.O.A.C.,[2] because it requires considerably fewer chemicals. The method is faster, since one worker can run 120 determinations easily in one day. It can also be used on materials very low in sugars and when material is at a premium, for example, on a leaf or the bark of a single plant, which would be practically impossible to analyze with Fehling's solution because, according to Phillips,[3] that reagent is not accurate for small quantities of glucose.

The Hagedorn-Jensen method,[4] using potassium ferricyanide, has many of these advantages. However, after a few trials in this laboratory it was discarded since variable blanks were obtained in the presence of invertase as reported previously by Schlenker.[5] Munday and Seibert[6] found that the Hagedorn-Jensen reagent was very sensitive to amino acids and other

* Published with the approval of the Director as Paper 69, Journal Series, Georgia Agricultural Experiment Station.

[1] *J. Biol. Chem.*, **100**, 695 (1933).
[2] *Methods of Analysis, A.O.A.C.*, 1935, 135.
[3] *J. Biol. Chem.*, **95**, 735 (1932).
[4] *Biochem. Z.*, **135**, 46 (1923).
[5] *J. Biol. Chem.*, **102**, 29 (1933).
[6] *Ibid.*, **100**, 277 (1933).

substances besides reducing sugars. Phillips,[3] Schlenker,[5,7] and other workers have shown that practically all the sugar reagents they studied gave different results for reducing sugars in plant materials, probably owing to varying reactions with non-fermentable reducing substances.

EXPERIMENTAL

Before comparing the Shaffer-Somogyi and the Quisumbing-Thomas methods on plant materials, the writer ran a few experiments on the Shaffer-Somogyi reagent. The reagent used was the "Copper Iodometric Reagent 50",[1] with the following composition:

	grams per liter
Na_2CO_3 (anhydrous)	25
$NaHCO_3$	20
Rochelle salt	25
$CuSO_4 \cdot 5H_2O$	7.5
KI	5
KIO_3, 0.1 *N* as to I_2, ml.	250

Briefly the method is as follows:

5 ml. of sugar solution is measured into a Pyrex test tube (25×200 mm.), 5 ml. of the reagent is added, and the solutions are mixed. The tubes are covered with glass or lead caps, placed in a boiling water bath for 15 minutes, and then cooled to about 30° C. 2 ml. of 2.5% each of KI and $K_2C_2O_4$, and 5 ml. of H_2SO_4 are added. The contents are well mixed, allowed to stand 5–10 minutes, and titrated with 0.005 *N* thiosulfate, starch being used as indicator. The starch solution was prepared according to Hanes.[8]

A copper-reduction-sugar curve was made on each batch of reagent. In some cases the curves varied slightly with each solution, but each curve remained constant for months.

Copper Reducing Power of Various Substances.—A few compounds that might be present in plant material were treated with the Shaffer-Somogyi reagent. When 5 mg. of each was used, the following materials showed no reducing action: Acetamide, asparagin, calcium gluconate, citric acid, fumaric acid, glutamic acid, levulinic acid, malic acid, mannite, oxalic acid, raffinose, starch, sucrose, thymol, and toluene. Amygdalin and formaldehyde gave slight reducing actions. D-arabinose, ascorbic acid, catechol, cellobiose, fructose, d-galactose, glucose, lactose, d-lyxose, maltose, and l-xylose had various reducing powers. For example, with 1 mg. of the following sugars, the milligrams of copper formed were as follows: lactose 0.85, cellobiose, 1.00, d-arabinose, 1.45, d-galactose, 1.67, l-xylose, 2.38, and glucose 2.69. The copper reduction curves should be determined on each sugar.

Effect of Plant Juices on pH of the Reagent.—The Shaffer-Somogyi reagent has a *p*H of approximately 9.2, and as its constituents form a strong

[7] *Ibid.*, **117**, 727 (1937).
[8] *Biochem. J.*, **23**, 99 (1929).

buffer, the *p*H should theoretically be difficult to change. Shaffer and Somogyi[1] indicated that varying the bicarbonate-carbonate ratio (which affected the *p*H of the solution) changed the quantities of reduced copper. The effect of various plant juices on the *p*H of the reagent was found by adding 5 ml. of juices to 5 ml. of reagent. The tubes were heated for 15 minutes in a boiling water bath, and cooled, and the *p*H values were determined with a glass electrode. The reagent had a *p*H of 9.2, and with 5 ml. of the following juices the *p*H values were as follows: Grape 8.25, cotton leaf 8.8, clarified cotton leaf 9.2, sweet potato 8.4, and clarified sweet potato 9.15. Unclarified juices did have a pronounced effect on the *p*H values, while those clarified with neutral lead acetate and deleaded with sodium oxalate did not.

