WEDNESDAY—MORNING SESSION

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Referee*

No new collaborative work having been done on the methods, it was recommended* at the 1946 meeting that the subjects be continued, as listed below:

(1) *Flavors and Non-alcoholic Beverages:* To continue the collaborative study of the reflux method for peel oil in citrus fruit juices, using the modified oil separation trap.

(2) *Beta-Ionone:* To continue collaborative work on small amounts of beta-ionone.

(3) *Lemon Oils and Extracts:* To continue collaborative study of the Ripper method for aldehydes in lemon oils and extracts; and

(4) To continue collaborative study of methods for esters in lemon extract; and

(5) To continue collaborative study of the Seeker-Kirby methods for esters in lemon and orange oils.

(6) *Organic Solvents in Flavors:* To continue collaborative study of extracts containing both isopropyl alcohol and acetone.

(7) *Glycerol, Vanillin, and Coumarin in Vanilla and Imitation Vanilla:* To continue collaborative study of the photometric methods for vanillin and coumarin; and

(8) To continue work on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla with special reference to automatic extraction of vanillin and coumarin.

(9) *Emulsion Flavors:* To continue study of emulsion flavors.

(10) *Maple Flavor Concentrates and Imitations:* To continue studies of maple concentrates and imitations.

(11) *Diacetyl:* To continue the study of the method described in *This Journal*, **25**, 255 (1942).

No reports were given on beta-ionone; lemon oils and extracts; organic solvents in flavors; glycerol, vanillin, and coumarin in vanilla and imitation vanillas; emulsion flavors; maple flavor concentrates and imitations; and diacetyl.

* For report of Subcommittee D and action by the Association, see *This Journal*, **30**, 59 (1947).

REPORT ON FEEDING STUFFS

By L. S. WALKER (Agricultural Experiment Station, Burlington, Vt.), *Referee*

In reviewing the method for "Hydrocyanic Acid Formed by Hydrolysis of Glucosides in Beans," it was found that the work was not completed, and no associate referee was appointed to continue the work done by R. A. Green (resigned).

RECOMMENDATIONS*

It is recommended that the following official, first action, methods be made official final action:

Sampling, *Methods of Analysis*, 1945, **27.1,** p. 404. Water-soluble acidity, **27.41,** p. 414. Rice hulls in rice bran, **27.43,** p. 414. Oat hulls in oats and oat feeds, **27.44,** p. 414.

It is recommended that the two tentative methods for "Iodine in Mineral Mixed Feeds," *Methods of Analysis*, 1945, p. 417, be checked by the Associate Referee on mineral mixed feed, with the method discussed by Johnson and Frederick (*This Journal*, **23,** 688, 1940), which is reported to give higher recovery of iodine.

It is recommended that an Associate Referee be appointed to study methods for crude protein with respect to catalysts.

It is recommended that further study be made on the following:

Lactose in mixed feeds
Fat in fish meal
Adulteration of condensed milk by-products
Fat in cooked animal feeds containing cereals
Crude fat or ether extract in feeds
Activity of yeast
Microscopic examination
Fluorine
Mineral constituents of mixed feeds
Crude fiber
Protein evaluations in fish and animal products
Sampling and analysis of condensed buttermilk

No reports were given on sampling (feeding stuffs); mineral mixed feeds (calcium and iodine); lactose in mixed feeds.

* For report of Subcommittee A and action by the Association, see *This Journal*, **30,** 41 (1947).

REPORT ON FAT IN FISH MEAL

By M. E. STANSBY (Seattle Technological Laboratory, U. S. Fish and Wildlife Service, Seattle 2, Washington), *Associate Referee*

The work on fat in fish meal has been discontinued during the War and this is the first report since 1942. Accordingly, the following summary is given to indicate the status of this problem up to the current year.

Fat contents of fish meal as determined by the usual means (ether extract of vacuum oven dried meal) are usually less than fifty per cent of the true value. A much more accurate measure of the fat content is obtained by making an acetone extract of the undried meal. This acetone extract, as shown by hundreds of experiments is substantially all soluble in ethyl ether. The acetone extract does not represent all the fat content of fish meal, values obtained usually being between 75 and 100 per cent of the total fat content. Meals held for prolonged storage periods tend to give low acetone extract values. An additional amount of extract can be obtained by digesting the residue from the acetone extraction with hydrochloric acid, drying the meal and re-extracting with ethyl ether. The sum of the acetone and hydrochloric acid digest-ether extraction gives values approaching the total oil content. However, in some samples, the fat content found by this method is still quite low. Some early experiments indicated that certain mixed solvents, especially mixtures of acetone, benzene (C_6H_6), and methanol gave higher extraction values than did acetone alone.

During the past year attention has been directed toward use of mixed solvents as a substitute for acetone in extracting fish meal for fat content. The mixtures of acetone, benzene, and methanol previously used in preliminary work have been studied in detail. It was found, early in this work, that certain mixtures of methanol and benzene gave higher extractive values than did any mixture of these solvents containing acetone. Fortunately the binary azeotropic mixture (39.6% methanol and 60.4% benzene) gave substantially peak extraction values. There is considerable advantage in working with such a constant boiling mixture since composition of the vapor phase is independent of volume of solvent used, or size of extraction cup. Hence composition of the solvent extracting the meal does not vary and is known under all conditions, thus giving consistent and reproducible results.

A series of California pilchard meals were examined by: (a) ether extractions of vacuum dried meal followed by ethyl ether extraction of hydrochloric acid digested meal; (b) acetone extraction of undried meal followed by ethyl ether extraction of hydrochloric acid digested meal residues; (c) binary methanol benzene extraction of undried meal followed by ethyl ether extraction of hydrochloric acid digested meal residue. In the case of (b) and (c) above, the original solvent extract was "purified"

TABLE 1.—*Comparative extractions of fat from pilchard meal with different solvents*

SAMPLE	APPROXIMATE AGE OF MEAL	SOLVENT	ORIGINAL EXTRACT[1]	HYDRO-CHLORIC ACID DIGEST[2]	TOTAL EXTRACT
number	*months*		*per cent*	*per cent*	*per cent*
1	1	ethyl ether	4.2	3.3	7.5
		acetone	8.3	1.8	10.1
		benzene-methanol	13.2	0.6	13.8
B	24	ethyl ether	2.2	2.6	4.8
		acetone	4.3	2.4	6.7
		benzene-methanol	6.1	0.9	7.0
3	24	ethyl ether	5.1	3.1	8.2
		acetone	8.4	2.1	10.5
		benzene-methanol	10.3	0.8	11.1
4	24	ethyl ether	4.6	3.7	8.2
		acetone	8.0	2.4	10.4
		benzene-methanol	10.5	0.8	11.3
5	24	ethyl ether	4.8	3.6	8.4
		acetone	8.0	2.6	10.6
		benzene-methanol	12.2	0.9	13.1
6	24	ethyl ether	4.6	3.7	8.3
		acetone	8.0	2.5	10.5
		benzene-methanol	11.9	1.0	12.9
7	24	ethyl ether	4.0	3.4	7.4
		acetone	7.6	2.3	9.9
		benzene-methanol	10.4	1.0	11.4
8	24	ethyl ether	4.3	3.2	7.5
		acetone	7.4	2.3	9.7
		benzene-methanol	9.7	1.0	10.7
11	24	ethyl ether	4.2	3.3	7.5
		acetone	7.3	2.1	9.4
		benzene-methanol	9.6	1.0	10.6
12	24	ethyl ether	4.0	3.3	7.3
		acetone	6.8	2.4	9.2
		benzene-methanol	8.5	0.9	9.4
13	24	ethyl ether	4.2	3.3	7.5
		acetone	6.9	2.3	9.2
		benzene-methanol	9.1	1.0	10.1
14	24	ethyl ether	4.4	3.2	7.6
		benzene	7.2	2.2	9.4
		benzene-methanol	9.5	1.1	10.6

[1] Original extract when solvent other than ethyl ether was used, is on "purified" basis; *i.e.*, solvent was removed and residue treated with ethyl ether and only such ether soluble fraction is given in this column.
[2] Ethyl ether extract of previously extracted meal which had then been digested one hour with 4 *N* hydrochloric acid.

by removal of solvent and dissolving in ethyl ether, filtering, and removing the ether.

Results of these experiments are shown in Table 1.

Consistently higher results are obtained using the mixed solvent than with acetone. Furthermore, this solvent removes a much higher per cent of the total fat on the first extraction with a correspondingly lower portion by the acid digestion-ether extraction.

In the early experiments, numerous tests showed that the gross acetone extract was substantially identical with the ether purified extract at least on freshly prepared meals. Further work is needed to determine whether the same is true of the benzene-methanol extract. The latter extract is of a gummy consistency which makes it exceedingly difficult to work with in the ether purification step of the process. Unless subsequent tests show that this purification is unnecessary, the benzene-methanol method does not appear to be very promising for a routine method.

SUMMARY

The binary azeotropic mixture of benzene and methanol gives higher fat content values for fish than does acetone or other solvents previously investigated. Further investigation is required to determine whether "purification" of the gross mixed solvent extract (removal of mixed solvent and separation of oil soluble in ethyl ether) is necessary. This procedure is very difficult to carry out with the mixed solvent and unless it can be eliminated the method does not lend itself to routine analyses.

The writer wishes to acknowledge the assistance of William Clegg, Chemist at the Seattle Fishery Technological Laboratory, Fish and Wildlife Service, in the conduct of the work.

No reports were given on adulteration of condensed milk products or on fat in cooked animal feeds containing cereals.

REPORT ON CRUDE FAT OR ETHER EXTRACT

By J. J. TAYLOR (Department of Agriculture, Tallahassee, Fla.), *Associate Referee*

Following up the preliminary work done last year, which indicated that results on fat determinations using the official methods gave high results if non-protein nitrogen was present, our collaborative project was the comparison of results of fat determinations using the official procedure and solvent with fat determinations using the same procedure but using petroleum ether as the solvent.

Sixteen collaborators were chosen who had signified their willingness to collaborate, and two samples of dairy feed were used for collaborative

REPORTS OF COLLABORATORS

FAT OR ETHER EXTRACT, 1946

Sample #1—Mixed Feed without Urea

COLLABORATOR'S NUMBER	MOISTURE	FAT EXTRACTION 16 HOURS WITH PETROLEUM ETHER	FAT EXTRACTION 16 HOURS WITH ETHYL ETHER
1	8.78	3.44	3.66
2	8.26	3.53	3.45
3	7.98	3.35	3.65
4	8.78	3.53	3.60
5	8.28	3.48	3.85
6	8.59	3.47	3.74
7	8.50	3.65	4.12
8	8.90	3.17	3.53
9	8.01	3.20	3.95
10	—	3.20	3.40
11	7.15	3.52	3.62
12	—	—	—
13	9.69	3.61	3.57
14	8.37	3.13	3.41
15	8.88	3.33	3.62
16	8.65	3.35	3.65
Average	8.50	3.39	3.65

Sample #2—with Urea added

COLLABORATOR'S NUMBER	MOISTURE	FAT EXTRACTION 16 HOURS WITH PETROLEUM ETHER	FAT EXTRACTION 16 HOURS WITH ETHYL ETHER
1	8.83	3.12	4.14
2	8.06	2.89	3.67
3	7.70	3.15	3.85
4	8.71	3.24	3.64
5	7.86	3.32	3.82
6	8.49	3.34	3.66
7	*8.13	*3.47	*4.81
8	8.70	3.12	3.62
9	*7.44	*2.83	*5.23
10	—	3.10	3.30
11	6.98	3.48	4.18
12	—	—	—
13	9.15	3.33	3.55
14	8.51	3.08	3.43
15	9.10	3.22	3.66
16	9.50	3.25	3.60
Average	8.46	3.25	3.70

* Not included in average.

study. The two samples were identical except that three per cent of urea was added to Sample #2. Collaborators were instructed to determine crude fat or ether extract using the official method (Fifth Edition 1940, p. 356 and determining Moisture as directed in par. 2, under Moisture, page 353) and then make the same determination using the same procedure, but using petroleum ether as the solvent (skelly-solve or a grade of equal quality). Collaborators were requested to report the average of not less than three determinations by each procedure.

A tabulation of results is submitted herewith. It is noted that in nearly all cases the extraction with ethyl ether (official method) gave higher results in both samples than the extraction with pertoleum ether. The average in the case of Sample #1 for petroleum ether extraction was fat 3.39 per cent; for ethyl ether extraction, fat 3.65 per cent; a difference of 0.26 per cent. In Sample #2, which contained the three per cent urea, the average was, for petroleum ether extraction, fat 3.25 per cent; for ethyl ether extraction, fat 3.70 per cent; or a difference of 0.45 per cent.

It is worthy of comment that apparently the introduction of non-protein nitrogen into feed mixtures complicates the fat determination, as evidenced by the more uniform results on Sample #1. The high result for petroleum ether extraction was 3.65 per cent, and the low 3.13 per cent, or a maximum deviation of 0.52 per cent; for ethyl ether extraction, the high result was 4.12 per cent, and the low 3.40 per cent, or a maximum deviation of 0.72 per cent; as compared with Sample #2, where the high for petroleum ether extraction was 3.48 per cent, and the low 2.83 per cent, or a maximum deviation of 0.65 per cent; and for ethyl ether, a high of 5.23 per cent, and a low of 3.30 per cent, or a maximum deviation of 1.93 per cent.

It is the opinion of your Associate Referee that petroleum ether of a suitable grade is a more suitable solvent for determining fat or ether extract, where non-protein nitrogen is present in the feed mixture, than ethyl ether; and it is suggested that the Association consider making petroleum ether tentative official as the solvent in feed mixtures containing non-protein nitrogen.

REPORT ON ACTIVITY OF YEAST

INVESTIGATION OF METHODS OF TESTING

By H. C. Schaefer (Ralston Purina Company, Nutrition Research Laboratories, St. Louis, Mo.), *Associate Referee*

Yeast testing has been carried out quite generally by measurement of the carbon dioxide produced from sugar by a definite quantity of yeast acting in a liquid medium. Hayduck's method (1881–1882) (1) consisted of measuring volumetrically the amount of carbon dioxide liberated by

yeasts growing in a 10% glucose solution to which buffer and salt were added. Meissl (2) in 1884 employed a sucrose solution, a ventilation value and concentrated sulfuric acid to absorb water as carbon dioxide was allowed to escape. The apparatus was weighed before fermentation and again after six hours, and loss in weight calculated. Subsequent methods of measuring carbon dioxide production have all been adaptations of these two.

Attempts were made in this laboratory to measure the fermentative power of yeast in three ways: 1. By pressureometer. 2. By adsorption of carbon dioxide by ascarite, a sodium hydrate asbestos adsorbent. 3. By weight loss after fermentation, an adaptation of the early Meissl method. A volumetric method was considered unnecessarily cumbrous if fermentation could be measured as efficiently by any of the three methods mentioned above, any of which require simpler equipment and manipulation.

Two samples of feed yeast and one sample of baker's yeast were used as test samples in the experiments. Five-gram samples of feed yeast and one-gram samples of yeast were suspended in 50 ml of 10 % glucose solution, and incubated for five hours at 30°C.

SAMPLE NO.	ASCARITE ADSORPTION	LOSS IN WEIGHT IN MG BY ALLOWING CO_2 TO ESCAPE	PRESSUREOMETER
1. Feed Yeast	0.4783	0.4884	Sample too large for gauge
2. Feed Yeast	0.0257	0.0120	0.0098
3. Yeast		0.7723	

It was decided that rates of fermentation could be measured by any of the three methods. The weight-loss method presented fewer difficulties and required a minimum of equipment. Selecting this method, experiments were carried out on variables in media, temperature and duration of incubation, sample size, shaking of samples, and type of incubation.

The apparatus consists of a 125 ml Erlenmeyer flask fitted with a rubber stopper in which is fitted an outlet for the escape of carbon dioxide and a trap for holding sulfuric acid which removes any moisture from the escaping gas. The trap is one which was devised in Purina Laboratories(3) and which has been used as an ammonia trap in analytical procedures for many years.

EXPERIMENTAL PROCEDURE AND DATA

It was found over the period of experimentation that the feed yeasts being used as test samples weakened from day to day in their fermenting ability. Therefore, values cannot be compared from table to table in the the following pages. Values within each table may be compared, however.

1. Duration of Incubation.—A one-gram sample of yeast and a five-gram sample of Feed Yeast No. 1 were suspended in 50 ml of 10% glucose, incubated at 30°C. for five hours, and weights recorded before and after incubation.

TIME	YEAST LOSS IN WEIGHT	FEED YEAST NO. 1 LOSS IN WEIGHT
	mg	*mg*
First hour	0.0463	0.0151
Second hour	0.0635	0.0464
Third hour	0.0789	0.0874
Fourth hour	0.1374	0.1205
Fifth hour	0.1318	0.1885
Sixth hour	0.1758	0.1406
Seventh hour	0.1651	

Results after five hours indicated that that amount of time was sufficient to show an appreciable amount of fermentation, and in all succeeding experiments five hours were used. This amount of time fits conveniently into a working-day schedule.

2. Temperature of Incubation.—Samples were set up as in the preceding experiment but were incubated at three temperatures.

SAMPLE	30°C LOSS IN WEIGHT AFTER 5 HOURS	34°C LOSS IN WEIGHT AFTER 5 HOURS	37°C LOSS IN WEIGHT AFTER 5 HOURS
	mg	*mg*	*mg*
Feed Yeast No. 1	0.5610	0.6578	0.7253
Yeast	0.4579	0.4857	0.5565

A temperature of 34°C. is most conveniently and easily maintained.

3. Type of Incubation.—Samples were set up as in the preceding experiments. One of each pair of duplicates was incubated for five hours at 34°C. in a water bath and the other for the same period of time in a 34°C. oven.

SAMPLE	WATER BATH, 34°C LOSS IN WEIGHT	OVEN, 34°C LOSS IN WEIGHT
	mg	*mg*
Yeast	0.4719	0.4785
Feed Yeast No. 1	0.7463	0.7416
Feed Yeast No. 2	0.0357	0.0435

Results do not show significant differences. All subsequent tests were run by incubating in a 34°C. oven.

4. Sample Size.—Graduated amounts of yeast were suspended in 50 ml of 10% glucose and incubated at 34°C. for five hours.

NO.	WEIGHT IN GMS. OF SAMPLE	LOSS IN WEIGHT AFTER 5 HOURS	RATIO BETWEEN WEIGHT OF SAMPLE AND CO_2 PRODUCTION
		mg	
1	0.25	0.0805	3.1
2	0.50	0.1662	3.0
3	0.75	0.2652	2.8
4	1.00	0.3630	2.8
5	1.25	0.4376	2.9
6	1.50	0.5306	2.8

One-gram yeast samples and five-gram feed yeast samples were used in subsequent experiments. Larger amounts by greater activity often produce too much foam for a 124 ml flask.