Temperature of Bath.—At the altitude of Experiment (950 feet), the temperature of the boiling water bath varied between 98.0° and 99.0° C. A series of determinations was run on pure sugars in which the boiling point of the bath was raised with calcium chloride. Within practical limits not enough difference in the quantities of reduced copper was found to warrant a correction factor.

Temperature of Tubes.—A few trials were made to determine the rate of heating of the solution in the tubes and the effect of position of the tubes in the container, a wire basket. Pyrex test tubes containing 5 ml. of reagent and 5 ml. of sugar solution, covered with a lead cap, were placed in a boiling water bath. Temperatures of the solutions were determined with a thermocouple. The results are shown in Figure 1.

These curves show that about 10 of the 15 minutes that the test tubes were kept in the bath were needed for the contents to reach the maximum temperatures. The tubes spaced closely together (about 1 cm. apart) took slightly more time to reach 95° C. than did those tubes spaced farther apart (2–3 cm.). The difference in the rate of heating was not enough to make a perceptible difference in reduced copper.

Determination of Fructose in Presence of Glucose.—Fructose has always been difficult to determine in the presence of glucose; however, it is known that fructose is a more active sugar than glucose (reduces copper faster). The tentative Jackson-Mathews modification of Nyns selective method,[9] using a copper carbonate reagent, and Strepkov's method[10] with a potassium ferricyanide microchemical reagent, determine fructose in the presence of glucose by using relatively long reaction periods and low bath temperatures, at which temperatures fructose reacts much more vigorously than glucose. A few trials were made with the Shaffer-Somogyi reagent. Some of the results are given in Table 1.

Neither fructose nor glucose had much copper reducing power at 50° C. At 55° C. fructose showed some activity if the concentration was com-

[9] *Methods of Analysis, A.O.A.C.*, 1935, 483.
[10] *Z. anal. chem.*, 111, 57 (1937).

paratively large or the reaction period was long, while glucose had very little activity even for long periods of time except at high concentrations. At 60° C. fructose was active at all concentrations and reaction periods, while small quantities of glucose were relatively inactive. Glucose became too reactive to warrant using higher temperatures unless modifications in the method were made. From the figures given in Table 1 and other data,

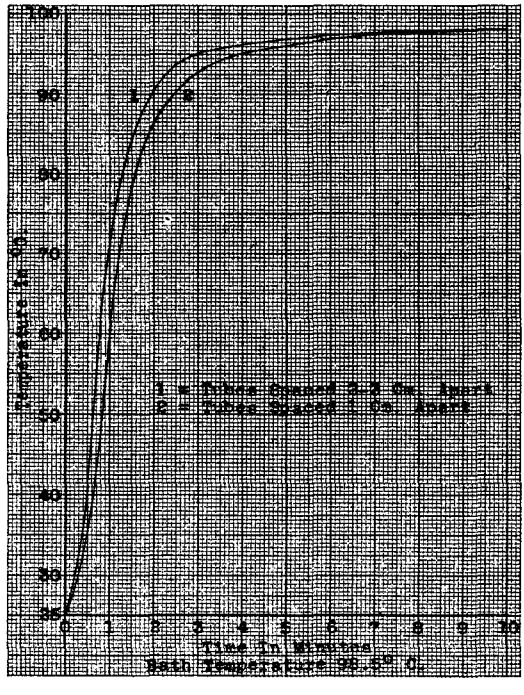

FIG. 1. HEATING RATES OF SOLUTIONS

55°–60° C. is the optimum temperature for the bath. In materials in which the concentration of glucose is many times larger than that of the fructose, complications do occur. However by setting up copper reduction curves for glucose and fructose at 60° C., preliminary data indicate that at least an approximate estimate of the fructose content should be obtained by this micro method.

Comparison of Shaffer-Somogyi and Quisumbing-Thomas Methods on Plant Materials.—Twenty-four plant materials were extracted with 80 per cent alcohol, dealcoholized, taken up in water, cleared with neutral lead acetate, deleaded with sodium oxalate, and made to volume. Three plant saps were also cleared with lead, deleaded, and made to volume.

The reducing and total sugars (sucrose being inverted by invertase) were determined in duplicate by the Shaffer-Somogyi method and by the Quisumbing-Thomas method, the volumetric permanganate modification[2]

TABLE 1.—*Effect of temperature on reactions of glucose and fructose*

	MINUTES IN BATH	MG. OF REDUCED COPPER AT BATH TEMPERATURE OF	
		55° C.± .1°	60° C.± .1°
Glucose			
0.5 mg.	30	0.00	0.00
0.5 mg.	60	0.00	0.00
0.5 mg.	180	0.00	0.00
1.0 mg.	30	0.00	0.02
1.0 mg.	60	0.00	0.13
1.0 mg.	180	0.14	0.54
2.0 mg.	30	0.00	0.02
2.0 mg.	60	0.00	0.52
2.0 mg.	180	0.57	1.63
Fructose			
0.5 mg.	30	0.00	0.04
0.5 mg.	60	0.02	0.43
0.5 mg.	180	0.30	0.61
1.0 mg.	30	0.25	0.28
1.0 mg.	60	0.34	1.18
1.0 mg.	180	1.59	1.99
2.0 mg.	30	0.14	0.24
2.0 mg.	60	0.97	2.79
2.0 mg.	180	3.60	4.35

being used. In addition, non-fermentable reducing substances were determined by yeast fermentation[7] with the Shaffer-Somogyi reagent. The results are given in Table 2.

The reducing sugars determined by the Shaffer-Somogyi reagent were uniformly higher than those found by the Quisumbing-Thomas method. The average for the former was 1.46 gram per 100 grams of material, compared to 1.24 grams for the latter. By subtracting the non-fermentable reducing substances from the reducing sugars determined by the Shaffer-Somogyi method, the fermentable sugars were calculated. These values, average of 1.30 grams, compared closely with the reducing sugar values, average of 1.24 grams, determined by the Quisumbing-Thomas method, showing that the discrepancies were principally due to the Shaffer-Somogyi reagent reacting with the non-fermentable substances,

while the Quisumbing-Thomas reagent did not react. The average sucrose values were 1.25 grams for the Shaffer-Somogyi method and 1.22 grams for the Quisumbing-Thomas method. As a group the results from the pasture plant samples showed a rather large discrepancy between the two

TABLE 2.—*Grams sugar in terms of glucose per 100 grams of material*

MATERIAL ANALYZED	QUISUMBING-THOMAS METHOD		SHAFFER-SOMOGYI METHOD		
	REDUCING SUGARS	SUCROSE	REDUCING SUGARS	FERMENTABLE SUGARS	SUCROSE
Tung leaves	1.94	0.71	2.20	1.98	0.69
Corn leaves	0.74	0.88	0.84	0.74	0.86
Cotton leaves	0.72	0.36	0.83	0.69	0.37
Grape leaves	1.54	0.02	1.72	1.44	0.07
Pecan leaves	1.06	0.73	1.10	0.90	0.80
Tobacco leaves	0.79	0.64	0.83	0.69	0.77
Bermuda grass	0.06	0.32	0.24	0.24	0.68
Carpet grass	0.05	0.26	0.24	0.24	0.23
Dallis grass	0.05	0.24	0.22	0.22	0.21
Blue grass	0.06	1.39	0.31	0.31	1.31
Bahia grass	0.09	0.41	0.28	0.10	0.32
Dutch clover	0.03	0.07	0.26	0.26	0.05
Lespedeza	0.12	0.05	0.29	0.29	0.27
Lima beans	0.00	2.43	0.18	0.09	2.63
String beans	2.19	0.33	2.44	2.37	0.29
Green pimientos	1.50	1.73	1.65	1.55	1.67
Red pimientos	4.99	0.36	5.38	5.15	0.31
Tomatoes	1.40	0.73	1.62	1.44	0.73
Squash	1.55	0.60	1.80	1.64	0.67
Corn	1.23	2.34	1.34	1.10	2.36
Sweet potatoes	1.09	4.16	1.53	1.20	4.22
Watermelon	5.00	3.71	5.38	5.00	3.55
Peaches	3.60	4.01	3.88	3.56	4.13
Peanuts	0.00	2.73	0.24	0.05	2.85
Cotton leaves	0.67*		0.77*	0.65*	
Cotton stems	0.54*		0.61*	0.50*	
Sweet potatoes	2.55*		3.13*	2.73*	
Average	1.24	1.22	1.46	1.30	1.25