5. Effect of Shaking.—Suspensions of Feed Yeast No. 1 in medium were prepared as in "1." One sample was shaken after each half hour during the five hour incubation period, and the other was left undisturbed. Periodic shaking did not facilitate the fermentation process.

Sample	Loss in weight in mg. when sample shaken each ½ hour	Loss in weight in mg. when sample left undisturbed
Feed Yeast No. 1	0.722	0.733

Periodic shaking did not facilitate the fermentation process.

6. Medium.—

(*a*) *Volume.*—A five-gram sample of Feed Yeast No. 1 was suspended in each of six graduated volumes of 10% glucose solution and incubated for five hours at 34°C.

NO.	AMOUNT OF 10% GLUCOSE SOLUTION	LOSS IN WEIGHT AFTER FIVE HOURS
	ml	*mg*
1	10	0.3399
2	20	0.4651
3	30	0.5026
4	40	0.4820
5	50	0.4763
6	60	0.4919

After sufficient quantity of medium was attained, it was apparent that any further increase did not produce any significant changes in amount of fermentation. Forty ml volumes were used in subsequent experiments.

(*b*) *Concentration of Glucose.*—A one-gram sample of yeast was suspended in each of five concentrations of glucose and incubated for five hours at 34°C.

NO.	CONCENTRATION OF GLUCOSE	LOSS OF WEIGHT AFTER FIVE HOURS
	per cent	*mg*
1	5	0.4118
2	10	0.4104
3	20	0.3870
4	25	0.4010
5	Saturated	0.2925

A 10% solution was adequate for maximum fermentation and was used throughout subsequent experiments.

(c) *Use of a Malt-extract Solution as a Medium.*—One-gram samples of yeast were suspended in 40 ml portions of 10% glucose and 10% malt-extract solutions, incubated for five hours at 34°C., and the amounts of fermentation compared.

Sample	Loss in weight in mg in malt-extract medium	Loss in weight in mg in glucose medium
Yeast	0.2297	0.7253

A glucose medium was superior to one of malt extract.

(d) *Use of Buffer and Effects of pH.*—On checking the *p*H of yeast and feed yeast suspensions before and after the fermentation process, it was indicated that there is an optimum *p*H for fermentation and that ingredients other than yeast in the test materials influenced *p*H to a large extent. Therefore, buffers were added to suspensions and experiments were carried out in which the *p*H was controlled. The following table shows the response of yeast in suspension when buffers are used.

SAMPLE	BUFFER	LOSS IN WEIGHT IN MG AFTER FIVE HOURS
1	None	0.4920
2	Phosphate	0.7030
3	Sodium Acetate	0.3473

Better results were obtained when the initial *p*H of suspensions was adjusted to 5.5. It was considered reasonable that values were more reliable when *p*H was controlled. The following table shows the effect of adjusting the initial *p*H with normal orthophosphate acid and improving stability by the addition of a phosphate buffer.

SAMPLE	INITIAL pH OF SOLUTION	pH OF SOLUTION AFTER FERMENTATION	LOSS IN WEIGHT IN MG AFTER 5 HRS.
(1) Yeast	5.95	4.7	0.7030
(2) Feed Yeast No. 1	5.95	5.17	0.6686
(3) Feed Yeast No. 2		5.6	0.0290

SAMPLE	pH ADJUSTED TO 5.5	pH OF SOLUTION AFTER FERMENTATION	LOSS IN WEIGHT IN MG AFTER 5 HRS.
(Duplicate of (2) & (3) above)			
(1) Feed Yeast No. 1	5.5	4.9	0.7364
(2) Feed Yeast No. 1	5.5	4.7	0.7143
(3) Feed Yeast No. 2	5.5	5.3	0.0260
(4) Feed Yeast No. 2	5.5	5.3	0.0272

7. *Reproducibility of Results.*—Values obtained on five different dates, upon samples of feed yeast set up identically, are shown in the following table.

DATE	LOSS IN WEIGHT IN MG AFTER 5 HRS. INCUBATION				
	6-18-46	6-19-46	6-21-46	6-24-46	7-15-46
(1) Feed Yeast No. 1	0.736	0.631	0.554	0.454	0.409
(2) Feed Yeast (Dup.)	0.714	0.582	0.548	0.437	
(3) Feed Yeast No. 2	0.021	0.032	0.025		0.004
(4) Feed Yeast (Dup.)	0.027	0.021	0.026	0.025	

It was obvious from the above results that the test materials were deteriorating in their fermentative ability. It was then desired to see what effect the type of storage of the test materials had on the speed of this deterioration. Samples were set up in the usual way from portions of the materials which had been stored at ice box temperature and portions of the same materials which had been retained at room temperature over a three week period. These results are compared in the following table to results obtained in the last experiment preceding the storage period.

SAMPLE	LOSS IN WEIGHT PRECEDING STORAGE PERIOD	LOSS IN WEIGHT AFTER 3 WKS. STORAGE IN ICE BOX	LOSS IN WEIGHT AFTER 3 WKS. STORAGE IN ROOM
	mg	*mg*	*mg*
Feed Yeast No. 1	0.454	0.409	0.140
Feed Yeast No. 2	0.025	0.004	(plus) 0.002

8. *Efficiency of method in measuring yeast activity as determined by testing mixtures containing varying amounts of Feed Yeast No. 1 and 2.*

MIXTURE	LOSS IN WEIGHT IN MG AFTER FIVE HOURS INCUBATION
(1) 25% No. 1 plus 75% No. 2	0.180
(2) 50% No. 1 plus 50% No. 2	0.322
(3) 75% No. 1 plus 25% No. 2	0.488

It may be seen that the method will measure efficiently the differences in quality of yeast feeds. The ratios in the above table may be represented graphically by a straight line.

SUMMARY AND CONCLUSIONS

The weight-loss method is an efficient method of measuring yeast activity if it is desired to measure fermentative power as evidenced by carbon dioxide production. The use of buffer and control of *p*H of suspensions of yeasts and feed yeasts in medium are considered advantageous for uniformity of testing and comparing results. A 10% glucose solution is of adequate concentration to afford adequate fermentation for testing. A temperature of 34°C. promotes adequate fermentation and may be conveniently maintained in the laboratory. Incubation may be carried out in either water bath or oven at 34°C. Since feed yeasts deteriorate in their fermentative ability over a rather short period of time, results are not reproducible on successive days. However, values on duplicate samples tested on the same day are in close agreement. Fermentative ability is retained longer when the test materials are kept at ice-box temperature.

The following method, comprising the details of the procedures of the foregoing experiments which were considered significant to the testing and control of testing of the activity of yeast in feed yeasts is suggested.

METHOD FOR TESTING OF YEAST ACTIVITY

Prepare a medium made up of equal portions of solns A and B, soln A containing 20 g of glucose in 100 ml of distilled water, soln B containing 5 g Na_2PO_4 and 20 g of KH_2PO_4 in 500 ml of distilled water.

Place a 5-gram sample of the material to be tested in a 125 ml Erlenmeyer flask, add 40 ml of the above medium and thoroly mix the soln. Adjust the *p*H to 5.5 with normal H_3PO_4. Stopper the flask with a trap fitted in a rubber stopper. Add to the trap a few ml of H_2SO_4 of a concentration of 5 parts conc. H_2SO_4 to 7.5 parts distilled water. Then weigh the complete apparatus and record the weight and time of weighing. Place immediately in a 34°C. oven or water bath and allow to remain undisturbed for 5 hours. At the end of this incubation period weigh again, and calculate the loss in weight. Take care not to shake suspensions unduly during handling and weighing.

The value of the yeast activity may be expressed in number of mg of carbon dioxide produced per gram of material in five hours.

REFERENCES

(1) Tanner, F. W., *The Microbiology of Foods*, 2nd. ed., Ill., Garrard Press, 1944, p. 148.
(2) *Ibid.*
(3) Potts, T. J., *Ind. Chem., Anal. Ed.*, **18**, 78 (1946).

No reports were given on microscopic examination or on fluorine.

REPORT ON MINERAL CONSTITUENTS OF MIXED FEEDS

By J. L. St. John (*Associate Referee*) and Edith Eng* (Division of Chemistry, Agricultural Experiment Station and State Chemist's Laboratory, Pullman, Washington)

Following the completion of the work on ash which resulted in the official method for ash in grain and stock feeds, No. **27.9**, page 405, of the 1945 edition of the A.O.A.C. Methods of Analysis, the Associate Referee has continued on a study of the mineral constituents in feeds. Samples were sent to collaborators for a study of the method of sample preparation for the determination of calcium and phosphorus, and for the study of methods for the determination of cobalt in feeds. Sufficient data have been received on calcium and phosphorus for a preliminary report.

Samples for calcium and phosphorus were prepared by the nitric-perchloric acid method developed by Gerritz (1) as follows:

Weigh a sample of suitable size (2 grams) into a Kjeldahl flask (500 or 300 ml). Add 20–30 ml of concentrated nitric acid and boil gently until all of the easily oxidizable matter is oxidized (30–45 minutes). Cool the soln somewhat and add 10 ml of perchloric acid (70–72%). Boil very gently, adjusting the flame so that the loss of perchloric fumes is a minimum. Continue boiling until the soln is water-white or nearly so, and dense white fumes appear. Use particular care not to boil to dryness at any stage in the procedure. Cool slightly, add 50 ml of distilled water, and boil to drive out any remaining nitrogen fumes. Cool, dilute, filter, and make to volume in a 250 ml flask.

The calcium determination was completed by method **27.47**, page 415, of the 1945 edition of Methods of Analysis, starting at the point where it states: "Pipet 25 ml of clear liquid . . . ." The phosphorus was completed on a separate aliquot by the volumetric method **2.12 (a)**, page 23.

These results are compared with those obtained by the utilization of the official method No. **27.47**, for calcium, and method No. **12.37**, for phosphorus, preparing the samples by **12.5**, followed by the volumetric procedure No. **2.12 (a)**.

Three samples of feed were submitted to the collaborators, including a chick starting mash (sample No. 1), a turkey starting mash (No. 2), and a chick developing mash (No. 3), recommended by the Agricultural Experiment Stations at the State College of Washington, at Pullman. These mashes were ground through a Wiley mill, thoroughly mixed, and sampled for shipment to collaborators.

Table 1 contains averages from data submitted by 12 collaborators which summarize the results available. The results will be presented in more detail at a later date after further data have been received from other collaborators. These average results show very close agreement be-

† Now Mrs. Edith Eng Huey.

tween the A.O.A.C. method, and the nitric-perchloric acid digestion method of sample preparation for both calcium and phosphorus. The difference between the two methods is slightly less for calcium than for phosphorus. A study (not presented in this summary) of the data shows that the maximum variation between the results of the different collaborators was less for the nitric-perchloric acid samples preparation than for the A.O.A.C. method in the case of calcium, although it was slightly more for the phosphorus determinations. Using the average of all three samples and disregarding the fact that each collaborator does not always find the difference between the methods in the same direction, it is found that the average of the difference between the two methods found by each collaborator was .08 per cent for calcium and .07 per cent for phosphorus. While the number of collaborators may be too small for statistical significance of the results, it has been found that the average deviation of each collaborator's results from the mean is practically the same for each of the two methods for both calcium and phosphorus, and is of the same magnitude as the values in the preceding sentence. Since the average deviation from the mean for each method of sample preparation and the average differences between the two methods are essentially the same, it would appear that the difference in results given by the two different methods of sample preparation are not significant.

TABLE 1.—*Averages showing agreement of the two methods of sample preparation*

SAMPLE	CALCIUM			PHOSPHORUS		
	A.O.A.C.	N—P*	DIFFERENCE	A.O.A.C.	N—P*	DIFFERENCE
1	1.99	1.96	0.03	0.83	0.79	0.04
2	2.17	2.16	0.01	0.95	0.93	0.02
3	2.04	2.02	0.02	1.00	0.96	0.04

* Nitric-perchloric sample preparation.

These results, taken in addition to other published results by Gerritz (1) and by Marshall (2), indicate that the nitric-perchloric acid method may be satisfactorily used for sample preparation for calcium and phosphorus determination. It is more economical of time. It is recommended that the nitric-perchloric acid method of sample preparation be adopted as a tentative method of the A.O.A.C.

Some analysts hesitate to use perchloric acid because of the danger of explosion, which may be involved. Methods have been developed in this laboratory using perchloric acid for the determination of not only calcium and phosphorus, but for the determination of manganese, sulfur, and potassium (3) (4) (5). It has been the opinion of this laboratory that there is perhaps little unusual danger in the use of perchloric acid if the samples

of organic matter are sufficiently digested with nitric acid so that all of the easily oxidizable organic matter is oxidized *before* the addition of the perchloric acid. Reference is also made to the papers mentioned above presenting the perchloric method for use with different elements.

REFERENCES CITED

(1) GERRITZ, H. W., *Ind. Eng. Chem., Anal. Ed.*, **7**, 116, and **7**, 167 (1935).
(2) MARSHALL, C. V., *This Journal*, **26**, 429 (1943).
(3) COOK, J. W., *Ind. Eng. Chem., Anal. Ed.*, **13**, 48 (1941).
(4) ST. JOHN, J. L., and MIDGLEY, M. C., *Ibid.*, **14**, 301 (1942).
(5) EVANS, R. J., and ST. JOHN, J. L., *Ibid.*, **16**, 630 (1944).

REPORT ON CRUDE FIBER

By WILLIAM L. HUNTER, (State Department of Agriculture, Sacramento 14, Calif.) *Associate Referee*

The analysis of crude fiber is one of the older measures of quality applied to feeding stuffs. It has been used in substantially its present form since late in the last century; and while the importance attached to the results obtained has been subject to modification, it remains a valuable control of importance to those who must consider feed composition. It is an empirical determination and must be closely followed if concordant results are to be obtained. Since laboratory equipment and convenience devices are continually changing, the procedure is subject to continuous review with a purpose of standardization. Uniformity of method and critical points of technique are the essence of such a procedure.

There have been numerous complaints because of unsatisfactory agreement of the results obtained by different laboratories. Many of these are probably due to departure from the official determination. This investigation is for the purpose of examining the general techniques to determine the revisions necessary to take into account present practices and to arrive at a new mode as the standard. It is also necessary to review the method for faults which may have resulted from changes in the composition of the object materials which introduce errors not originally contemplated. The present procedure is quite explicit as to both method and technique, but it is known to be honored more in the breach than in the following.

First attention will be given to standardization of technique. This year's work has consisted of a survey of procedures used by various laboratories, including equipment and conveniences for use. We have had the cooperation of those who participate in the collaborative sample of the Association of American Feed Control Officials. Aside from the fact that these laboratories are a good cross section of those regularly doing crude

fiber analyses, it was believed that the analysis data available from these collaborations might result in a considerable economy of investigational work. Seventy-one replies were received on questionnaires sent to official and industry laboratories. It is a bit surprising but not entirely unexpected, that we find among them almost seventy variations from the official procedure. These are mostly differences in equipment, but in some cases include departures from the method. The information obtained in the questionnaires has been studied in connection with the results on several collaborative samples. There appears to be no correlation. Deviations from the average are generally as great in one case as in another and are completely lacking in direction, in that the results of any laboratory do not fall consistently on one side of the general average. They may be either above or below. This fact would seem to indicate an allowable latitude in establishing a new procedure; but before such a conclusion is reached there must be further collaboration under closely controlled conditions which reduce the number of variables entering the consideration.

Further study will be given to the information on laboratory practices for the purpose of arriving at the most acceptable technique before this work is continued. It is recommended* that study of the crude fiber determination be continued.

REPORT ON PROTEIN EVALUATION IN FISH AND ANIMAL PRODUCTS†

By FRANK J. KOKOSKI (Division of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York), *Associate Referee*

The usual methods of determining the total protein in fish and animal concentrates do not give an index of true nutritional value of these products. This fact has led to the study of possible chemical methods which will better approximate true feeding value of such protein concentrates. This paper is in the nature of a progress report on such a study.

H. J. Almquist, E. L. R. Stockstad, and E. R. Holbrook,[1] at the University of California, developed a chemical method for evaluating the quality of the protein present in animal protein concentrates. Experiments conducted by these authors have demonstrated that the protein value obtained by these methods, which are fully described in their paper, shows a close correlation with evaluation obtained by growth data of chick tests. The method described in Dr. Almquist's paper consists of separating the total protein into several fractions which vary greatly in

* For report of Subcommittee A and action of the Association, see *This Journal*, 30, 41 (1947).

† Approved by the Director of the New York State Agr. Exp. Station for publication as Journal Paper No. 723.

[1] Almquist, H. J., E. L. R. Stockstad, and E. R. Holbrook. "Supplementary Values of Animal Protein Concentrates in Chick Rations." *The Journal of Nutrition*, Vol. 10, No. 2, Aug. 1935.

nutritive value. Following is a brief explanation of the various fractions separated.

A. *Copper Precipitable Protein.* The cupric ion in the presence of dilute hydroxide ion is one of the most specific reagents for intact protein. This precipitate includes intact protein, the undigestible protein, the water soluble protein, and to some extent the protein of decomposition products. Consequently it is necessary to determine the amount of undigestible and water soluble protein in order to measure the content of water insoluble, digestible, and copper precipitable protein which has the highest nutritive value.

B. *Phosphotungstic Acid Precipitable Proteins.* The peptones, peptides, and amino acids resulting from protein decomposition are precipitated in this fraction. This fraction shows the extent of decomposition.

C. *The Undigestible Protein.* This is a measure of protein derived from horn, hoof, hair, and protein rendered indigestible by high temperatures in the processing plant.

D. *The Hot-Water Soluble Protein.* The chief hot-water soluble protein present in animal products is gelatin, which is derived mainly from bone, cartilage, connective tissue, and skin. The nutritive value of this fraction is low.

Dr. Almquist states in his paper: "It may be pointed out that the method of analysis employed provides strong evidence of the condition and type of raw materials and of the effect of the manufacturing process on the quality of the product. Decomposition caused by putrefaction, autolysis, or poor methods of manufacture is reflected in a decrease of the copper-precipitable fraction and an increase in the phosphotungstic-acid-precipitable fraction. The inclusion of undigestible nitrogenous substances and the effect of excessive temperatures introduce an unduly large undigestible fraction. An increase in the cartilage, connective tissue, skin, and bone content causes a corresponding increase in the hot-water-soluble protein nitrogen. The same is true when the 'stick' is included in the final product. Finally, the presence of much nitrogen in the form of ammonia, amines, urea, and similar types of non-protein nitrogen causes a wide discrepancy to appear between the summation of the nitrogen accounted for by the copper and phosphotungstic acid treatments and the total nitrogen."

Dr. Almquist further states, "When analyses of protein concentrates are made for the estimation of (A) Intact protein, (B) Protein decomposition products, (C) Undigestible protein, and (D) Hot-water soluble protein, the analytical characteristics were found to exhibit a good correlation with nutritive value for chicks, when assigned the relative values of 100, 40, 0, and 40 respectively. Such analytical methods offer a possibility for the rapid laboratory determination of protein quality in commercial concentrates."