* Per 100 ml. of sap.

methods, probably because the sugar content of the aliquots analyzed was too small for accurate determination with the Quisumbing-Thomas method. If larger aliquots had been taken, presumably the results would have been closer.

SUMMARY

The Shaffer-Somogyi method offers possibilities for determining sugars in plant materials because it is economical, fast, and utilizable on material having a low sugar content.

The results from the analyses of 27 samples with the Shaffer-Somogyi and Quisumbing-Thomas methods show fairly close agreement between the fermentable sugar values as determined by the former method and the reducing sugar values as determined by the latter method. The sucrose values obtained with the two methods show close agreement.

DETERMINATION OF SULFIDES IN DEPILATORIES*

By EDWARD M. HOSHALL (Cosmetic Division, U. S. Food and Drug Administration, Washington, D. C.)

Most chemical depilatories on the American market contain sulfides. The sulfides of barium, strontium, and sodium, and the hydrosulfide of calcium are used most extensively in the trade. A few others are also employed, as shown in Table 1.

TABLE 1.—*Components of chemical depilatories—sulfides*

AGENT	USED AS—		
	LIQUID	POWDER	PASTE
Ammonium sulfide	x*		
Arsenic sulfide		x*	x*
Barium polysulfide		x	x
Barium sulfide	x	x	x
Calcium saccharo-sulfide	x		x
Calcium sulfide			x
Calcium sulfhydrate	x		x
Lithium sulfide	x		x
Potassium sulfide	x*		x*
Sodium hydrosulfide	x*		
Sodium polysulfide	x		
Sodium sulfide	x		x
Strontium hydrosulfide			x
Strontium sulfide		x	x
Zinc sulfide		x	

* Limited use.

The alkali sulfides are usually found in liquid depilatories in concentrations of 2 per cent to 10 per cent, calculated as the anhydrous salts. The sodium salt is generally used because the ammonium salt has too strong an odor and the potassium salt is too caustic. Lithium sulfide has been introduced recently as the active component of a liquid depilatory.

The less caustic sulfides of barium and strontium, in concentrations varying from 20 per cent to 35 per cent, calculated as the pure sulfide, are used in powder depilatories more frequently than is the calcium salt, because of its low solubility. A zinc sulfide-lime combination has

* Abstracted from a report to the Cosmetic Division, Food and Drug Administration, "Depilatories, Their Composition and Analysis," December 18, 1939, pages 1–72.

also been used. Powder depilatories, kept in well-stoppered containers, are usually more stable than other types.

From the standpoint of distribution and sales the pastes constitute the largest class, and it is conservatively estimated that over 75 per cent of chemical depilatories are sold in this form. Barium and strontium sulfides are generally found in pastes, and a limited use is made of calcium hydrosulfide and calcium saccharo-sulfide. The active ingredients in pastes vary from 5 per cent to 20 per cent, calculated as the pure sulfide.

EXPERIMENTAL

Since most of the sulfides used in the depilatories are technical grades, or only partially purified, many impurities and reaction by-products are present.

An even greater source of analytical difficulty is the instability of depilatories in this group. Owing to hydrolysis, oxidation by air, and reaction with other components of the preparation, many products are formed of which few, if any, possess keratolytic activity. An example, illustrative of sulfide instability, is a barium sulfide depilatory paste made from a good grade of barium sulfide, starch, talc, glycerin, water, and perfume. Two weeks after preparation, qualitative analysis showed that the following ions were present:

carbonate	sulfide	sulfite	barium
hydroxyl	hydrosulfide	sulfate	iron (BaS impurity)
	free sulfur	thiosulfate	manganese (BaS impurity)

Even the purest sodium sulfide obtainable is unstable in a solution of freshly distilled water, the percentage of sodium sulfide decreasing gradually.