On the basis of Dr. Almquist's work, it was decided to test these methods in our laboratory on some commercial animal and fish concentrates selected at random from our 1945 official feeding stuffs samples. Nothing was known of the history or the nutritional value of these products. These samples were all analyzed in duplicate for the four fractions A, B, C, and D, and the results obtained are recorded in Table 1.

Acknowledgement is hereby made for the valuable assistance given by Miss J. M. Smith in the analyses of these products.

It must be understood that the quality index does not represent percentage of protein. It is simply a figure which shows the quality of the protein present, on a comparative basis.

In studying the results tabulated in Table 1, it will be noted that the maximum variation between duplicate determinations under

A	B	C	D	and quality index, respectively, was
7.4	1.8	1.4	1.7	7.8 and the average variation, respectively, was
1.945	0.365	0.34	0.46	2.0

The copper precipitable protein results in sample No. 15 vary by 7.4 per cent of the total protein and this is again reflected by a difference of 7.8 in the quality index. Since the determinations of the sample were not repeated, it may be assumed that this discrepancy was due to error. On the remainder of the samples the results sustain the claim that relatively good checks can be obtained by these methods.

According to J. W. Kuzmeski and A. F. Spelman,[2] "A meat or fish concentrate of excellent quality should have an index value of 70–75, one of good quality 65–70, and one of average quality 60–65. A product with an index value of less than 55 may be considered of low quality. Fish meal of highest quality may run as high as 80–87."

Using these as a criterion for judging the results reported in Table 1, we find that out of the twenty samples analyzed only two samples, namely, meat and bone sample No. 4 and fish meal sample No. 15, can be considered of low quality. Four meat and bone scraps and one fish sample are of average quality; six samples of meat and bone scraps are of good quality; three samples of meat and bone scraps and four samples of fish meal are of excellent quality. The highest quality index found was 81.3 on fish meal sample No. 20. From these results it must be judged that the animal and fish concentrates analyzed meet or exceed accepted standards of protein quality.

CONCLUSIONS

The methods of analysis, though fairly accurate as far as duplicate determinations are concerned, are nevertheless not rapid as compared to the

[2] Kuzmeski, J. W., and A. F. Spelman. "The Quality Index of Meat and Fish Products." Mass. Agr. Exp. Station, Control Series Bul. No. 113.

TABLE 1.—*Protein quality index of fish and animal products*

SAMPLE NUMBER*	TOTAL PROTEIN PER CENT	PERCENTAGE OF TOTAL PROTEIN AS—				PROTEIN QUALITY INDEX†	AVE.
		COPPER PRECIPITABLE	PHOSPHO-TUNGSTIC ACID PRECIPITABLE[1]	UNDIGESTIBLE	HOT-WATER SOLUBLE PROTEIN		
		A	B	C	D		
1a	55.1	87.6	4.7	11.8	16.3	67.9	66.5
b		85.5	4.7	12.2	17.0	65.0	
2a	53.4	85.0	4.7	9.2	21.9	64.6	63.8
b		82.6	4.9	9.4	20.2	62.9	
3a	46.9	87.6	3.8	10.7	17.7	67.8	69.6
b		91.3	3.2	10.9	17.1	71.4	
4a	55.6	67.8	17.6	11.0	26.6	47.8	47.6
b		68.5	18.0	12.4	26.6	47.3	
5a	50.6	87.8	4.3	9.9	17.4	69.3	70.3
b		88.5	4.3	9.1	16.4	71.3	
6a	55.4	81.0	4.5	8.7	19.9	62.3	63.8
b		83.9	4.5	8.8	19.1	65.3	
7a	48.4	82.9	8.1	12.8	19.8	61.4	60.3
b		79.8	7.9	12.2	19.4	59.1	
8a	42.3	94.3	2.4	9.7	19.4	74.0	72.8
b		92.0	2.4	9.5	19.8	71.6	
9a	45.5	87.5	6.0	10.5	19.6	67.6	67.5
b		87.0	6.0	9.9	20.2	67.4	
10a	47.4	88.8	4.0	15.6	14.7	66.0	65.4
b		88.2	3.6	16.0	14.7	64.8	
11a	48.4	86.6	3.7	12.0	15.9	66.7	66.7
b		86.6	4.3	12.0	15.7	66.7	
12a	41.9	87.6	5.3	11.7	21.2	65.3	63.7
b		84.7	5.3	11.7	21.7	62.1	
13a	42.9	88.3	4.4	10.5	21.9	66.5	66.7
b		88.1	5.8	10.5	21.9	66.8	
14a	52.5	87.0	7.8	6.7	16.8	73.3	73.1
b		85.5	8.4	6.5	15.8	72.9	
15a	60.8	94.9	1.5	38.2	5.8	54.1	50.2
b		87.5	1.3	38.2	5.8	46.3	
16a	57.8	90.1	1.2	9.2	7.1	77.1	75.7
b		87.5	1.2	9.2	7.6	74.3	
17a	62.6	86.7	2.6	7.0	12.6	73.1	73.1
b		86.4	2.2	7.0	12.3	73.0	
18a	62.5	90.2	1.9	11.8	7.4	74.8	76.2
b		93.0	1.4	11.5	7.5	77.6	
19a	61.4	79.3	8.8	5.7	23.6	62.9	62.3
b		77.2	10.6	5.7	23.5	61.6	
20a	63.2	92.7	1.4	8.5	7.4	80.4	81.3
b		92.8	1.4	7.1	7.1	82.1	

[1] Obtained from the filtrate from the copper precipitation.
* Samples 1–14, inclusive, are meat and bone scraps. Samples 15–20 are fish meals.
† Dr. Almquist's formula for calculating quality index: (A+.4B)−(C+.6D)=quality index.

ordinary chemical methods employed in feeding stuffs analyses. However, as compared to growth tests on chicks, which take six weeks time, the methods may be considered relatively rapid. To complete the analyses in duplicate on these twenty samples required the full time of two chemists for about four weeks. The methods at best are cumbersome and time consuming.

RECOMMENDATIONS*

There should be a continuation of study on chemical methods for evaluating protein in animal and fish concentrates. Certain improvements in technic may save much time. Upon completion of such improvements the methods should be tried by a number of collaborators on samples of known quality as evaluated by growth tests on chicks. Your Associate Referee on this project does not have the necessary facilities at his disposal to carry out this phase of the program. Assistance in such work must of necessity be found elsewhere.

REPORT ON SAMPLING AND ANALYSIS OF CONDENSED BUTTERMILK

By RAGNAR E. BERGMAN (State Department of Agriculture, St. Paul, Minnesota), *Associate Referee*

Because of the increasing use of semi-solid milk products in feeding practices and apparent difficulties of sampling and testing such materials, it was deemed necessary to first study available methods and develop new procedures suitable for the proper sampling and analysis of condensed buttermilk. The following is a paragraph taken from a letter written by H. A. Lepper, Sec.-Treas. of the A.O.A.C., to L. S. Walker, Referee on feeding stuffs:

"As you know, this product is shipped in barrels and the whey has a tendency of separating and floating on top. Obtaining a representative sample is quite a problem. This problem has also presented itself to State feed officials, and recently the question has arisen in connection with the enforcement of the Federal law. It would appear advisable for the Association to appoint someone to begin a study as soon as possible on methods of sampling condensed buttermilk and to develop methods of preparation of sample and analysis. The methods provided for solids in milk products should all be studied to find which one is most applicable to condensed buttermilk. We know that there is a variation in results depending upon the method chosen."

Although sampling of this type of material is the primary problem, it is impossible to determine the best sampling procedure if there is no proven analysis method for moisture by which we can determine the efficiency of a selected sampling procedure. Since total solids content is the basis

* For report of Subcommittee A and action by the Association, see *This Journal*, 30, 41 (1947).

TABLE 1.—*Collaborative results*
(Percentages)

CHEM.		VACUUM DRIED 100° C			AIR DRIED 100° C			VACUUM DRIED 70° C			AIR DRIED 70° C			TOLUENE
	ACID.	SOLIDS	ACID.	SOLIDS	SOLIDS	ACID.	SOLIDS	SOLIDS	ACID.	SOLIDS	SOLIDS	ACID.	SOLIDS	SOLIDS
	A	B(1)	B(2)	B(3)	C(1)	C(2)	C(3)	D(1)	D(2)	D(3)	E(1)	E(2)	E(3)	F
1	5.45	27.8	5.14	29.2	27.6	6.79*	28.0	29.8	7.21*	28.6	29.9	6.08*	29.9	
2	5.51	28.72	3.61	30.38	27.54	2.64	28.75	30.03	4.48	31.49	30.31	4.32	31.09	30.09
3	5.44	26.97	3.98	28.21	27.42	4.82	28.98	28.91	5.04	29.31	29.22	5.41	29.29	31.31
4 a.		28.39	4.07		28.26	3.51								29.99
5 b.	5.3	26.22	4.4	28.51	25.06	1.6*	26.65	29.39	4.7	28.85	29.28	4.3	29.66	
6	5.68	28.6	4.22	28.9	27.8	5.17	28.3	29.9	5.40	29.0	28.8	4.61	27.8	
7	5.45	27.43	3.92	28.25	25.45	2.21	27.04	29.48	4.90	28.60	28.72	5.00	28.43	28.7
8	5.22	27.46	4.44	28.00	27.23	4.59	27.90	30.43	4.49	28.83	29.62	4.78	28.50	29.81
9	5.61	27.82	4.59	27.90	28.61	3.10	28.67	28.77	5.39	28.83	29.56	5.30	29.09	
10 b.	5.49				27.17	4.26					27.94	4.74		
Ave.	5.46	27.71	4.26	28.67	27.21	3.79	28.04	29.59	4.91	29.19	29.26	4.81	29.22	29.98

* Omitted from average.
a. Average of 3 tests.
b. Average of 2 tests.

upon which condensed buttermilk is sold, it was decided to begin at this point in attacking the problem.

Except for the already established methods of the A.O.A.C. for solids determination on milk products, very little information was to be had from a literature search. However, two points were established: (1) On drying of buttermilk products, a charring of the sample is noted; (2) Lactic acid of buttermilk products decomposes when heated. A note by J. W. E. Harrisson appearing in *This Journal,* **18,** 645 (1935) recommends the use of an agent such as zinc oxide to prevent loss of lactic acid and any unfavorable action it might have on milk solids when drying.

Consequently, collaborators, who had shown their willingness to cooperate in a gratifying manner, were sent a semi-solid buttermilk sample with following instructions:

(A) Determine acidity of sample.

(B) Determine solids at 100°C. under vacuum. (A.O.A.C. method). Determine acidity of dried sample.

(C) Determine solids at 100°C. without vacuum. Determine acidity of dried sample.

(D) Determine solids at 70°C. with vacuum. Determine acidity of dried sample.

(E) Determine solids at 70°C. without vacuum. Determine acidity of dried sample.

(F) Determine solids by toluene distillation method.

The collaborators were further asked to repeat the above determinations, this time adding 2 grams of recently ignited zinc oxide, 5 ml of water, and mixing thoroughly. Results are shown in Table 1 under B(3), C(3), D(3), and E(3).

List of collaborators:

(1) Wm. L. Hunter and Van P. Entevistle, California
(2) Harry J. Fisher and Owen L. Nolan, Connecticut
(3) P. B. Curtis and A. E. Rihn, Indiana
(4) L. E. Bopst and H. R. Walls, Maryland
(5) Rodney C. Berry and Jas. A. Johnson, Virginia
(6) W. C. Geagley and Percy O'Meara, Michigan
(7) Howard Hammond, North Dakota
(8) Guy G. Frary and E. H. Zilliox, South Dakota
(9) J. F. Fudge and T. L. Ogier, Texas
(10) L. S. Walker, Vermont
(11) J. W. Kuzmeski and C. Tyson Smith, Massachusetts

COMMENTS OF COLLABORATORS

(1) Samples on drying darkened from light brown to black. Zinc oxide plus water addition shortened time to come to constant weight. Spattering noted when applying vacuum.

(3) Residue very dark and difficult to dissolve after drying at 100°C., but the ZnO residue is white. Residue is unchanged after drying at 70°C.

(5) In titration of charred samples end point is difficult to determine. 14 to 31 hours needed for drying at 70°C.

(8) ZnO samples spattered. Thirty minutes on steam bath before placing in oven prevented this. ZnO seemed to prevent charring.
(9) Residues brittle and almost black; difficult to read end point.
(10) Trouble reading indicator end point. Required 22 hours to reach constant weight at 70°C.
(11) It seems impractical to dry the type of material submitted to constant weight in the usually accepted sense of the term by any of the methods used to obtain the results reported.

Results and comments of the collaborators verify sample charring and lactic acid loss on drying of buttermilk products. However, this is eliminated somewhat at the lower temperature. Length of time required would be a definite disadvantage to the use of the lower temperature (70°C). The use of zinc oxide seems to aid the situation, indicating that the neutralization of the lactic acid tends to prevent the charring. Use of vacuum minimizes the lactic acid loss, as does also the use of the lower drying temperature. Three collaborators made the acidity test on the zinc oxide dried samples and their results, shown in Table 2, indicate that the lactic acid was not neutralized by the zinc oxide.

TABLE 2.—*Per cent lactic acid in ZnO dried samples*

CHEMIST	B(4)	C(4)	D(4)	E(4)
8	3.80	3.59	2.59	2.31
5	4.20	4.50	3.90	2.90
9	3.57	3.60	3.16	3.57
Ave.	3.86	3.87	3.22	2.93

If the loss in lactic acid is added to the total solids content in the vacuum and air dried samples the results in the case of 100°C. drying would be 28.91 per cent and 28.88 per cent, respectively; while in the case of 70°C. drying they would be 30.14 per cent and 29.91 per cent.

Considering the type of sample used the results of the solids content determinations are in good agreement. However, because of the charring, indicating an oxidation possibility, and the incomplete neutralization of the lactic acid in the zinc oxide experiment, further work on this problem is contemplated.

The sampling procedure now most frequently used is shaking of barrel or container, or the use of a wooden paddle in agitating the product and then taking a sample when the material is believed to be uniform. Another method is to use a special tool which draws a core, about three-fourths of an inch in diameter, from top to bottom of container. This tool is constructed especially for liquids and semisolid products. Samples drawn by these methods will be used in further collaborative work.

It is recommended* that collaborative work on the sampling and analysis of condensed buttermilk be continued.

* For report of Subcommittee A and action of the Association, see *This Journal*, 30, 41 (1947).

CONTRIBUTED PAPERS

THE DETERMINATION OF MOISTURE IN FERTILIZERS[1]

By WILLIAM H. ROSS[2] and KATHARINE S. LOVE (Division of Fertilizer and Agricultural Lime, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland)

The present official method for the determination of moisture in fertilizers was adopted by this Association at its second annual meeting in 1885 (4). The method consists in drying the sample at a temperature of 98°–100°C. for a period of 5 hours. The loss in weight includes free moisture, occasional volatile decomposition products from constituents of the sample, and usually more or less water of crystallization from monocalcium phosphate and other hydrated constituents. Inasmuch as all superphosphates and mixed fertilizers of the ordinary type contain some water of crystallization or hydration, and since many mixed fertilizers also contain easily decomposable materials, it must follow that this method cannot be depended upon to give accurate results for free moisture in all types of materials and mixtures.

At the 1931 meeting of the Association a second procedure was recommended for adoption as a tentative method (6) for the determination of moisture in fertilizers. This method consists in subjecting the sample to distillation with toluene for a period of 1–10 hours depending on the nature of the material being analyzed. The boiling point of toluene is 110.8°C. This temperature is still higher than that recommended by the official method and thus this method is also capable of liberating volatile reaction products and water of hydration from the sample. This tentative method is, therefore, likewise unsuited for the determination of free moisture in mixed fertilizers and many fertilizer materials.

A recommendation was accordingly adopted at the 1940 meeting of this Association that the Associate Referee on phosphoric acid make a study of the methods for the determination of moisture in fertilizers. As a preliminary to this study, Whittaker and Ross (7) in 1941 reviewed the various methods that were then in use or had been proposed for the determination of moisture. The view was expressed that the problem of determining free moisture in fertilizers could be solved by drawing heated air through the sample at a constant temperature of 60°C. At this temperature there is little or no decomposition of hydrated salts that commonly occur in mixed fertilizers and the decomposition or oxidation reactions that cause so much trouble in present methods are reduced. The equipment designed for this rapid removal of moisture at a relatively low temperature is de-

[1] Presented at the annual meeting of the Association of Official Agricultural Chemists, at the Thursday morning session, October 26, 1944.
[2] Deceased.

TABLE 1.—*Ammonium nitrate samples*

SAMPLE NO.	SOURCE	TYPE OF GRANULES	TREATMENT	MOISTURE IN SAMPLE
ANPL #1	Alberta Nitrogen Products, Ltd.	Sprayed	None	Original
ANPL #2	" " " "	"	0.5% WP[1]+3.5% Kittitas	Added
WCW #3	Welland Chemical Works	"	None	Original
TVA #4	Tennessee Valley Authority	Grained	"	Absorbed
TVA #5	" " "	"	1% PRP[2]+4% Celite	"
MCW #6	Military Chemical Works	Crystallized	None	Original
MCW #7	" " "	"	"	"

[1] Paraffin-petrolatum coating.
[2] Petrolatum-rosin-paraffin coating

scribed by Hardesty, Whittaker, and Ross elsewhere in this issue under the title of "The Air-flow Method for the Determination of Moisture in Fertilizers." (2).

During the past few years solid ammonium nitrate has become an important source of fertilizer nitrogen. Since small amounts of moisture have a profound effect on its physical condition, an accurate method of determining its moisture content is essential to the improvement and control of the mechanical condition of this material. Ammonium nitrate undergoes slow decomposition at 100°C. and it is desirable to know the adaptability of the official methods and other procedures for determining its moisture content.

With the view to finding a suitable method for this determination a set of ammonium nitrate samples was prepared and submitted to a number of laboratories accustomed to making moisture determinations in this material. The samples were accompanied with the request that they be analyzed for moisture by such methods as were considered to be best suited for ammonium nitrate.

The samples prepared for this collaborative study are given in Table 1. Sample ANPL #1 was selected from a commercial shipment that had been stored in a dry place. Sample ANPL #2 was also selected from a commercial shipment but its moisture content was increased by spraying it with water while being rolled in a rotating drum. Samples TVA #4 and TVA #5 were taken from commercial shipments that had been stored in bags for a period of three months at 55°F. and a relative humidity of 80 per cent. Sample WCW #3 was collected at the plant before it was dried to the point desired for shipment. Samples MCW #6 and MCW #7 were prepared on a pilot plant scale. The former was dried as completely as was considered practical under commercial conditions. Sample MCW #7 was represented as being only partially dried. The moisture in Samples ANPL #1 and ANPL #2 and in Samples TVA #4 and TVA #5 includes that which had been taken up by the samples subsequent to thorough drying at the factory. The moisture in Sample WCW #3 and in Samples MCW #6 and #7, on the other hand, represents original moisture that had not been eliminated by drying.