As materials for reference standards the following chemicals were obtained from commercial sources:

Sodium sulfide: An unopened bottle of "Sodium Sulfide, Purified Crystals." Although the crystals were well formed, moisture was present on the bottle walls and a small amount of a gray-green slime, later found to consist of iron impurities, was present within the crystal. Recrystallization failed to increase the percentage of sodium sulfide.

Barium sulfide: The best of three commercial grades was selected, "Barium Sulfide. . . . Yellow Powder . . . about 85%." The product was lumpy and required grinding before sampling. Difficulty was experienced in obtaining homogeneous samples without excessive grinding, which caused a loss of H_2S.

Strontium sulfide: The most homogeneous of three lots was selected. All lots available were of technical grade.

Calcium sulfide: A product labeled "Calcium Sulphide U.S.P." It appeared to be homogeneous and of good quality and met the requirements of the U.S.P. IX.

Zinc sulfide: A partially purified technical product contained large quantities of sulfite. Therefore freshly precipitated zinc sulfide from known amounts of reagent zinc was used as reference.

Lithium sulfide: None of this salt was available from commercial sources and attempts to prepare it in the laboratory yielded products of indefinite composition.

Several methods (Nos. 1–13), which appeared applicable to soluble and slightly soluble sulfides, were investigated. The results obtained by analysis of the selected reference sulfides are given in Table 2.

TABLE 2.—*Percentage of anhydrous sulfides by various methods*

METHOD	Na_2S	BaS	SrS	CaS	COMMENTS
1—Iodine Titration (1)	33.5 33.4 33.5	89.6 88.4	60.2 60.0	66.1 —	Values high, SO_3 and S_2O_3 included
2—Zinc Titration (2)	30.3 31.2 28.6 29.2	72.0 74.5	51.8 54.0	Too Insol.	Applicable to water-soluble sulfides only
3—Evolution Method (3) (Absorb in alkaline cadmium salts)	32.1 28.6 30.8	79.6 73.1 82.8	51.6 48.7 46.2	69.0 68.1 62.6	
4—(Absorb in neutral cadmium salts) (3)	24.5 21.5	62.0	—	—	Evolution methods not recommended owing to presence of CO_2, SO_2, and decomp. of sulfides to compounds other than H_2S in hot acid solution
5—(Absorb in ammon. zinc chloride) (3)	29.4 29.2	70.1	—	—	
6—(Absorb in sodium arsenate) (3)	28.1 27.8 28.5 28.8	—	—	—	
7—Silver Titration*	31.2 31.2	85.0 84.9	58.9	—	Application too limited
8—A.O.A.C. zinc chloride sulfide-sulfur method (4)	31.2 31.0 31.1 31.1	Not applicable			30.94 31.09 } for freshly prepared Na_2S solution
9—Copper-thio Titration (5)	30.1† 30.8† 29.0† 28.0†	47.6	35.4	50.6 76.8	
10—Arsenic Titration (6) (First modification)	30.1 29.6 29.6	59.3 70.6 73.3 73.5	47.5 49.5 52.7 52.1	63.6 63.9 63.6 63.7	ZnS = 97.8 & 98.1
11—Weil's Method (7) (Evolution)	28.4 29.0	—	—	—	Too cumbersome and tedious
12—Arsenic Gravimetric (8) (Weigh as As_2S_3)	30.2 30.4	Not applicable			
13—Arsenic Titration (8) (As_2S_3 sep'd & det'd)	30.6	75.0		63.5 63.7	Method satisfactory; but somewhat lengthy

* Add excess of 0.1 *N* $AgNO_3$ to the sulfide, acidify with HNO_3, make to volume, filter, and titrate an aliquot with 0.1 *N* KSCN. The method is accurate, but not applicable in presence of halogens, sulfates, and other interfering ions.

† Average of 4 determinations on each aliquot of different size.

Many of these methods were eliminated as being too tedious and cumbersome for routine analysis; some were limited in scope because of the slight solubility of the alkaline-earth sulfides, while others yielded variable and non-reproducible results.

Method 10 was selected as the most promising, and with it as a basis the following modified method was developed:

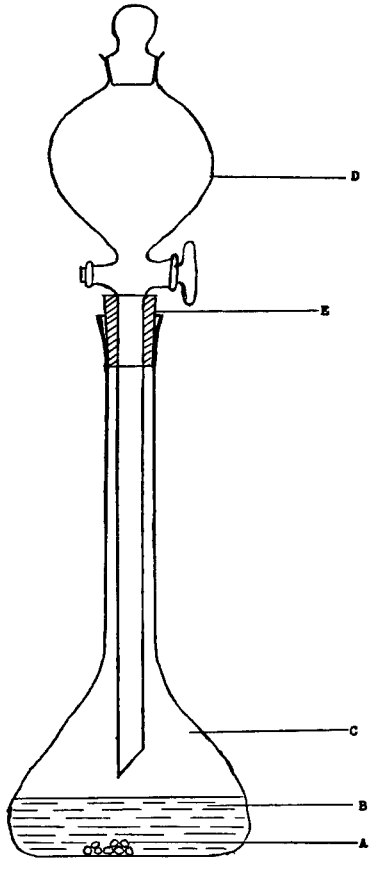

FIG. 1.—APPARATUS USED WITH METHOD 14, FOR THE DETERMINATION OF SULFIDES IN THE PRESENCE OF CARBONATES

METHOD 14

REAGENTS

(a) *Arsenious acid solution.*—0.1 *N*.

(b) *Standard Iodine solution.*—0.1 *N*, standardized against (a).

DETERMINATION

Pipet exactly 50 ml. of 0.1 *N* As_2O_3 solution into a 250 ml. g.s. volumetric flask. Weigh a sample of the depilatory containing less than 0.12 gram of sulfide calculated as H_2S and transfer to the flask (Note 1). Add 20 ml. of cooled H_2SO_4 (1+1) (Note 2), stopper, and shake vigorously until all reactions have subsided and the sample is decomposed. Cool to room temperature, dilute to volume with distilled water, and filter through a dry filter into a dry flask, rejecting the first 20 ml. of filtrate. Withdraw exactly 100 ml. of the clear filtrate, and add 5 ml. of starch solution and sufficient 0.1 *N* iodine solution to produce a blue tint. Make alkaline with solid $NaHCO_3$, adding 1–2 grams in excess. Titrate with 0.1 *N* iodine solution. Calculate net ml. of 0.1 *N* As_2O_3 used.

FACTORS

1 ml. 0.1 *N* As_2O_3 = 1 ml. 0.1 *N* Iodine

0.002556	gram	H_2S
0.005854	"	Na_2S
0.01802	"	$Na_2S+9\ H_2O$
0.003446	"	Li_2S
0.005411	"	CaS
0.01261	"	BaS
0.008977	"	SrS

NOTE 1.—The presence of small quantities of carbonates does not affect the results obtained by this method. If, however, appreciable quantities are present, the CO_2 liberated on acidification will force the stopper from the flask and cause loss of H_2S. The arrangement in Figure 1 will prove effective for use in the presence of carbonates. Add the sample (A) (Fig. 1) to the excess of 0.1 *N* As_2O_3 (B) in the 250 ml. flask (C). Place a 60 ml. separatory funnel (D) in the neck of the flask with the tip of the stem just above the surface of the liquid (B).

Place 20 ml. of cooled H_2SO_4 (1+1) and exactly 10 ml. of 0.1 *N* As_2O_3 in the separator, open the stopcock slightly, and allow the acid to flow into the solution. The liberated CO_2 and traces of H_2S will bubble up through the column of acid, and by gentle shaking the CO_2 can be removed. When the reaction has subsided wash the contents of the separator into the flask, make to volume with distilled water, and proceed as directed in the method, beginnning "filter through a dry filter."

NOTE 2.—Substitute 15 ml. of HCl if carbonization occurs or if the sulfide dissolves with difficulty.

With sodium sulfide as a reference, the effect of several factors in the above method was studied. The results are shown in Table 3.