The methods used by the collaborators for the determination of moisture in the samples and the results obtained are shown in Table 2. The results as a whole show good agreement considering the hygroscopic nature of the samples submitted for analysis. Good results were obtained with the Fischer method which consists of solvent-extraction of moisture from the sample and determination of the amount of moisture in the solvent by means of Karl Fischer Reagent (1, 5). This method is rapid and seems to be quite well adapted for the determination of moisture in ammonium nitrate. It, however, gives water of crystallization as well as free moisture when both are present and it is, therefore, not suited for the

TABLE 2.—*Moisture in ammonium nitrate samples*
Percentages

COLLABORATOR	METHODS	ANPL #1	ANPL #2	WCW #3	TVA #4	TVA #5	MCW #6	MCW #7
	Oven-drying							
Alberta Nitrogen Products, Ltd.	2 hrs. at 90°C.	0.13	2.97	0.74	0.75	0.92	0.17	0.75
Hercules Powder Company	2 hrs. at 95°C.	0.11	2.70	0.50	0.57	0.85	0.11	0.44
U. S. Department of Agriculture	20 hrs. at 70°C.	0.08	2.91	0.74	0.75	0.78	0.08	0.66
Consolidated Mining and Smelting Company	4 hrs. at 80°–85°C.	0.08	2.75	0.72	0.65	0.80	0.08	0.55
Military Chemical Works	5 hrs. at 70°C.	0.06	2.59	0.64	0.47	0.71	0.11	0.47
Military Chemical Works	2 hrs. at 100°C.	0.08	2.76	0.71	0.56	0.88	0.09	0.42
Eastern States Farmers' Exchange	5 hrs. at 100°C.	0.11	2.79	0.76	0.61	0.81	0.09	0.43
Tennessee Valley Authority	20 hrs. at 70°C.	0.09	2.84	0.82	0.71	0.83	0.09	0.73
	Air-flow							
U. S. Department of Agriculture	Air at 70°C. for 4 hrs.	0.07	2.89	0.80	0.79	0.80	0.08	0.60
Welland Chemical Works	Dry air at 65°–70°C. for 40 minutes	0.07	2.69	0.78	0.61	0.70	0.06	0.33
Eastern States Farmers' Exchange	Air at 60°C. for 3 hrs.	0.09	2.73	0.78	0.58	0.77	0.09	0.47
Tennessee Valley Authority	Dry air at 60°C. for 6 hrs.	0.07	2.72	0.75	0.78	0.81	0.08	0.67
Tennessee Valley Authority	Dry air at 70°C. for 5 hrs.	0.08	2.68	0.91	0.83	0.79	0.10	0.60
	Other Procedures							
Solvay Process Company	2 hrs. in vacuum at 80°C.	0.09	2.67	0.89	0.68	0.83	0.07	0.71
Alberta Nitrogen Products, Ltd.	Fischer	0.09	2.52	0.72	0.70	0.99	0.09	0.85
Mean		0.09	2.75	0.75	0.67	0.82	0.09	0.58

determination of free moisture in superphosphate and mixed fertilizers. The other methods used by the collaborators included ordinary oven drying at temperatures of 100°C. or below, and drying by the air-flow method for varying lengths of time and at different temperatures.

The rate at which ammonium nitrate undergoes drying by these two methods and by the method of drying at room temperature in a vacuum over "Anhydrone"[3] is shown by the data in Table 3. The results in the table indicate that drawing air at 70°C. through the sample for 3 hours by air-flow method is equivalent to 18–24 hours drying in a static air oven at

TABLE 3.—*Rates at which samples of ammonium nitrate decrease in weight on drying by different methods*

TIME OF DRYING, HOURS	PER CENT LOSS IN WEIGHT OF SAMPLE						
	ANPL		WCW	TVA		MCW	
	#1	#2	#3	#4	#5	#6	#7
	In Air Oven at 70°C.						
6	0.07	2.83	0.66	0.66	0.71	0.05	0.48
12	0.08	2.88	0.72	0.73	0.76	0.08	0.59
18	0.08	2.90	0.74	0.75	0.78	0.08	0.65
24	0.05	2.88	0.74	0.75	0.77	0.06	0.67
30	0.08	2.92	0.76	0.78	0.80	0.09	0.68
	In Vacuum over Anhydrone at Room Temperature						
6	0.00	2.79	0.36	0.65	0.70	0.00	0.28
12	0.00	2.80	0.36	0.69	0.74	0.00	0.28
18	0.01	2.82	0.38	0.70	0.75	0.01	0.28
24	0.01	2.85	0.40	0.73	0.79	0.02	0.29
30	0.01	2.85	0.39	0.73	0.79	0.02	0.29
	Air-flow at 70°C.						
1		2.82	0.58	0.73	0.68	0.03	0.41
2		2.87	0.72	0.77	0.74	0.07	0.57
3		2.90	0.82	0.81	0.79	0.09	0.71
4		2.90	0.85	0.81	0.79	0.10	0.75
5		2.93	0.89	0.83	0.85	0.12	0.78

the same temperature. The air-flow method thus has the advantage of being more rapid than the method of drying in an air oven and it is, therefore, better adapted for use in control work. The table further shows that samples, such as ANPL #2, TVA #4 and TVA #5, which contained only added or absorbed moisture, can be dried in a vacuum over Anhydrone at room temperature, but that this method of drying does not eliminate within a period of 30 hours all the original or occluded moisture in such samples as WCW #3 and MCW #7.

[3] Commercial name for anhydrous magnesium perchlorate.

OTHER FERTILIZER MATERIALS

The results obtained in a comparison of the official method, the vacuum-drying method and the air-flow method for the determination of moisture in other fertilizer materials are shown in Table 4. The three methods give essentially the same results for the stable materials, ammonium sulfate and potassium chloride. The higher values obtained by the official method on the other materials listed in Table 4 are attributable to a number of factors (a) decomposition of the compound such as may occur slowly with ammonium nitrate at 100°C., (b) loss of water of crystallization such as

TABLE 4.—*Moisture in fertilizer materials as determined by different methods*
Percentages

TIME OF DRYING	MONO-CALCIUM PHOSPHATE MONO-HYDRATE[1]	MONO-CALCIUM PHOSPHATE MONO-HYDRATE[2]	DICALCIUM PHOSPHATE DIHYDRATE	SUPER-PHOSPHATE	DOUBLE SUPER-PHOSPHATE	AMMONIUM SULFATE	POTASSIUM CHLORIDE	AMMONIUM NITRATE
			Official Method					
3	0.10	0.95	2.99	8.55	4.03	—	0.05	0.17
5	0.09	1.15	16.16	8.61	4.05	1.78	0.05	0.30
7	0.13	1.27	17.95	8.66	4.18	—	0.06	0.40
		In Vacuum over Anhydrone at Room Temperature						
16	0.00	0.56	0.00	8.30	3.08	—	0.00	0.00
21	0.00	0.58	0.00	8.33	3.09	1.80	0.00	0.00
26	0.00	0.58	0.32	8.41	3.14	—	0.00	0.00
			Air-flow Method at 60°C.					
1	0.03	0.32	0.02	7.25	2.60	—	0.01	0.14
2	0.02	0.33	0.02	7.42	2.76	1.72	0.00	0.23
3	0.03	0.33	0.04	7.39	2.75	—	0.01	0.32

[1] Washed with acetone.
[2] Contains slight amount of free acid.

may occur at 100°C. with C. P. monocalcium phosphate, dicalcium phosphate, or the superphosphates used in these tests, and (c) the vapor pressure of the liquid phase of monocalcium phosphate and the superphosphates containing free phosphoric acid is higher at 100°C. than at lower temperatures, which may account in part for the higher values obtained at 100°C.

The results in Table 4 also indicate that drying in vacuum over Anhydrone does not remove occluded moisture from ammonium nitrate. On the other hand, it gives higher results for moisture in dicalcium phosphate dihydrate after 26 hours in the desiccator than those obtained by the air-flow method. As compared with the air-flow method it also has a tendency to give considerably higher results for materials containing free phosphoric

acid, namely, the unwashed monocalcium phosphate monohydrate and the superphosphates used in these tests.

CONCLUSIONS

Under the conditions of these tests the following conclusions would seem valid for the materials and methods studied.

1. The official method usually gives higher results for moisture in fertilizer materials containing water of hydration than either of the other two methods.
2. The air-flow method gives lower results for moisture in materials that tend to lose water of hydration in vacuum over Anhydrone than either the official or the vacuum-drying methods.
3. The presence of free acid, as observed by Hill and Jacob (3), increases the rate at which monocalcium phosphate monohydrate loses water of hydration when dried as directed in the official method.
4. The air-flow method gives higher results than the vacuum-drying method for moisture in a material such as ammonium nitrate which contains occluded water.
5. Although ammonium nitrate is known to undergo decomposition at 100°C, the rate of decomposition is too slow to seriously interfere with the determination of moisture in this material by the official method.
6. All three methods give essentially the same results for moisture in stable materials that do not contain occluded water or water of crystallization.
7. One or two hours' drying by the air-flow method at 60°C. would seem to suffice for the determination of moisture in all samples used in the tests with the possible exception of crystalline ammonium nitrate containing occluded water.
8. The official method is not adapted to the determination of free moisture in the presence of easily oxidizable organic materials, as observed by Hardesty, Whittaker, and Ross in the accompanying paper (2), since a temperature of 100°C. may cause decomposition and loss in weight of the sample other than that representing the free-moisture content.

LITERATURE CITED

(1) Fischer, Karl, *Angew. Chem.*, **48**, 394 (1935).
(2) Hardesty, John O., Whittaker, Colin W., and Ross, William H., *This issue*, page 640.
(3) Hill, W. L., and Jacob, K. D., *This Journal*, **17**, 487–505 (1934).
(4) Jenkins, E. H., White, H. C., and Stubbs, W. C., *U. S. Dept. Agr. Div. Chem. Bull.* **7**, 14 (1885).
(5) Smith, D. M., Bryant, W. M. D., and Mitchell, J., Jr., *J. Am. Chem. Soc.*, **61**, 2407–2412 (1939).
(6) Smith, John B., *This Journal*, **15**, 272–277 (1932).
(7) Whittaker, Colin W., and Ross, William H., *This Journal*, **25**, 132–142 (1942).

FACTORS AFFECTING THE AVAILABILITY OF AMMONIATED SUPERPHOSPHATES. PART II.

THE EFFICIENCY OF AMMONIATED SUPERPHOSPHATES AS INDICATED BY POT TESTS IN THE GREENHOUSE[1]

By WILLIAM H. ROSS,[2] J. RICHARD ADAMS,[3] JOHN O. HARDESTY, AND COLIN W. WHITTAKER[4]

Superphosphates that have been ammoniated differ from those of the ordinary type in that they contain a higher proportion of phosphatic components soluble in neutral ammonium citrate solution. Inasmuch as the availability of a phosphatic material as determined by the official method depends on its solubility in neutral ammonium citrate solution, it would be expected, and has actually been found by analysis, that the method indicates a higher availability for the P_2O_5 in a mixture containing a low proportion of an ammoniated superphosphate than in one containing a high proportion of the same material. This failure to indicate a constant availability for a given material is a serious defect in the official method. In compliance with a recommendation that was adopted by the Association in 1942, a further study was undertaken of (a) the factors that affect the chemical availability of the P_2O_5 in ammoniated mixtures; and (b) the manner in which the same factors affect the efficiency of an ammoniated superphosphate in promoting crop growth.

The results of the study of the factors affecting the chemical availability of ammoniated mixtures, namely: (a) degree of ammoniation, (b) storage temperature following ammoniation, (c) moisture content, (d) presence of dolomite, and (e) presence of fluorides, were presented in Part I of this report (2). The present paper presents results of a collaborative study dealing with the effects of the foregoing factors on the efficiency of ammoniated mixtures in promoting crop growth in the greenhouse, and is a continuation of the work recommended by the Association in 1942.

PROCEDURE

The fertilizer used in these tests consisted of 5-6-8 grade prepared from 4-12-4 ammoniated base after 36 days storage of the base at temperatures of 20, 60, and 90°C. The dolomite, when present during ammoniation and storage, was added at the rate of 250 pounds per ton of the 4-12-4 base mixture prior to ammoniation. All of the 5-6-8 mixtures and the 5-0-8 check mixture, as applied to the soil, contained 303 pounds of dolomite per ton regardless of the dolomite status of the 4-12-4 base mixture. There-

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fore, in this paper, the references to the presence or absence of dolomite refer to the 4-12-4 base mixture during ammoniation and storage. The relationships of the results reported might be quite different if dolomite were not present in all of the 5-6-8 mixtures. The dolomite used had a fineness of 90% through a standard 100 mesh screen. The aqua ammonia added was varied to give ammoniated mixtures containing 9 per cent moisture and 0, 2, 3, 4, and 5 parts of ammonia per 100 parts of ammoniated superphosphate on the basis of 20 per cent available P_2O_5 in the superphosphate used. Anhydrous ammonia was used to ammoniate mixtures that were formulated to contain only 3 per cent moisture.

Nitrogen in the completed mixtures was supplied mainly as ammonium sulfate and sodium nitrate, the same amounts of these materials being used in all cases. Because, however, of the variable amount of nitrogen in the form of ammoniated superphosphate it was necessary to adjust the total amount of nitrogen in order to have the same percentages of nitrogen present in all mixtures. This was done by adding the required amounts of urea. Potash was supplied as high grade potassium chloride.

The test crops included millet, wheat, Sudan grass, and sorghum. The fertilizer was applied to the millet and wheat crops by mixing the required amount with 5 per cent of the soil in the pot and depositing this mixture in a horizontal layer halfway between the bottom and top of the pot. With the Sudan grass and sorghum the fertilizer was located in a circular area about 3 inches in diameter and 1.5 inches from the top of the soil. The seed were planted in a ring 5 inches in diameter concentric with the fertilized area. Both methods of placement were used with sorghum on the Atwood fine sandy loam.

Four different brands of superphosphate were compared in the tests. Three (A, B, and C) were made from Florida pebble and one (D) from Tennessee brown rock phosphate. Superphosphate A was used in most of the tests. The average yields obtained in the pot tests (with isolated exceptions) indicated no significant difference, either before or after ammoniation, in the availability to plants of the P_2O_5 in these superphosphates from different sources. Other superphosphates used in the tests of the effect of fluorine will be described later.

Seven different acid soils (*p*H range 4.6 to 5.9) at 5 locations, and an alkaline soil, the Houston clay (*p*H 8.2) at one of the locations, were used in the study. The conditions under which the tests were made with the different soils and crops, and the mean yields obtained in the nonammoniated and no-phosphorus check pots, are given in Table 1. These phosphorus deficient soils were chosen to afford accurate measurement of small differences in the efficiency of the phosphates. The relative efficiency of applied phosphates may be affected by the level of available phosphorus in the soil. If that be the case, the data secured on these soils may

TABLE 1.—*Conditions of pot experiments*

COOPERATING ORGANIZATION	SOIL			P_2O_5 RATE PER ACRE	CROP			AV. YIELD DRY WT. PER POT		REPLICATIONS
	TYPE	pH	AIR-DRY WT. PER POT		KIND	GROWING PERIOD	PLANTS PER POT	WITH[1] P_2O_5	WITHOUT P_2O_5	
			lbs.	*lbs.*		*days*		*grams*	*grams*	
Bur. Plant Industry	Hartsells fine sandy loam	5.5	11.2	80	Millet	45	5	11.7	0.7	5
" " "	Wooster silt loam	4.9	11.6	80	Millet	62	5	36.1	14.4	5
" " "	" " "	4.9	11.5	40	Wheat	137	5	31.3	9.3	3
" " "	Sassafras sandy loam	4.6	11.2	70	Wheat	124	5	32.3	0.7	3
Arkansas Experiment Station	Newtonia silt loam	5.9	15.0	120	Sudan grass	49[2]	16	25.9	3.9	6
Alabama Experiment Station	Cecil clay	5.7	—	—	Sudan grass	—	—	23.7	0.9	3
South Carolina Experiment Station	Grady sandy loam	5.5	—	—	Sudan grass	95	—	24.3	10.8	2
Mississippi Experiment Station	Atwood fine sandy loam	5.9	24.0	60	Sorghum	84	8	35.2	15.7	4
	Houston clay	8.2	15.0	60	Sorghum	58	8	21.7	15.7	4

[1] Non-ammoniated.
[2] First cutting.

not be readily interpretable in terms of soils higher in available phosphorus.

Average yields and differences required for significance are given in the Appendix, Sections 1 and 2. To facilitate discussion the data have been computed to relative yield increases and are used in that form in the tables in the body of the paper.

DEGREE OF AMMONIATION

The effect of degree of ammoniation on the availability to plants of the P_2O_5 in ammoniated superphosphate mixtures is indicated by the results in Table 2. This table shows the average relative increase in yield of millet, wheat, Sudan grass, and sorghum when fertilized with superphosphate mixtures that have been ammoniated to 0, 2, 3, 4, and 5 per cent on

TABLE 2.—*Effect of degree of ammoniation on the availability to plants of the P_2O_5 in ammoniated superphosphate mixtures. Dolomite present; moisture 9 per cent; temperature of storage 20°C.*

TEST MIXTURE	DEGREE OF AMMONIATION: NH_3 ADDED ON BASIS OF SUPERPHOSPHATE IN MIXTURE	AVERAGE RELATIVE INCREASE IN YIELD[1]
No.	*Per cent*	*Per cent*
1	0	100
2	2	100
3	3	88
4	4	82
5	5	65

[1] On the basis of 100 as the average relative increase for the non-ammoniated mixture.

the basis of the superphosphate in the mixture. The results show that a 2 per cent ammoniated superphosphate mixture is as available to plants as a non-ammoniated mixture but that the efficiency of the ammoniated superphosphate decreases as the degree of ammoniation is increased to and beyond 3 per cent.

TEMPERATURE OF STORAGE

The samples that were ammoniated from 2 to 5 per cent were stored at temperatures of 20°, 60°, and 90°C. for 36 days. The results in Table 3 show the average relative increases in yield obtained in pot tests with the mixtures stored at different temperatures. The data in the table are consistent in showing that the availability to plants of the P_2O_5 in mixtures containing dolomite decreases, not only with increase in the rate of ammoniation but also with increase in the temperature at which the samples are stored following ammoniation. The data further show that the effect of temperature in the range 60°–90°C. is more pronounced for the lightly than for the heavily ammoniated mixtures.