TABLE 3.—*Effect of various factors in the determination of sulfide by the proposed method*

Na_2S+9H_2O TAKEN *gram*	VOLUME *ml.*	Na_2S FOUND *per cent*	
	Sample Size		
0.2	250	30.8	
0.4	250	30.8	
0.6	250	30.78 (Av. 5)	
0.8	250	30.65 (Av. 4)	
2.0	250	30.7	
	Volume of Solution		
0.4	100	30.5	30.7
0.4	250	30.7	30.8
0.4	500	30.7	30.7
	Acidification		
		HCl	H_2SO_4
0.4	250	30.7	30.8
0.6	250	30.7	30.8
1.0	250	30.6	30.5
	Bicarbonate Excess		
0.6	250 0.5 g excess	30.75 (Av. 5)	
0.6	250 5.0 g excess	30.7 (Av. 2)	

Applied to the reference sulfides, previously discussed, the method yielded the results shown in Table 4.

TABLE 4.—*Percentage of anhydrous sulfide by Method 14*

REFERENCE SULFIDE	DETERMINATIONS						AVERAGE PER CENT
	1	2	3	4	5	6	
Sodium sulfide $+9H_2O$	30.8	30.8	30.5	30.8	30.8	30.7	30.7
Barium sulfide	75.1	74.9	75.5	73.5*	73.3*	75.4	75.2
Strontium sulfide	51.9	51.8	52.7	52.1	52.2	51.6	52.1
Calcium sulfide	63.6	63.7	63.5	64.0	64.0	63.9	63.8
Zinc sulfide	97.8	98.1	—	—	—	—	98.0

* Undissolved particles present.

The average of the results obtained for sodium sulfide in Table 4 are in reasonable agreement with the values obtained by Methods 2, 3, 8, 9,

and 10 given in Table 2. There is marked variation in the sulfide content of the reference sulfides when assayed by various methods (Tables 2 and 4).

An attempt was made to determine the actual sulfide-sulfur content in barium sulfide by determining the total sulfur and correcting for free sulfur, sulfite sulfur, thiosulfate sulfur, and sulfate. The complexity of the product precluded satisfactory results.

APPLICATION

One liquid, two powders, and a paste depilatory were prepared according to somewhat modified commercial formulas, from the reference sulfides previously described. Analysis of these preparations by Method 14 produced the results given in Table 5.

TABLE 5.—*Application of Method 14 to depilatories of known sulfide content*

PRODUCT	TAKEN ANHYDROUS SULFIDE	FOUND ANHYDROUS SULFIDE	RECOVERY	COMMENTS
	gram	*gram*	*per cent*	
Liquid No. 1 (Na_2S)	0.1724	0.1640	95.1	Preparation 6 hours old
	0.1724	0.1660	96.3	
	0.1721	0.1569	91.2	Preparation 48 hours old
	0.1721	0.1599	92.9	
	0.1742	0.1547	88.8	Preparation 144 hours old
	0.1742	0.1545	88.7	
Powder No. 1 (BaS)	0.4512	0.4627	102.5	
	0.4512	0.4608	102.1	
	0.4374	0.4380	100.1	
	0.4374	0.4284	97.9	
	0.4512	0.4597	102.5	
	0.4512	0.4585	102.3	
Powder No. 3 (CaS)	0.2233	0.2207	98.8	
	0.2233	0.2211	99.0	
	0.3350	0.3320	99.1	
	0.3350	0.3317	99.0	
Paste No. 1 (SrS)	0.2600	0.2582	99.3	Preparation 6 hours old
	0.2600	0.2594	99.8	
	0.2600	0.2515	96.7	Preparation 3 days old
	0.2600	0.2511	96.6	

The low recoveries shown in Table 5 for Liquid No. 1 raise the question of the stability of Na_2S in solution. Table 6 shows the results of experi-

ments conducted to determine what effect aging has on the sulfide content of a solution containing Na_2S and water only.

TABLE 6.—*Effect of aging on the sulfide content in a water solution of Na_2S (8 grams Na_2S+9H_2O per 100 ml.)*

Na_2S TAKEN	Na_2S FOUND	AGE OF SOLUTION	RECOVERY
gram	*gram*	*hours*	*per cent*
0.1866	0.1848	(solid Na_2S)	99.0
0.1866	0.1858	(solid Na_2S)	99.6
0.2488	0.2383	0.5	95.8
0.2488	0.2383	0.5	95.8
0.1866	0.1780	1	95.4
0.2488	0.2372	1	95.3
0.1866	0.1761	24	94.4
0.2488	0.2347	48	94.3

DISCUSSION

Liquid No. 1.—Table 6 indicates that the low recoveries shown in Table 5 resulted from decomposition of the sulfide rather than from errors in the analytical method. This conclusion is strengthened by the further lowering in the sulfide content, found after 48 and 144 hour periods (Table 5).