TABLE 3.—*Effect of temperature of storage on the availability to plants of the P_2O_5 in ammoniated superphosphate mixtures, dolomite present; moisture 9 per cent*

TEST MIXTURE	DEGREE OF AMMONIATION: NH_3 ADDED ON BASIS OF SUPERPHOSPHATE IN MIXTURE	STORAGE TEMPERATURE	AVERAGE RELATIVE INCREASE[1]
No.	*Per cent*	°C.	*Per cent*
2	2	20	100
3	3	20	88
4	4	20	82
5	5	20	65
6	2	60	93
7	3	60	68
8	4	60	62
9	5	60	55
10	2	90	76
11	3	90	59
12	4	90	53
13	5	90	53

[1] On the basis of 100 as the average relative increase for the non-ammoniated mixture.

EFFECT OF MOISTURE

The effect of the moisture content of an ammoniated superphosphate mixture on the availability of its P_2O_5 to plants is shown by the data in Table 4. The samples used in this series of tests were all ammoniated to 5 per cent on the basis of the superphosphate in the sample. The average results reported by all the collaborators were consistent in showing that the P_2O_5 in samples ammoniated at a moisture content of only 3 per cent is somewhat more available to plants than that in samples that were am-

TABLE 4.—*Effect of moisture on the availability to plants of the P_2O_5 in ammoniated superphosphate mixtures. Degree of ammoniation, 5 per cent; dolomite present*

TEST MIXTURE	MOISTURE	TEMPERATURE OF STORAGE	AVERAGE RELATIVE INCREASE IN YIELD[1]
No.	*Per cent*	°C.	*Per cent*
14	3	20	78
5	9	20	65
15	3	60	66
9	9	60	55
16	3	90	75
13	9	90	53

[1] On the basis of 100 as the average relative increase for the non-ammoniated mixture.

moniated at a moisture content of 9 per cent, and that the effect of the moisture was most pronounced at the highest temperature, 90°C.

EFFECT OF DOLOMITE

The effect of the presence of dolomite during ammoniation and storage on the availability to plants of the P_2O_5 in ammoniated mixtures was determined by pot tests with two sets of samples. The values in Table 5 indicate that dolomite tends to reduce the availability of P_2O_5 to a considerable extent in the mixtures stored at 60 and 90°C. and to a lesser extent in those stored at 20°C. The greater proportion of the results, however, showed the presence of dolomite to have no significant effect on the availability to plants of the P_2O_5 in the samples stored at 20°C. Two series of tests, those with millet and sorghum, produced such drastic differences in results between the samples containing dolomite and those not containing it, that the average of all results shows some reduction in availability of the samples containing dolomite when stored at 20°C. Table 5 also shows, that in mixtures without dolomite, the temperature of storage has little or no influence on the availability of P_2O_5 to plants.

TABLE 5.—*Effect of the presence of dolomite during ammoniation and storage on the availability to plants of the P_2O_5 in ammoniated superphosphate mixtures. Degree of ammoniation, 5 per cent; moisture 9 per cent*

TEST MIXTURE	DOLOMITE	TEMPERATURE OF STORAGE	AVERAGE RELATIVE INCREASE IN YIELD[1]
No.		*°C.*	*Per cent*
17	Absent	20	75
5	Present	20	65
18	Absent	60	76
9	Present	60	55
19	Absent	90	72
13	Present	90	53

[1] On the basis of 100 as the average relative increase for the non-ammoniated mixture.

EFFECT OF FLUORINE

The three superphosphates used in the preparation of the samples for this series of tests were prepared in the laboratory. One was a synthetic superphosphate consisting of a mixture containing one mol of C.P. monocalcium phosphate to each 2 mols of calcium sulfate, with sufficient filler to reduce the P_2O_5 content to 20 per cent. Three sets of 3 samples each were prepared from the synthetic superphosphate. One set contained no fluorine; another contained fluorine as calcium fluoride; and the third contained fluorine as sodium fluoride, the fluorine added in each case being equivalent to 10 per cent of the P_2O_5 present. The second superphosphate was prepared from Tennessee phosphate rock containing 3.22 per cent fluorine, and the third from the same rock after it had been defluorinated.

One sample of each set (not ammoniated) was stored at 20°C.; a second

was ammoniated to 5 per cent and stored at 20°C.; and the third was ammoniated to the same degree and stored at 60°C.

The values shown in Table 6 indicate that the presence of added soluble fluorides has, on the average, little effect on the availability to plants of the P_2O_5 in either the ammoniated or non-ammoniated mixtures containing synthetic superphosphate, but that some effect is apparent when the fluorine is present as superphosphate from Tennessee rock. Bartholomew (1) observed that soluble fluorine compounds added to the soil did not

TABLE 6.—*Effect of the presence of fluorine during ammoniation and storage on the availability to plants of the P_2O_5 in ammoniated superphosphate mixtures. Dolomite absent; moisture 9 per cent*

TEST MIXTURE	FLUORINE	DEGREE OF AMMONIATION: NH_3 ADDED ON BASIS OF SUPERPHOSPHATE IN MIXTURE	TEMPERATURE OF STORAGE	AV. RELATIVE INCREASE IN YIELD[1]
No.		*Per cent*	*°C.*	*Per cent*
	Synthetic Superphosphate			
26	Absent	0	20	100
27	Absent	5	20	75
28	Absent	5	60	80
29	Present as CaF_2	0	20	100
30	Present as CaF_2	5	20	94
31	Present as CaF_2	5	60	71
32	Present as NaF	0	20	100
33	Present as NaF	5	20	89
34	Present as NaF	5	60	77
	Superphosphate from Tennessee Rock			
35	Present	0	20	103
36	Present	5	20	63
37	Present	5	60	59
	Superphosphate from Defluorinated Tenn. Rock			
38	Absent	0	20	100
39	Absent	5	20	83
40	Absent	5	60	82

[1] On the basis (test mixtures 26–34) of 100 as the average relative increase for the non-ammoniated synthetic superphosphate mixture and of 100 (test mixtures 35–40) as the average relative increase for the non-ammoniated superphosphate made from defluorinated Tennessee rock (No. 38).

affect the availability of phosphorus in the soil or that added as monocalcium phosphate, but the presence of fluorine in naturally occurring phosphate rock greatly influenced the availability of their phosphorus to plants. MacIntire and coworkers (6) observed that no toxic effect on plant growth was caused "by the component fluorides of superphosphates used judiciously in relation to the need for liming."

SHORT-SEASON VERSUS LONG-SEASON CROPS

Long experience has shown that crops possess marked differences in their ability to acquire phosphorus from water-insoluble sources. This difference in the response that crops make to phosphates of relatively low

TABLE 7.—*Percentage availability to short- and to long-season crops of the P_2O_5 in ammoniated superphosphate mixtures. Dolomite present; moisture 9 per cent; temperature of storage 20°C.*

TEST MIXTURE	DEGREE OF AMMONIATION: NH_3 ADDED ON BASIS OF SUPERPHOSPHATE IN MIXTURE	AVERAGE RELATIVE INCREASE IN YIELD	
		SHORT-SEASON CROPS[1]	LONG-SEASON CROPS[2]
No.	*Per cent*	*Per cent*	*Per cent*
1	0	100	100
2	2	98	106
3	3	84	101
4	4	76	106
5	5	55	98

[1] Millet, sorghum, and one cutting of Sudan grass.
[2] Wheat and two cuttings of Sudan grass.

solubility varies with their growing season, their root development and other inherent genetic characteristics (3, 10).

In the present investigation the crops of millet, sorghum, and one cutting of Sudan grass were harvested on an average of about 55 days from the time of planting. The period between seeding time and harvest for wheat and two cuttings of Sudan grass amounted to about 125 days. Millet, sorghum, and one cutting of Sudan grass may thus be considered as short-season crops, while wheat and two cuttings of Sudan grass are relatively long-season crops. The relative increases in yield shown in Table 2 represent the average of the values found for all crops grown on the acid soils. In Table 7 the values found for these crops are broken down into the average relative increases in yield found for (a) the short-season crops: millet, sorghum, and one cutting of Sudan grass; and (b) the long-season crops: wheat and two cuttings of Sudan grass. The data in Table 7 are in agreement with the observation that, when other factors are the same, long-season crops are able to utilize water-insoluble phosphates to better advantage than those having a shorter growing season. The values in the table indicate that the ammoniation of a superphosphate, to 3 per cent or more, causes a significant decrease in the availability of its P_2O_5 content to short-season crops. The results obtained with the long-season crops indicate, on the other hand, that there is no material difference between the efficiency of the non-ammoniated samples and any of the ammoniated samples that had been stored at 20°C. It is felt, however, that the data submitted are too limited to justify any definite conclusions on the relative response of long- and short-season crops to heavily ammoniated superphosphates.

CHEMICAL AVAILABILITY IN RELATION TO CROP RESPONSE ON ACID SOILS

In Table 8 the availability of the ammoniated superphosphate samples as determined chemically by the official method is compared with their

availability in promoting crop growth as indicated by pot tests on the acid soils. The results indicate that the official method can be used to evaluate accurately the availability to plants of the P_2O_5 in non-ammoniated goods, and in ammoniated mixtures containing a maximum of about 2 per cent ammonia on the basis of the superphosphate present. The table shows, however, that in more heavily ammoniated mixtures the official method gives higher availability values than those indicated by the pot tests for all crops, and that the spread between the two methods increases with increase in the ammoniation of the mixture.

TABLE 8.—*Effect of various treatments on the availability of the P_2O_5 in ammoniated superphosphate mixtures. Dolomite present; moisture 9 per cent*

TEST MIXTURE	DEGREE OF AMMONIATION	TEMPERATURE OF STORAGE	PERCENTAGE AVERAGE AVAILABILITY OF P_2O_5 IN SAMPLE		
			AS DETERMINED CHEMICALLY	AS DETERMINED BY POT TESTS ON ACID SOILS	
			ON BASIS OF A 1-GRAM SAMPLE[1]	ON BASIS OF 100 FOR NON-AMMONIATED SAMPLE	ON BASIS OF 95 FOR NON-AMMONIATED SAMPLE
No.	*Per cent*	°*C.*			
1	0	20	95	100	95
2	2	20	94	100	97
3	3	20	94	88	84
4	4	20	94	82	78
5	5	20	93	65	62
6	2	60	92	93	88
7	3	60	91	68	65
8	4	60	90	62	59
9	5	60	90	55	52
10	2	90	75	76	72
11	3	90	74	59	56
12	4	90	74	53	50
13	5	90	84	53	50

[1] Chemical availability determined on a 4-12-4 mixture after ammoniation and storage.

The principal phosphatic components of a superphosphate ammoniated to 2 per cent on the basis of 20% P_2O_5 are monoammonium phosphate, dicalcium phosphate, and small amounts of monocalcium phosphate and undecomposed rock (2, 4). As the quantity of added ammonia is increased beyond 2.3 per cent, less soluble phosphate is formed at the expense of the more soluble phosphatic components of the mixture (4, 5). This less soluble phosphate may consist of one or more basic phosphates such as tricalcium phosphate, hydroxyl apatite, and more or less fluorinated apatites (6). In this report the term "reverted P_2O_5" will refer to the P_2O_5 in these basic phosphates.

The increase in the spread of the results obtained by the two methods with increase in the rate of ammoniation is to be expected if it is assumed

that the percentage availability to plants of the reverted P_2O_5 in heavily ammoniated superphosphates remains essentially the same irrespective of the degree of ammoniation. In the official chemical method for evaluating P_2O_5 availability, the proportion of the reverted P_2O_5 in heavily ammoniated superphosphates which dissolves in 100 ml of the neutral ammonium citrate solution does not remain the same, but varies with the proportion of the reverted P_2O_5 in the sample taken for analysis.

TABLE 9.—*Per cent availability of the P_2O_5 in ammoniated superphosphate mixtures. Dolomite present; moisture 9 per cent; temperature of storage 20°C.*

TEST MIXTURE	DEGREE OF AMMONIATION	AVAILABILITY FOUND BY CHEMICAL METHOD		AVAILABILITY BY POT TESTS; MEAN OF ALL CROPS	
		1-GRAM SAMPLE[1]	2-GRAM SAMPLE[1]	FOUND[2]	CALCULATED[3]
No.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	0	95	93	95	95
2	2	94	91	95	95
3	3	94	87	84	85
4	4	94	85	78	75
5	5	93	80	62	68

[1] As determined in a 4-12-4 mixture.
[2] On basis of 95 as the availability for the non-ammoniated mixture.
[3] On assumption that the P_2O_5 in the reverted phosphate is 50 per cent available to plants and in undecomposed rock, 15 per cent available.

If the proportion of reverted P_2O_5 in a sample is small, the action of the citrate solution may be such as to dissolve completely the P_2O_5 present, indicating complete availability. However, if the reverted P_2O_5 in the sample is large, the citrate solution may then be capable of dissolving only a small proportion of the P_2O_5 and a low availability will be indicated. This explains the action of the official method, already referred to, in giving a high availability rating for ammoniated mixtures of low P_2O_5 content, and a low availability rating for mixtures of high P_2O_5 content (2, 8).

According to Keenen (4) the reverted P_2O_5 in an ammoniated superphosphate containing 20 per cent of total P_2O_5 amounts to about 4.0 per cent of the sample at 3 per cent ammoniation, 8.2 per cent at 4 per cent ammoniation, and 12.0 per cent at 5 per cent ammoniation. The corresponding P_2O_5 contents of ammoniated 4-12-4 mixtures prepared from the same superphosphate would be 2.4, 5.0, and 7.2 per cent, respectively.

If it be assumed that the P_2O_5 in the reverted phosphate, and in the undecomposed rock, of ammoniated goods are respectively 50 and 15 per cent available to all crops on acid soils, then the availability to plants of the P_2O_5 in the ammoniated samples may be calculated as shown by the data in Table 9. The table also shows the availability of the samples as found (1) by pot tests, and (2) by the official chemical method when (a) 1-gram, and (b) 2-gram samples are taken for analysis. The close agree-

ment between the calculated availability values and those found by the pot tests indicates that the percentage availability to plants of the reverted P_2O_5 in heavily ammoniated superphosphate mixtures does remain essentially the same irrespective of the degree of ammoniation.

Table 9 further indicates that, as compared with pot tests on all crops with acid soils, the chemical method using a 2-gram sample for analysis may give slightly low results for non-ammoniated and 2 per cent ammoniated samples, and high results for those ammoniated in excess of 3 per cent. The official method in which a 1-gram sample is taken for analysis, also gives high results for heavily ammoniated mixtures but it can apparently be used to accurately evaluate non-amoniated goods and ammoniated mixtures containing a maximum of 2 per cent of ammonia.

CROP RESPONSE ON ALKALINE SOIL

Two series of pot tests were conducted to determine the availability of P_2O_5 in ammoniated superphosphate mixtures, to sorghum grown on an alkaline soil. This soil was Houston clay with a *p*H of 8.2. There is evidence that heavily ammoniated superphosphates are less available to plants grown on alkaline soils than to those grown on acid soils (7, 9). It was considered desirable therefore not to include the data obtained on the Houston clay soil in the same tabulation with that reported for the acid soils.

TABLE 10.—*Effect of various treatments on the availability of the P_2O_5 in ammoniated superphosphate mixtures to sorghum grown on alkaline soil.*[1]
Dolomite present; moisture 9 per cent

TEST MIXTURE	DEGREE OF AMMONIATION	TEMPERATURE OF STORAGE	PERCENTAGE AVERAGE AVAILABILITY OF P_2O_5 IN SAMPLE	
			AS DETERMINED CHEMICALLY	AS DETERMINED BY POT TESTS
			ON A BASIS OF A 1-GRAM SAMPLE[2]	ON BASIS OF 100 FOR NON-AMMONIATED SAMPLE
No.	*Per cent*	*°C.*		
1	0	20	95	100
2	2	20	94	63
3	3	20	94	69
4	4	20	94	38
5	5	20	93	5
9	5	60	90	−13
13	5	90	84	+27

[1] Houston clay, *p*H 8.2.
[2] Chemical availability determined on a 4-12-4 mixture after ammoniation and storage.

Table 10 gives the results of various treatments on the availability of the P_2O_5 in ammoniated superphosphate mixtures to sorghum grown on an alkaline soil. The data indicate that as little as 2 per cent ammoniation

of the superphosphate in the mixture decreases the availability of the P_2O_5 to the crop grown on this soil. The data further indicate that the spread between the percentage average availability of the P_2O_5 as determined chemically and by pot tests increases with increase in the degree of ammoniation, and with increase in temperature of storage after ammoniation. The results of these pot tests show that the P_2O_5 in the ammoniated mixtures is considerably less available to plants grown on this alkaline soil than to those grown on acid soils.

SUMMARY

A collaborative study was made of the factors that affect the efficiency of ammoniated superphosphate mixtures in promoting crop growth in the greenhouse. The results of pot tests with 7 different acid soils in 5 separate locations indicate that:

(a) Superphosphate mixtures that have been ammoniated to 2 per cent on the basis of the superphosphate in the mixture and stored at ordinary temperatures, with or without dolomite, are as available to plants on acid soils as the corresponding non-ammoniated mixtures.

(b) The availability to plants of the P_2O_5 in ammoniated mixtures containing dolomite tends to decrease as the rate of ammoniation is increased beyond 3 per cent, and as the temperature of storage is increased above normal. The presence of dolomite apparently causes a reduction in the availability to plants of the P_2O_5 in ammoniated mixtures stored at elevated temperatures and, to a lesser extent, in such mixtures stored at ordinary temperatures.

(c) The availability to plants of the P_2O_5 in heavily ammoniated mixtures is less, for all three of the temperatures at which they were stored, when the moisture content of the mixture was 9 per cent than when it was 3 per cent.

(d) The addition of fluorides to an ammoniated or a non-ammoniated mixture has little or no effect on the availability of its P_2O_5 content to plants.

(e) Long-season crops appear to be more responsive to water-insoluble phosphates than those having a shorter growing season.

(f) The percentage availability to plants of the reverted P_2O_5 in heavily ammoniated superphosphate mixtures remains essentially the same irrespective of the degree of ammoniation. As determined by the official chemical method, the percentage availability of the reverted P_2O_5 in ammoniated mixtures does not remain the same, but varies with the proportion of the reverted P_2O_5 in the sample taken for analysis. The spread between the percentage availability of the P_2O_5 in ammoniated mixtures as determined chemically, and by pot tests, increases therefore as the degree of ammoniation is increased beyond 2 per cent.

(g) Ammoniated superphosphates are less effective in promoting crop growth on alkaline than on acid soils.

APPENDIX

SECTION 1.—*Effects of degree of ammoniation, temperature of storage, presence of dolomite, moisture and source of P_2O_5 on response of millet, wheat, sudan grass, and sorghum to ammoniated superphosphates on various soils*

TEST MIXTURE	SUPERPHOSPHATE	NH_3 ADDED	STORAGE TEMP.	H_2O[2]	DOLOMITE	YIELDS[1] OF VARIOUS CROPS ON INDICATED SOILS: MILLET[3] HARTSELLS	MILLET[3] WOOSTER	WHEAT[3] WOOSTER	WHEAT[3] SASSAFRAS	SUDAN GRASS[4] NEWTONIA[5]	SUDAN GRASS[4] CECIL[6] A	SUDAN GRASS[4] CECIL[6] B	SUDAN GRASS[4] GRADY[6]	SORGHUM ATWOOD[4]	SORGHUM ATWOOD[3]	SORGHUM HOUSTON[4]	AVERAGE RELATIVE INCREASE IN YIELD[7]
No.		*per cent*	°*C.*	*per cent*	*lbs./ton*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*per cent*
						Effect of Degree of Ammoniation											
Check	None	—	—	—	—	0.7	14.4	9.3	0.7	3.9	0.9	0.0	10.8	22.1	15.8	15.7	—
1	A	0	20	9	250	11.7	36.1	31.3	32.3	25.9	23.7	17.3	24.3	41.7	35.2	21.7	100
2	A	2	20	9	250	11.0	39.3	34.2	33.2	26.2	24.9	17.2	24.5	39.3	32.1	19.3	100
3	A	3	20	9	250	11.3	27.5	32.9	30.0	23.9	23.3	18.1	19.5	39.8	32.8	20.4	88
4	A	4	20	9	250	11.2	25.1	33.5	31.9	20.2	23.1	21.0	21.6	37.0	26.6	17.5	82
5	A	5	20	9	250	9.5	25.0	33.1	28.6	15.7	21.0	19.2	16.1	32.9	19.2	14.5	65
						Effect of Temperature of Storage (Tests 2–5 vs 6–9 and 10–13)											
6	A	2	60	9	250	11.1	33.0	33.3	29.9	24.9	25.6	16.8	19.7		33.0		93
7	A	3	60	9	250	8.6	27.1	31.4	29.9	16.2	21.0	20.3	16.5		22.1		68
8	A	4	60	9	250	9.3	24.0	29.8	29.6	15.9	21.8	20.0	15.5		17.2		62
9	A	5	60	9	250	7.0	20.6	30.4	30.1	12.9	18.2	16.5	15.2		18.1	14.2	55
10	A	2	90	9	250	9.2	27.3	29.9	26.9	24.8	24.2	18.1	17.5		25.4		76
11	A	3	90	9	250	7.6	25.0	30.0	26.1	14.4	18.9	17.3	14.5		21.3		59
12	A	4	90	9	250	7.7	21.8	29.6	26.3	12.0	18.3	19.5	15.1		17.7		53
13	A	5	90	9	250	8.2	20.8	30.5	28.0	9.0	18.1	19.7	15.3	32.4	19.0	13.3	53

Effect of Moisture (Tests 5, 9, and 13 vs 14, 15, and 16, respectively)																	
14	A	5	20	3	250	10.0	26.9	32.8	32.9	19.2	23.1	18.7	19.2	37.3	24.2	14.0	78
15	A	5	60	3	250	8.7	28.8	31.7	30.4	14.3	22.1	18.7	15.8	27.6	18.8	14.3	66
16	A	5	90	3	250	8.9	26.0	30.3	27.0	19.6	20.7	16.5	21.5	35.8	26.7	18.0	75
Effect of Dolomite (Tests 5, 9, and 13 vs 17, 18, and 19, respectively)																	
17	A	5	20	9	none	9.8	27.6	33.5	28.3	15.3	20.2	19.9	17.1		27.4		75
18	A	5	60	9	none	8.6	22.6	30.5	30.1	15.7	22.0	17.7	22.1		24.1		76
19	A	5	90	9	none	8.0	26.2	30.8	30.8	18.1	22.0	17.1	15.5		25.3		72
Effect of Source of P_2O_5 (Test 1 vs 20, 22, or 24 and test 8 vs 21, 23, or 25)																	
20	B	0	20	9	250	11.7		32.6	34.3	27.5	40.5[8]	17.0	28.5		38.0		110
21	B	4	60	9	250	8.0		31.5	28.9	9.3	26.4	17.3	18.2		16.2		70
22	C	0	20	9	250	11.8		33.2	35.6	27.2	37.2	15.1	27.1		36.5		109
23	C	4	60	9	250	8.2		30.4	31.5	9.7	25.4	17.1	17.4		21.2		67
24	D	0	20	9	250	12.3		32.0	35.2	28.4	38.5	15.9	24.5		40.9		106
25	D	4	60	9	250	9.2		31.7	33.2	17.1	30.4[8]	16.6	17.3		21.7		78
Difference required for significance:[9]																	
5% level						3.3	5.4	2.5	4.7	6.2	[10]	[10]	4.1	6.1	7.6	3.8	
1% level						4.4	7.2	3.4	6.2	8.2			5.5	8.2	10.0	5.0	

[1] Average weight per pot of oven-dried aerial portions of crop harvested a little before maturity.
[2] Moisture content during ammoniation and storage.
[3] The fertilizer was mixed with 5 per cent of the soil and placed in a horizontal layer half way between the bottom and top of the pot.
[4] The fertilizer was placed in a circular area 3 inches in diameter and 1.5 inches from the top of the soil. The seed were planted in a 5-inch ring concentric with this rtilizer area.
[5] First cutting.
[6] First cutting (A), Second cutting (B).
[7] On the basis of 100 as the average increase for the non-ammoniated superphosphate; results on alkaline soil not included.
[8] Planted a month later than other members of the series.
[9] Between treatments but not between treatments and checks.
[10] Individual pot yields not reported.

APPENDIX

SECTION 2.—*Effect of presence of fluorine during ammoniation and storage on response of crops to ammoniated superphosphates on various soils*[1]

TEST MIXTURE	NH_3 ADDED	STORAGE TEMP.	YIELDS[2] OF VARIOUS CROPS ON INDICATED SOILS							AVERAGE RELATIVE INCREASE[5]
			MILLET[3]	WHEAT[3]		SUDAN GRASS[4]			SORGHUM[3]	
			HARTSELLS	WOOSTER	SASSAFRAS	NEWTONIA	CECIL	GRADY	ATWOOD	
No.	*per cent*	*°C.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*per cent*
Check	—	—	4.5	9.3	0.7	3.9	0.9	10.8	15.8	—
Synthetic Superphosphate, Fluorine free										
26	0	20	27.3	35.8	33.9	28.5	26.3	23.2	35.4	100
27	5	20	21.2	34.4	33.6	15.3	22.3	17.1	31.5	75
28	5	60	26.2	35.0	35.8	16.8	28.7	16.5	27.1	80
Synthetic Superphosphate, Fluorine added as CaF_2										
29	0	20	27.4	36.6	38.0	26.3	25.2	23.7	33.9	100
30	5	20	26.8	33.9	37.6	22.2	25.9	23.1	30.4	94
31	5	60	20.1	29.8	28.5	18.5	25.5	18.9	25.4	71
Synthetic Superphosphate, Fluorine added as NaF										
32	0	20	26.6	32.9	35.7	27.0	29.9	21.8	38.0	100
33	5	20	25.6	33.8	36.4	26.3	25.5	18.5	32.5	89
34	5	60	20.5	33.8	32.3	16.3	22.4	22.0	27.2	77

Superphosphate made from Tennessee Rock Phosphate										
35	0	20		29.6	30.2	28.7	37.6[6]	22.3	37.6	103
36	5	20		29.7	29.3	14.2	27.5	17.8	19.4	63
37	5	60		28.6	29.1	15.8	28.9	13.9	20.6	89
Superphosphate made from Defluorinated Tennessee Rock Phosphate										
38	0	20		33.9	35.0	24.8	38.4	20.5	34.3	100
39	5	20		33.0	31.7	19.6	33.2	18.9	29.0	83
40	5	60		31.5	32.9	14.4	36.8[6]	21.6	25.5	82
Difference Required[7]										
		5% level	1.7	2.5	4.7	6.2		4.1	7.6	
		1% level	2.3	3.4	6.2	8.2		5.5	10.0	

[1] All mixtures contained 9 per cent of water and dolomite was absent.
[2] Average weight per pot of oven-dried aerial portions of crop harvested near maturity.
[3] The fertilizer was mixed with 5 per cent of the soil and placed in a horizontal layer half way between the bottom and the top of the pot.
[4] The fertilizer was placed in a circular area 3 inches in diameter and 1.5 inches from the top of the soil. The seed were planted in a 5-inch ring concentric with this fertilized area.
[5] On the basis (test mixtures 26–34) of 100 for the non-ammoniated fluorine-free synthetic superphosphate (No. 26) and of 100 (test mixtures 35–40) for the non-ammoniated superphosphate made from defluorinated rock (No. 38).
[6] Planted a month later than other members of the series.
[7] Between treatments but not between checks and treatments.

LITERATURE CITED

(1) BARTHOLOMEW, R. P., *Soil Sci.*, **40**, 203–17 (1935).
(2) HARDESTY, JOHN O., ROSS, WILLIAM H., and ADAMS, J. RICHARD, *This Journal*, **26**, 203–11 (1943).
(3) JORDAN, W. H., N. Y. Agr. Expt. Station Bull. 358, 11–30 (1913).
(4) KEENEN, FRANK G., *Ind. Eng. Chem.*, **22**, 1378–82 (1930).
(5) KEENEN, FRANK G., *Ibid.*, **24**, 44–9 (1932).
(6) MACINTIRE, W. H., WINTERBERG, S. H., THOMPSON, J. G., and HATCHER, B. W., *Ibid.*, **34**, 1469–79 (1942).
(7) ROSS, WILLIAM H., JACOB, K. D., and BEESON, K. C., *This Journal*, **15**, 227–66 (1932).
(8) ROSS, WILLIAM H., *Ibid.*, **25**, 318–21 (1942).
(9) SALTER, ROBERT M., and BARNES, E. E., Ohio Agr. Expt. Sta., Bull. 553 (1935).
(10) TRUOG, E., Wis. Agr. Expt. Station, Res. Bull. 41 (1916).

THE AIR-FLOW METHOD FOR THE DETERMINATION OF MOISTURE IN FERTILIZERS*

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Water in fertilizer materials and mixtures may exist as free moisture; water of crystallization, such as that occurring in gypsum, $CaSO_4 \cdot 2H_2O$; or water of constitution as in phosphoric acid or sundry organic materials that are often components of mixed fertilizers. The free moisture and its burden of dissolved salts forms a liquid phase on the surface of the solid particles and determines, to a large extent, the physical condition of the fertilizer. An accurate knowledge of the free moisture content is thus of prime importance to the fertilizer manufacturer and chemist. Unfortunately, such knowledge is not always afforded by methods commonly used.

In a review of the problem of determining moisture in fertilizers, Whittaker and Ross (4) pointed out that the decomposition of organic materials and of crystalline hydrates and the liberation of water of constitution, that may occur when samples are dried at 100°C., could be largely avoided if the moisture were removed rapidly at a low temperature as by passing a current of air, heated to about 60°C., directly through the sample. Such a technique would take advantage of the slower rate of liberation of water of crystallization and of constitution, than of free moisture, and of the greater stability of crystal hydrates and

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organic materials at the lower temperature. These workers also indicated that drying over a suitable desiccant for at least two hours at room temperature in a well evacuated desiccator approached the ideal procedure more closely than most of the other methods studied. However, some of the commonly used powerful drying agents like anhydrous magnesium perchlorate are capable of reducing the partial vapor pressure of moisture surrounding the sample to such an extent that some of the crystalline hydrates like magnesium ammonium phosphate hexahydrate, that may be present in mixed fertilizers, may lose water of crystallization. Three essentials of a method for free moisture would thus appear to be (a) rapid removal of the moisture, (b) low temperature, and (c) a not too low partial vapor pressure of water vapor in the atmosphere surrounding the sample. The present official method (2) for determining moisture by oven drying for 5 hours at 100°C. does not meet any of the foregoing conditions. The present paper describes a method, designated as the air-flow method, in which the necessary conditions are met more adequately.

Elsewhere in this issue (3) Ross and Love have compared the air-flow method with the official method and the vacuum-drying procedure for the determination of moisture in various types of fertilizer materials. They found that the principal salt hydrates that are likely to occur in mixed fertilizers can be dried by the air-flow method without the loss of water of crystallization that occurs when the same materials are dried by the official method. The present paper extends these results to mixed fertilizers with special attention to those that may lose water of constitution at 100°C. or in which thermal reactions generating volatile reaction products may occur.

AIR-FLOW METHOD

Apparatus

The equipment designed for the rapid removal of moisture at a relatively low temperature is shown in Figures 1 and 2. It consists of a metal manifold (Figure 1) $10\frac{1}{2}\times2\frac{1}{2}\times1\frac{1}{4}$ inches in size with a $\frac{1}{4}$-inch nipple centrally located on one side for attachment to the vacuum line, and six $1\frac{1}{4}$-inch tapered stopper seats evenly spaced along the top to accommodate a No.-6, one-hole, rubber stopper. A $1\frac{1}{4}$-inch length of light metal tubing, $\frac{1}{2}$-inch in diameter, extends through the rubber stopper to a height of approximately $\frac{1}{4}$-inch above the surface. This extension of the metal tubing above the surface of the stopper is for the purpose of centering a fritted glass crucible over the hole in the stopper. Since the crucible is held in place by suction, it is necessary to grind a smooth surface on the lower edge of each fritted glass crucible and the surface of the stopper in order to insure an air-tight connection between the edge of the crucible and the stopper when air is being drawn through the sample in the crucible. Figure 2 shows the manifold with crucibles in position in a constant temperature oven and connected to a gage in the vacuum line. The most suit-

able type of drying oven for the determination is one in which the incoming air is drawn over the heating coils before it contacts the samples. With this equipment the amount of air passing through the sample and the temperature of the sample can be maintained constant. A manifold to accommodate 12 or more crucibles may be used providing sufficient vacuum is maintained to keep approximately the same quantity of air passing through each sample.

The fritted glass crucible is the type ordinarily used for filtration. Those

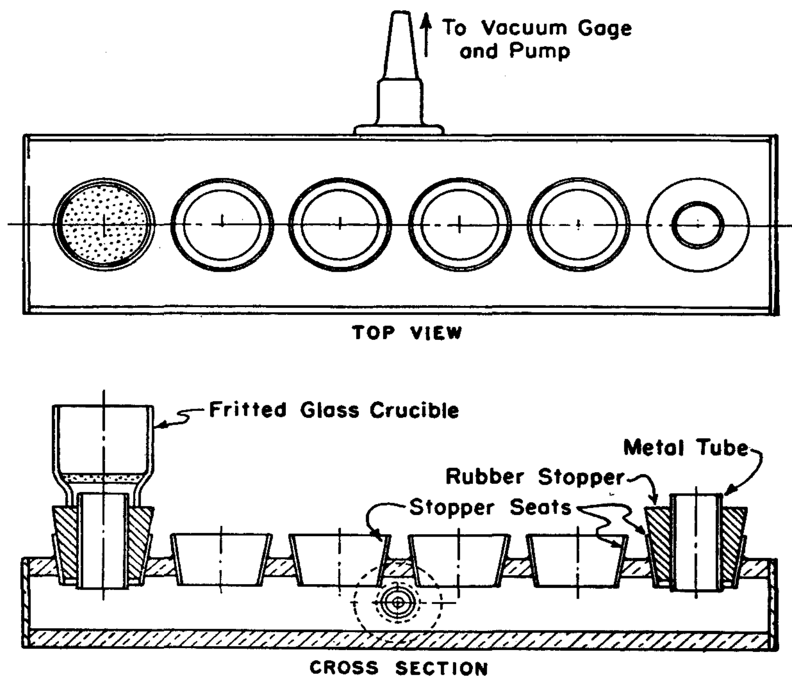

FIG. 1.—Manifold for use in determining moisture by the air-flow method.

used in this work were of Pyrex glass, $1\frac{3}{4}$ inches tall, $1\frac{3}{16}$ inches in diameter at the top and fitted with a $\frac{7}{8}$-inch fine-porosity fritted glass plate. For accurate work a set of crucibles of matched porosity should be used. Such a set may be obtained by selecting several that pass a given quantity of air at a constant pressure in about the same length of time. For this purpose the rate of air flow may be determined with a gas meter or by fitting the crucible to the tight-fitting rubber stopper of a large separatory funnel filled with water and selecting crucibles which give approximately the same length of time for the funnel to empty.

Tests have shown that matched fine-porosity crucibles under constant

vacuum allow the passage of air through each crucible on the manifold at approximately the same rate (0.1 cu. ft. in 60–108 seconds at 60°C. and 63 cm. vacuum), and, since the resistance to air flow afforded by the fritted disk is large relative to that afforded by the sample, variations in

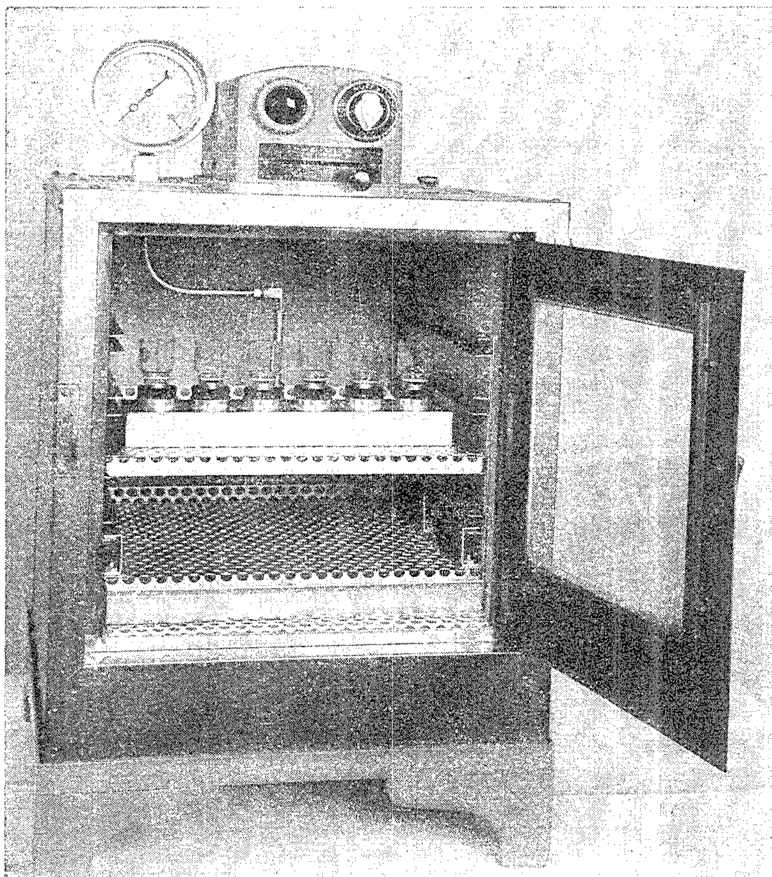

FIG. 2.—The air-flow apparatus in place in a drying oven.

the amount and particle size of the sample have no appreciable effect on the amount of air passed through the sample in a given length of time. A vacuum of about $\frac{1}{2}$ atmosphere has been found suitable for most moisture determinations. This facilitates routine procedure in that all samples may be removed at the same time with the reasonable assurance that they have been subjected to the same drying conditions.