Powder No. 1.—The recoveries are somewhat high. Blank determinations on each component of the powder failed to account for these high results. Sampling difficulties, lack of homogeneity of the product, and the necessity for careful grinding of the barium sulfide have been previously discussed. These factors may be responsible.

Powder No. 3.—Satisfactory yields were obtained for calcium sulfide in the presence of 50 per cent of calcium carbonate.

Paste No. 1.—The method gave satisfactory results when applied to a paste containing about 5 per cent of strontium sulfide and 9 inactive depilatory components. The instability of the preparation is evident.

If, owing to interfering substances not encountered during this investigation, the method is found inapplicable, Method 13 may apply.

SUMMARY

A modification of Mohr's arsenious acid method for the determination of hydrogen sulfide is tentatively proposed for the determination of the sulfides or hydrosulfides of sodium, barium, strontium, calcium, potassium, ammonium, and lithium in liquid, powder and paste depilatories. A supplementary method is described, which is applicable in the presence of carbonates.

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BOOK REVIEW

The Merck Index. Fifth edition. Merck & Co., Rahway, N. J. 1940. 1060 pp. Price $3.00.

This book is described as an encyclopedia for the chemist, pharmacist, physician, dentist, and veterinarian. It contains useful scientific data and other information on the physical, chemical, and medicinal properties, as well as the various uses of chemicals and drugs; more than 4,500 chemical, clinico-chemical reactions, tests, and reagents; formulas for preparations of culture media, fixatives, and staining solutions; useful tables; antidotes for poisons; literature references; and descriptions of more than 5900 individual substances. It also includes descriptions for 113 colors, 126 indicators, and 187 minerals.

The format remains the same as in previous editions, but the new matter has increased the size from 585 pages in the fourth edition to 1060 in this.

Users of the earlier editions will notice one marked difference in the arrangement of the new edition. In the previous editions, for example, the acids were grouped together alphabetically: acid, abietic; acid, acetic; acid, acetylsalicyclic, and so on to acid, wolframic. In this edition the acids are arranged individually and alphabetically: acetic acid, phosphoric acid, valeric acid, etc. On the other hand, the subject "oils" is treated as in former editions, "oil of allspice, oil of almond, etc." This will probably be slightly confusing to the older chemists.

Ten years ago, after an interval of 23 years, the fourth edition of the index appeared. During the last decade many new drugs have been synthesized (larocaine, merthiolate, sulfapyridine, etc.); new uses have been discovered for old drugs (such as nicotinic acid and sulfanilamide); and refinements in technic have been developed for the dispensing and administration of drugs. The new edition of the index includes these advances. Much of the new material is of particular interest to analytical chemists.

In the description of a salt, for example, in addition to its appearance, solubilities, stability, and uses, the formula, molecular weight, percentage of metal (or metallic oxide), acid radical, water of hydration, anhydrous salt if pertinent, and occasionally other essential factors, are given. As illustrations, the factors for ammonium sulfate and quinine sulfate are quoted in part: $(NH_4)_2SO_4$, mol. wt. 132.14; NH_3, 25.78%; H_2SO_4, 74.22%; SO_3, 60.59%; N, 21.2%; $(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O$, mol. wt., 782.51; anhyd. salt, 95.4%; H_2O, 4.6%; anhydrous quinine, 82.86%, H_2SO_4, 12.53%.

It is also indicated whether a substance is used as a medicine, chemical reagent, indicator, or in technology. The less commonly used synonyms are given and cross-indexed to the better known names.

Considering the vast amount of encyclopedic information contained in this book and the typographical difficulties in publishing information of this character, the number of errors is surprisingly small. The publishers supply a list of about 30 and the reviewers have noted a few more.

Workers in every phase of agricultural chemistry will find useful information in this book. To the analytical chemist the book is well nigh indispensable.—L. E. WARREN.