Procedure

A two-gram sample of the fertilizer or fertilizer material is weighed into tared crucible and spread evenly over the fritted glass disk. When six crucibles (or the manifold capacity) have been prepared and the oven temperature adjusted to 60°C., the vacuum is turned on the manifold and the crucibles are put in place on the rubber stoppers, making certain that each is tightly seated as indicated by its resistance to removal. If less than six crucibles are to be used the other openings in the manifold must be tightly stoppered. The vacuum is adjusted to about $\frac{1}{2}$ atmosphere and the air flow continued for two hours, or longer if necessary. The oven thermometer should be located with its bulb near the top of the manifold to insure that the temperature of the air entering the crucibles is being measured. The oven temperature should be checked a few minutes after the vacuum is turned on and again later. Some ovens do not readily take up the additional load because of the cool air being drawn in, so that adjustment is sometimes necessary. The determinations usually proceed smoothly and few precautions are necessary. The fact that the air passes downward through the sample prevents any loss of fine particles through dusting. Loss in weight is computed after cooling the crucibles in a desiccator and weighing.

APPLICATION TO MIXED FERTILIZERS

Loss of Volatile Matter Other Than Free Moisture

In a previous paper (1) it was shown that fertilizer mixtures containing superphosphate, inorganic nitrates, and organic matter may undergo decomposition at 100°C. This is the result of the oxidation of organic matter by nitric acid, which is formed in the reaction between nitrate and monocalcium phosphate, or free phosphoric acid, in the presence of moisture. Such mixtures heated at temperatures of 85 to 100°C. for 2 hours were found to lose between 6 and 7 per cent of their weight in the form of volatile matter, including oxides of nitrogen, carbon dioxide, and water of constitution. While these reactions may occur slowly at much lower temperatures, they are greatly accelerated at temperatures in the neighborhood of 100°C. No appreciable loss of volatile matter other than free moisture was observed to occur below 85°C. Therefore it would be expected that moisture determinations on such mixtures would give widely variant results depending on whether the determinations were made above or below 85°C. Mixtures were prepared and analyzed for moisture by 3 different methods according to the formulation and analytical data presented in Table 1. The mixtures were formulated to contain approximately 7 per cent free moisture according to analysis of the materials by vacuum desiccation; free moisture was added when necessary. At this moisture content the mixtures containing large amounts of am-

monium nitrate were slightly clammy after 30 days of storage in moisture-proof containers, while the other mixtures were dry and friable. Gross weight of the samples remained constant during storage, showing that no loss by evaporation of moisture occurred. During the moisture determination by the official method at 100°C., mixtures containing large amounts of ammonium nitrate in conjunction with unammoniated superphosphate

TABLE 1.—*Effect of fertilizer formulation on moisture values by different methods*

MATERIAL	FORMULAS OF FERTILIZER MIXTURES (POUNDS PER TON)						
	8-24-0	8-24-8			4-24-8	3-12-6	8-16-16
	No. 1	No. 5	No. 7	No. 9	No. 13	No. 47004	No. 957
Ammonium Sulfate	385	385	—	385	385	—	310
Ammonium Nitrate	240	240	480	240	—	—	67
Nitrogen Solution 2A	—	—	—	—	—	171	190
Triple Superphosphate	1000	1000	1000	1000	1000	—	690
Normal Superphosphate	—	—	—	—	—	1200	—
Potassium Chloride	—	270	270	—	270	200	530
Potassium Sulfate	—	—	—	310	—	—	—
Hydrated Lime	25	25	25	25	25	—	—
Magnesium Limestone	—	—	—	—	—	116	113
Cocoa Shell Meal	100	80	100	40	100	—	100
Sand Filler	250	—	125	—	220	313	—
METHOD	MOISTURE VALUES AFTER CURING (PER CENT)						
	SAMPLE NUMBERS						
	1	5	7	9	13	47004	957
A.O.A.C. (5 hrs., 100°C.)	10.81	9.93	14.83	8.79	7.19	6.86	7.30
Vacuum Dessication[1]	4.48	4.73	7.18	4.48	2.23	6.04	5.92
Air-Fow (2 hrs., 60°C.)[2]	4.26	4.36	7.07	4.24	2.07	5.89	5.83

[1] With 27 inches vacuum, over anhydrous magnesium perchlorate for 48 hours at 25°C.
[2] Aspiration with 15 inches vacuum on manifold containing 6 samples in matched fine-porosity crucibles.

and organic matter (Nos. 1, 5, 7, and 9) had a marked tendency to liberate more volatile matter than that represented by the free moisture content (7 per cent) of the original mixture. They had an appreciable odor of nitric acid, and mixture No. 7 liberated noticeable brown fumes during the early stage of heating. This loss of volatile matter other than free moisture is reflected in the values obtained by the official method on mixtures 1, 5, 7, and 9. As compared with No. 7 containing no sulfate, mixtures 1, 5, 9, 13, and 957, containing large amounts of ammonium sulfate, gave evidence of hydrate formation as indicated by their low free moisture content according to the vacuum-drying and the air-flow methods of analysis. The results obtained by these two methods on mixture No. 7 show that little or no hydration occurred since the amount of free mois-

ture recovered after curing was essentially the same as the original free moisture content (7 per cent) of the sample.

The vacuum procedure gave results on all mixtures that were consistently higher, by small amounts, than those obtained by the air-flow method. This may be attributed to a slight, partial loss of water of crystallization when using the vacuum method, and suggests that the air-flow method gives a slightly better estimate of free moisture when crystal hydrates are present.

The results given in Table 1 demonstrate the applicability of the air-flow and vacuum-drying methods to the problem of following the course

TABLE 2.—*Loss in weight of cocoa shell meal during drying by different methods*

METHOD OF DRYING	DURATION OF DRYING	LOSS IN WEIGHT
	Hours	*Per cent*
Oven at 100°C.	3	6.29
" " "	5	6.49
" " "	7	6.60
Vacuum Drying[1]	16	3.28
" "	21	4.05
" "	26	4.22
Air-Flow[2]	1	3.57
" "	2	3.59
" "	3	3.59

[1] Over anhydrous magnesium perchlorate at 30°C.
[2] At 60°C.

of hydration reactions in fertilizers during the curing stage. They also indicate that the present official method of drying for 5 hours at 100°C. is not applicable to mixtures containing large amounts of ammonium nitrate in conjunction with organic matter and superphosphate.

Effect of Atmospheric Humidity

The tenacity with which water is held by some organic fertilizer conditioners, *e.g.*, cocoa shell meal, is well known. With materials of this kind it would be expected that the partial pressure of moisture in the air passing through the sample would influence results by the air-flow method. A comparison of the results obtained by different methods of drying cocoa shell meal (Table 2) shows that oven drying at 100°C. gives considerably higher values than either the vacuum-drying or the air-flow method. This is attributed to the slow decomposition of the organic matter at this temperature. The data also show that drying in vacuum over anhydrous magnesium perchlorate for 21 to 26 hours gives somewhat higher values for moisture in this material than were obtained by the air-flow procedure.

When the material dried over the desiccant was further treated by the air-flow method, it regained an amount of moisture equivalent to the difference between the original results obtained by these two methods. This indicates that the moisture vapor in the air drawn through this highly adsorptive material prevented as complete drying as was obtained by the vacuum-drying procedure.

The relative humidity of the air in the laboratory during the course of experiments described in this paper varied between 23 and 70 per cent. However, with the exception of the cocoa shell meal and the phosphatic materials containing free phosphoric acid as described in the accompanying paper by Ross and Love (3), it will be observed (Table 1) that the results obtained by the air-flow method are in fair agreement with those obtained by the vacuum-drying method in which the humidity of the air in the laboratory is not a factor.

Known methods for determining moisture in fertilizer materials and mixtures are necessarily empirical when considered from the standpoint of their application to many types of samples. When extremely small changes in moisture content are of interest, as in the control analysis of ammonium nitrate, it may be expedient to either dry the air which passes through the sample during the determination of moisture by the air-flow method, or to maintain the air at a selected constant relative humidity. This would also offer the possibility of shortening the time required for the determination. However, the feasibility of modifying the method, to include such practice for routine analytical work on fertilizers, depends upon whether or not extreme accuracy is desired. The fertilizer chemist is interested in obtaining moisture results that reveal the causes for variation in either the physical condition of the fertilizer or in the results of analyses for other constituents. Possibly no single method is sufficient for all materials and mixtures. The present official methods are not applicable to many of our present day mixtures and it would seem necessary to replace or supplement them with more reliable methods. The air-flow and vacuum-drying methods show considerable promise in this respect since neither of them causes any appreciable decomposition of the organic matter or hydrates commonly occurring in fertilizers.

SUMMARY AND CONCLUSIONS

A procedure designated as the air-flow method of determining free moisture in fertilizer materials and mixtures is described. It consists briefly in drawing air heated to 60°C. through the sample for 2 hours.

Comparative results were obtained on fertilizer samples containing easily oxidizable components using three different methods of determining moisture, namely: the present official method in which the sample is oven dried for 5 hours at 100°C., the method of drying in vacuum over anhydrous magnesium perchlorate for varying periods of time at room

temperature, and the air-flow method. The results show that the present official method is not adapted to the determination of free moisture in such samples, since a temperature of 100°C. may cause decomposition and consequent loss in weight other than that representing the true free moisture content. These decomposition reactions do not occur during the determination by either the vacuum-drying or air-flow methods, which offer considerable promise for routine evaluation of free moisture in fertilizers.

LITERATURE CITED

(1) Hardesty, John O., and Davis, R. O. E., *Ind. Eng. Chem.*, **38**, 1298–1303 (1946).
(2) *Official and Tentative Methods of Analysis, A.O.A.C.*, page 20, 6th Edition, 1945.
(3) Ross, William H., and Love, Katharine S. *This Issue*, page 617.
(4) Whittaker, Colin W., and Ross, William H., *This Journal*, **25**, 132–42 (1942).

A COMPARISON OF COPPER SULFATE AND MERCURIC OXIDE AS CATALYST IN THE DETERMINATION OF PROTEIN IN FISH MEAL

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Several different catalysts have been used for the measurement of protein in feeding stuffs by the Kjeldahl procedure. Among those used are copper, mercury, and selenium. It has been observed by several laboratories that the use of copper as a catalyst resulted in lower protein content in vegetable oil meals than when mercury was used as the catalyst (1) (2).

In our laboratory we have used copper sulfate as a catalyst in nitrogen digestions for protein determinations for more than thirty years. In this report we are presenting data in which we compared the use of mercuric oxide with copper sulfate as a catalyst in the determination of protein in fish meal.

First, we investigated the effect of the kind of catalyst used on the minimum time required for complete digestion. Twenty-eight 1-gram portions of a fish meal sample were weighed into digestion flasks. To fourteen of then, 11 grams of anhydrous sodium sulfate, 0.2 gram anhydrous copper sulfate, and 25 ml of sulfuric acid were added. To the remainder, 11 grams of anhydrous sodium sulfate, 0.7 gram red mercuric oxide, and 25 ml of sulfuric acid were added.

Two portions were digested for each catalyst, and for each of the periods of time shown in Table 1.

The balance of the official procedure was then followed (3) (4). In the case of copper sulfate the solution was made alkaline with sodium hydroxide and distilled into N/5 sulfuric acid. In the case of mercuric oxide,

sodium thiosulfate, and sodium hydroxide were added before distillation according to the Kjeldahl-Gunning-Arnold procedure. The resulting ammonia was in every case distilled into the same quantity of N/5 sulfuric acid.

The work was repeated with five additional samples of fish meal.

TABLE 1.—*Comparison of digestion time for copper and mercury catalysts*

SAMPLE	TIME IN MINUTES AFTER CLEARING						
	20	40	60	80	100	120	140
	Per cent†	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
		*(a) Using Copper Sulfate**					
B5610	65.00	65.72	65.77	65.85	65.80	65.75	66.03
B5634	57.96	59.50	59.62	59.59	59.59	59.66	59.59
B5638	63.42	64.60	64.59	64.63	64.65	64.66	64.66
B5646	67.17	68.15	68.04	68.22	68.41	68.24	68.34
B5670	62.51	63.42	63.77	63.86	63.82	63.79	63.70
B5680	64.44	65.68	65.54	65.63	65.52	65.70	65.52
Average	63.41	64.51	64.56	64.63	64.63	64.63	64.64
		*(b) Using Mercuric Oxide**					
B5610	65.12	65.77	65.72	65.75	65.77	65.80	65.86
B5634	58.24	59.74	59.71	59.76	59.62	59.92	59.74
B5638	63.53	64.59	64.93	64.65	64.96	64.73	64.59
B5646	67.06	68.18	68.36	68.17	68.31	68.24	68.39
B5670	62.79	63.88	63.81	63.96	63.84	64.03	64.07
B5680	64.70	65.72	65.64	65.57	65.64	65.52	65.77
Average	63.57	64.65	64.70	64.64	64.69	64.71	64.74

* Results are average of two determinations.
† Protein protein ($N \times 6.25$)
Total digestion time for:
$CuSO_4$ = 35 minutes + digestion time in table
HgO = 20 minutes + digestion time in table.

After determining the minimum digestion time, the information was used in determining the protein in forty samples of fish meal.

Four 1-gram portions of each sample were weighed out at the same time to make certain that comparisons were at the same moisture basis. Duplicate determinations were made using copper sulfate for a catalyst, and also duplicates using mercuric oxide. Results showing the average of these duplicate determinations are given in Table 2.

OBSERVATIONS

The results recorded in Table 1 show that the minimum time required for complete digestion is 40 minutes after the solution becomes clear. This time was used for the samples analyzed in Table 2. The data show that continued digestion up to 140 minutes does not cause any appreciable change.

TABLE 2.—*Per cent protein in fish meal*

SAMPLE NUMBER	COPPER SULFATE	MERCURIC OXIDE	COMPARISON OF Hg WITH Cu PER CENT PROTEIN
B5568	64.27	65.18	+0.91
B5569	63.56	64.34	+0.78
B5570	63.66	64.65	+0.99
B5581	66.35	66.37	+0.02
B5596	63.62	65.93	+2.31
B5597	64.80	64.89	+0.09
B5707	66.18	66.03	−0.15
B5708	60.63	61.29	+0.66
B5781	63.27	64.00	+0.73
B0014	70.31	70.81	+0.50
B0015	53.79	54.08	+0.29
B0116	65.10	66.08	+0.98
B0154	61.46	61.83	+0.37
B0132	59.25	59.62	+0.37
B0133	62.40	62.61	+0.21
B0188	69.17	69.03	−0.14
B0270	68.48	68.38	−0.10
B0328	68.74	68.73	−0.01
B0330	65.73	65.84	+0.11
B0331	69.87	71.62	+1.75
B0350	63.06	63.39	+0.33
B0383	63.29	63.61	+0.32
B0362	61.44	62.66	+1.22
B0464	64.05	65.07	+1.02
B0465	62.81	63.13	+0.32
B0475	59.39	59.45	+0.06
B0493	65.18	65.23	+0.05
B0547	63.72	64.34	+0.62
B0563	63.41	63.84	+0.43
B0729	61.56	61.76	+0.20
B0658	63.13	63.78	+0.65
B0733	59.85	59.78	−0.07
B0728	56.20	56.98	+0.78
B0848	67.61	68.21	+0.60
B0849	65.62	65.62	0.00
B0850	65.07	66.28	+1.21
B0865	68.74	69.07	+0.33
B0866	65.21	65.37	+0.16
B0953	60.95	64.74	+3.79*
B1044	67.09	67.09	0.00
Average	63.95	64.52	+0.57

* This sample was normal in physical appearance.

It was noted in all these determinations that the copper digestion cleared after approximately 35 minutes and the mercury after 20 minutes.

The digestions were heated with open 500-watt electric heating elements.

In reviewing the results of Table 2 it will be noted that in the 40 samples analyzed all gave higher results with mercury except five. These samples show results from 0.01–0.15 per cent lower. The difference is so small that it is well within the experimental error.

Of the remaining 35 samples, 19 are from 0–.50 per cent higher for mercury, 10 are .50–1.00 per cent higher, 4 from 1.00–2.00 per cent higher, 1 is 2.31 per cent higher, and 1 is 3.79 per cent higher.

The average protein of these 40 samples is 0.57 per cent higher with mercuric oxide than with copper sulfate.

DISCUSSION

The results secured with fish meal are in agreement with those of other protein concentrates investigated in this laboratory. Following the same chemical procedure the average difference obtained for 40 samples of soybeans and soybean meal was +0.25 per cent in favor of mercury. In 40 samples of cottonseed meal it was +0.23 per cent. In 20 samples of tankage it was +0.28 per cent, and in 40 samples of meat scrap +0.36 per cent. Thus, of the 719 determinations made in comparing these catalysts, the results secured with fish meal are the most impressive.

CONCLUSIONS

Our observations indicate that when mercuric oxide is used as a catalyst results tend to be higher than when 0.2 gram anhydrous copper sulfate and 11 grams of sodium sulfate are used to determine protein in fish meal.

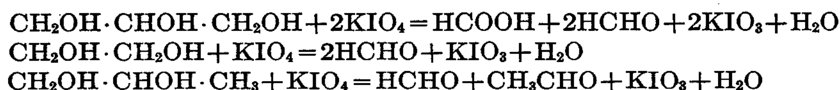
REFERENCES

(1) Kenzo Nakajima and Masami Ikede, *Chem. Abstracts*, **32**, 4101 (1937).
(2) Michael Lauro, *Ind. Eng. Chem., Anal. Ed.*, **3**, 401 (1931).
(3) *Methods of Analysis, A.O.A.C.*, 6th Ed., 27, **2.25** (1945).
(4) *Ibid.*, 27, **2.26**.

THE ESTIMATION OF GLYCEROL IN THE PRESENCE OF PROPYLENE AND ETHYLENE GLYCOLS

By S. H. Newburger and C. F. Bruening (Food and Drug Administration, Federal Security Agency, Baltimore, Maryland)

Shupe[1] utilizes the Malaprade periodate oxidation to determine glycerol in the presence of propylene and ethylene glycols.



$$CH_2OH \cdot CHOH \cdot CH_2OH + 2KIO_4 = HCOOH + 2HCHO + 2KIO_3 + H_2O$$
$$CH_2OH \cdot CH_2OH + KIO_4 = 2HCHO + KIO_3 + H_2O$$
$$CH_2OH \cdot CHOH \cdot CH_3 + KIO_4 = HCHO + CH_3CHO + KIO_3 + H_2O$$

[1] Shupe, I. S. *This Journal*, **26**, 249 (1943).

Since formic acid from glycerol is the only acid oxidation product yielded by any of these substances, a titration of the acid furnishes a method of determining glycerol. Each mol of glycerol provides one mol of formic acid.

Shupe makes the following recommendations:

(a) That appropriate correction for any acidity in the oxidizing 0.02 *M* potassium periodate solution be made.

(b) That the oxidized solution be titrated to a clear yellow end point with 0.02 *N* sodium hydroxide and methyl red indicator.

We found these recommendations difficult to follow. Potassium periodate solutions when titrated with 0.02 *N* sodium hydroxide and methyl red indicator do not show a sharp end point, but change slowly from a red to a salmon yellow color. On further addition of alkali, it is not possible to obtain the yellow color which is observed in the titration of the oxidized glycerol solutions. Nine collaborators[2] who determined glycerol in a vanishing cream reported blanks from 0.05 to 0.3 ml of 0.02 *N* sodium hydroxide for equal volumes of the same potassium periodate solution.

When oxidized glycerol solutions are titrated, the color changes from red, through a salmon yellow, to a yellow. However, the change from salmon yellow to clear yellow is so gradual that it is extremely difficult to ascertain when the end point has been reached. Experiments on solutions of known glycerol content have convinced us that the correction for the acidity of the potassium periodate must be omitted, and the titration continued until no perceptible color change is caused by the addition of one drop of 0.02 *N* sodium hydroxide. Otherwise, the results will be low.

The aforementioned collaborators[2] obtained recoveries averaging 100 per cent of the glycerol present in the vanishing cream when calculated from the periodate consumed, but their recoveries averaged only 96.4 per cent calculated from alkali titration of the same solutions. The individual results varied from 93.1 to 99.8 per cent.

The following observations provided the experimental basis for a modified method:

Potassium periodate solution is acid to methyl red, and the addition of 0.02 *N* sodium hydroxide produces a gradual color change. The solution never becomes a clear yellow color. Likewise, potassium iodate solution is acid to methyl red but changes sharply to a clear yellow on the addition of 0.05 ml of 0.02 *N* sodium hydroxide. That the poor end point with methyl red is probably due to the acidic properties of the potassium periodate itself was demonstrated in the following manner:

To a large excess of propylene glycol in aqueous solution were added a few drops of methyl red indicator and enough 0.02 *N* sodium hydroxide to make the solution a clear yellow. When potassium periodate was added

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[2] Bruening, C. F., *This Journal*, **30**, 507 (1947).

the solution turned red. However, as the periodate was reduced to iodate by the propylene glycol, the solution reverted to a yellow color.

The above experiments indicated that if excess potassium periodate were destroyed by propylene glycol, before attempting the titration of formic acid, better results would be obtained in the alkalimetric determination of glycerol.

An effort was also made to substitute for methyl red an indicator showing a readily detected end point. Both bromcresol purple (*p*H 5.2–6.8) and chlorphenol red (*p*H 4.8–6.4) seemed to be more satisfactory than methyl red. Bromcresol purple was chosen since its color change is more easily seen.

The following modified method was developed:

REAGENTS

Bromcresol purple indicator soln.—Dissolve 0.1 gram in 100 ml of alcohol.

Potassium periodate.—0.02 *M*. Dissolve 4.6 grams KIO_4 in about 500 ml hot water. Dilute to about 900 ml with water, cool to room temperature, and make to 1000 ml.

Sodium hydroxide.—0.02 *N*. Dilute 1 volume of 0.1 *N* NaOH to 5 volumes with water.

Propylene glycol.—Dilute 0.5 ml of commercial product to 25 ml with H_2O. Add 25 ml of 0.02 *M* KIO_4 and allow to stand for 10 minutes. Titrate with 0.02 *N* NaOH using 3 drops of the indicator soln. If not more than 0.05 ml of the base is consumed, the product is suitable for use.

PROCEDURE

Transfer a sample containing not more than 45 mg of glycerol, or its equivalent, to a 110 ml volumetric flask. Add 1 drop of bromcresol purple indicator solution and neutralize. Make the final adjustment to a light purple color with 0.02 *N* NaOH. Add 50 ml of 0.02 *M* KIO_4, dilute to volume with water, mix and allow to stand for one hour.* Pipet a 50 ml aliquot into a flask, add 10 drops of propylene glycol (ca 0.5 ml), mix well, wash down sides of flask with water, and allow to stand for 10 minutes. Add 3 drops of indicator and titrate with 0.02 *N* NaOH to a light purple end point. The color change is from yellow, through neutral gray, to a light purple color.

1 ml 0.02 *N* NaOH = 1.84 mg glycerol.

This procedure was applied to samples containing varying amounts of glycerol. A specially purified glycerol (99.55%), assayed by the periodate consumption method and specific gravity determination, was used to prepare a standard solution, from which aliquots containing known amounts of glycerol were taken. The results of the analyses are given in Table 1.

All recoveries in Table 1 are within 100 ± 0.9 per cent.

The titration of formic acid with 0.02 *N* sodium hydroxide using bromcresol purple indicator proceeds quite smoothly if the excess potassium periodate is destroyed. The iodates produced in the reduction have no

* Excess periodate must be present. Test for periodate by adding $NaHCO_3$ and KI to test portions; if excess is present, iodine will be liberated.

effect upon the titration. This was demonstrated by the following experiments:

A 0.1 gram sample of potassium iodate (approximately the amount formed by the reduction of the 22.7 ml of 0.02 *M* potassium periodate which was originally present in the 50 ml aliquot taken for titration) was dissolved in 30 ml of water and titrated with 0.02 *N* sodium hydroxide using 3 drops of the indicator. One drop (0.05 ml) of 0.02 *N* sodium hydroxide was sufficient to produce a sharp color change.

A solution of 22.7 ml of 0.02 *M* potassium periodate was diluted with 25 ml of water, 10 drops of propylene glycol added, and the resulting solution allowed to stand for 10 minutes. Three drops of indicator were then added. Again 0.05 ml of 0.02 *N* sodium hydroxide was sufficient to titrate the acidity of the solution.

TABLE 1.—*Application of method to known amounts of glycerol*

SAMPLE	TITER FOR 50 ml ALIQUOT—ML OF 0.02 *N* NaOH	GLYCEROL FOUND	RECOVERY
mg.		*mg.*	*per cent*
40.29	9.95	40.28	100.0
	9.91	40.12	99.6
30.22	7.47	30.24	100.1
	7.46	30.20	99.9
20.14	4.99	20.20	100.3
	4.96	20.08	99.7
10.07	2.51	10.16	100.9
	2.51	10.16	100.9

The above experiments indicate that no acidity is contributed by either the reduced potassium periodate or the oxidation products of the propylene glycol. On the other hand, the addition of as much as 2 ml of 0.02 *N* sodium hydroxide to 22.7 ml of 0.02 *M* potassium periodate containing bromcresol purple did not cause the indicator to change from a yellow to a purple color.

An oxidized glycerol solution was titrated to the selected light purple end point. The *p*H of the resulting solution when measured with a Beckman *p*H meter was found to be 6.4.

The method was then applied to a number of solutions containing known amounts of glycerol, propylene glycol, and ethylene glycol. The results are presented in Table 2.

All the recoveries in Table 2 are within 100 ± 0.3 per cent.

The following test showed that neither the ethylene glycol or its oxidation products gave an acid blank:

Ten drops of ethylene glycol were diluted to 25 ml with water, 22.7 ml of 0.02 *M* potassium periodate added, and the solution allowed to stand for 10 minutes. Three drops of indicator were added and the solution titrated with 0.02 *N* sodium hydroxide. The titer was less than 0.05 ml of 0.02 *N* sodium hydroxide.

It has already been shown that the propylene glycol does not interfere with the procedure.

TABLE 2.—*Application of method to solutions containing known amounts of glycerol, propylene glycol, and ethylene glycol*

SAMPLE		TITER FOR 50 ML ALIQUOT—ML OF 0.02 N NaOH	GLYCEROL FOUND	RECOVERY
	mg		*mg*	*per cent*
Glycerol	20.14	4.99	20.20	100.3
Propylene glycol	33.16	4.97	20.12	99.9
Glycerol	20.14	4.98	20.16	100.1
Ethylene glycol	27.56	4.98	20.16	100.1
Glycerol	20.14	4.98	20.16	100.1
Propylene glycol	16.58	4.96	20.08	99.7
Ethylene glycol	13.78			
Propylene glycol	33.16	0.04	—	—
Ethylene glycol	27.56	0.04	—	—

CONCLUSION

The alkali titration procedure of Shupe for the determination of glycerol has been modified. Excess periodate is reduced before the titration, and bromcresol purple is substituted for methyl red as the indicator.

By this method glycerol can be accurately determined alone, and in solutions containing propylene and ethylene glycols.

RESIDUAL CHLORINE IN MILK AFTER THE ADDITION OF HYPOCHLORITE

By FERRIN B. MORELAND* (Chemistry Department, Division of Public Health Laboratories, Kansas State Board of Health, Topeka, Kans.)

In interpreting the results of the Rupp test[1] on milk samples examined in the laboratory, the question arose as to the amount of residual free chlorine actually still present in the milks. A cursory examination of the

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[1] U. S. Dept. Agr. Bull. 1114 (1922); *A.O.A.C, Methods of Analysis*, 6th ed. (1945), p. 317, 22.36–22.38, Hauser and King, *This Journal*, 28, 417–24 (1945).

RESULTS OF RUPP TESTS

Milk Kept at Room Temperature

Cl_2 ADDED (P.P.M.)	ELAPSED TIME	TESTS			
		a	b	c	d
1000	27 hrs.	Pale yellow	Yellow	Brown	Dark blue
	50 hrs.	—	Pale yel.	Brown	Dark blue
	5 days	—	—	Brown	Dark blue
500	27 hrs.	—	Pale yel.	Yel.-brn.	Dark blue
	50 hrs.	—	—	Yel.-brn.	Dark blue
	5 days	—	—	Yel.-brn.	Dark blue
200	27 hrs.	—	—	Yellow	Dark blue
	50 hrs.	—	—	Yellow	Dark blue
	5 days	—	—	Yellow	Dark blue
100	27 hrs.	—	—	Pale yel.	Dark blue
	50 hrs.	—	—	Pale yel.	Dark blue
	5 days	—	—	Pale yel.	Purple
	2 hrs.	—	Pale yel.	Yellow	Dark blue
	6 min.	Very pl. yel.	Pale yel.	Yellow	Dark blue
	18 hrs.	—	—	Yellow	Dark blue
	41 hrs.	—	—	Yellow	Dark blue
	4 days	—	—	Yellow	Dark blue
	1½ hrs.	—	Pale yel.	Yellow	Dark blue
	18 hrs.	—	—	Yellow	Dark blue
50	6 min.	—	Very pl. yel.	Yellow	Dark blue
	18 hrs.	—	—	Pale yel.	Purple
	41 hrs.	—	—	Pale yel.	Purple
	4 days	—	—	—	—
	10 min.	—	Pale yel.	Yellowish	Dark blue
	2 hrs.	—	—	Yellowish	Dark blue
	19 hrs.	—	—	Yellowish	Dark blue
10	6 min.	—	—	Very pl. yel.	Bluish
	18 hrs.	—	—	—	—
	41 hrs.	—	—	—	—
	4 days	—	—	—	—
5	6 min.	—	—	—	Pale purple
	18 hrs.	—	—	—	—
	41 hrs.	—	—	—	—
	4 days	—	—	—	—

Milk Kept in Refrigerator

Cl_2 ADDED (P.P.M.)	ELAPSED TIME	TESTS			
		a	b	c	d
100	12 min.	Pale yellow	Pale yel.	Yellow	Dark blue
	1 hr.	Pale yellow	Pale yel.	Yellow	Dark blue
	2 hrs.	—	Pale yel.	Yellow	Dark blue
	6 hrs.	—	Very pl. yel.	Yellow	Dark blue
	1 day	—	—	Yellow	Dark blue
	2 days	—	—	Yellow	Dark blue
	3 days	—	—	Yellow	Dark blue
	5 days	—	—	Yellow	Dark blue
	10 days	—	—	Yellow	Dark blue
	¾ hr.	Pale yellow	Pale yel.	Yellow	Dark blue
	21 hrs.	—	—	Yellow	Dark blue
50	¾ hr.	—	—	Yellowish	Dark blue
	21 hrs.	—	—	Yellowish	Dark blue
	3 days	—	—	Yellowish	Dark blue
20	1 hr.	—	—	Yellowish	Bl.-purple
	21 hrs.	—	—	Yellowish	Dark blue
	3 days	—	—	Yellowish	Dark blue
5	1 hr.	—	—	Very pl. yel.	Purple
	21 hrs.	—	—	—	—
	3 days	—	—	—	—

literature did not shed any light on the question, and the experiments herein described were therefore undertaken.

Random samples of raw market milk were used. The chlorine was added in the form of a commercial bleaching solution (Purex) containing, at the time of use, enough sodium hypochlorite to yield about 1.5 per cent of available chlorine. The Rupp test was performed as described in the A.O.A.C. Methods of Analysis. Residual chlorine was determined by titrating a 25 ml sample of the milk with 0.01 *N* sodium thiosulfate after the addition of 5 ml of 7% potassium iodide solution, in the presence of starch added near the end point.

The residual chlorine of the milk decreased rapidly as shown in the tables. At a chlorine dosage calculated to produce 100 p.p.m. in the milk, the value dropped to zero in around a quarter of an hour. The response to tests *a* and *b* decreased correspondingly; however, tests *c* and *d*, at this dosage, continued positive long after the residual chlorine, as indicated by the thiosulfate titration, reached zero. There was some falling off after several days at 50 p.p.m., and in a few hours at lower concentrations.

The term "available chlorine" as used in the heading of the table inter-

TITRATION OF FREE CHLORINE

Milk Kept at Room Temperature

Cl_2 ADDED (P.P.M.)	ELAPSED TIME	Cl_2 FOUND (P.P.M.)
1000	31 min.	244
	2⅓ hrs.	167
	26 hrs.	7
500	22 min.	<150
	2¼ hrs.	87
	26 hrs.	0
200	12 min.	41
	2¼ hrs.	14
	26 hrs.	0
100	7 min.	17
	49 min.	0
	5 min.	<24
	2 hr.	0
	1 min.	14
	1 hr.	0
	5 min.	<10
	15 min.	0
50	5 min.	0
	6 min.	0
10	6 min.	0
5	6 min.	0

Milk Kept in Refrigerator

Cl_2 ADDED (P.P.M.)	ELAPSED TIME	Cl_2 FOUND (P.P.M.)
100	1 min.	14
	6 min.	17
	11 min.	7
	15 min.	4
	20 min.	3
	30 min.	1
	60 min.	1
	2 min.	17
	4 min.	15
	6 min.	14
	10 min.	11
	21 min.	8
	30 min.	8
	60 min.	3
	123 min.	0
50	2 min.	0
20	1 min.	0
5	2 min.	0

Note: Horizontal lines set off separate experiments.

preting the reactions to the Rupp test (*Methods of Analysis*, **22.38**) is apt to be misleading since the values given are for the amount of available chlorine which is present at the instant of its addition to the milk, rather than when the Rupp test is actually performed, perhaps much later.

A CRITICAL STUDY OF THE MODIFIED AYRE-ANDERSON METHOD FOR THE DETERMINATION OF PROTEOLYTIC ACTIVITY[1]

By BYRON S. MILLER (Federal Hard Wheat Quality Laboratory, Manhattan, Kansas)

The determination of proteolytic activity of flour and flour products is somewhat difficult because of the small amount of proteolytic enzymes present in these materials. A survey of the literature indicates that of all proposed methods the one by Ayre and Anderson (1), as modified by Landis (5) and Redfern (10), is the simplest and least troublesome. This procedure is based on the semi-autolytic digestion of the sample in the presence of an auxiliary substrate (Bacto-hemoglobin) at controlled temperature and *p*H. The hemoglobin provides additional readily attackable protein material to supplement that present in the flour. The undigested protein is precipitated with trichloroacetic acid after an initial short time as well as after a final long digestion period, and the unprecipitated non-protein nitrogen is determined by a Kjeldahl procedure. The increase in non-protein nitrogen determined from the two digestions is a measure of the proteolytic activity of the sample.

Hildebrand (3) compared the Ayre-Anderson precipitation method with the Landis and Frey rate-of-gelation method (6) and found that both methods gave essentially similar results and had approximately the same experimental error and ability to differentiate between samples. The modified Ayre-Anderson method has been subjected to a collaborative study, and on the basis of this study Redfern (11) recommended that the semi-autolytic method be discontinued. The data from different laboratories and those from the same laboratory obtained on different days showed poor agreement. The present investigation deals with the conditions necessary to provide a reproducible and convenient method of determining proteolytic activity based on a modified Ayre-Anderson procedure.

METHODS

Digestion Procedure: A 5-g sample of flour is weighed into a 125 ml Erlenmeyer flask and to this is added 0.625 g (moisture-free basis) of Bacto-hemoglobin.[2] The mixture is agitated by rotating the flask until the flour and substrate are intimately mixed. This procedure facilitates complete suspension of the hemoglobin and flour. A volume of 25 ml of attempered 0.1 *M* sodium acetate-0.1 *M* acetic acid buffer solution at *p*H 4.7 is added. After agitation and stirring with a glass rod until a uniform

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[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Department of Milling Industry, Kansas State College. Published as Contribution No. 137, Department of Milling Industry, Kansas State College.

[2] The hemoglobin used in these studies was obtained from the Difco Laboratories, Detroit, Michigan.

suspension is obtained, the flask is tightly stoppered and placed in an automatic shaking device fitted in a constant temperature water bath held at 40.0±0.1°C. The automatic device is not absolutely essential, however, since manual agitation of the digestion flasks each hour gave results identical to those obtained by employing continuous agitation.

A separate flask corresponding to a single proteolytic determination is used for each point of activity. After a definite reaction time five ml of 36% trichloroacetic acid is added to each flask and shaken in the bath for an additional five minutes. The contents of each flask are then poured into a centrifuge tube and centrifuged for five minutes at 1,800 r.p.m. The centrifugate is filtered through filter paper and the solid residue is discarded. A 5-ml portion of this solution is pipetted directly into a Kjeldahl flask and analyzed for nitrogen. A precipitation is made on each sample after 15 minutes of digestion, which takes the place of a blank determination. This time is sufficiently long to allow the original soluble constituents of the flour time to dissolve completely. The final digestion period is for five hours.

Kjeldahl Procedure: The standard Kjeldahl-Gunning method (7) is used; however, a digestion time of 20 minutes is sufficient. Each still is checked for leaks and duplicability by digesting aliquots of ammonium oxalate solution. A definite volume of water (350 ml) is used to dilute the acid and is added in such a way as to wash down all of the trichloroacetic acid which has condensed in the neck of the flask during the digestion process. The concentrated alkali is also added in such a manner as to lave the neck of the flask and thus neutralize any portions of acid remaining on the neck of the flask. The unneutralized standard acid is back-titrated with 0.0714 N sodium hydroxide.

Calculation of Activity: Proteolytic activity is calculated on the basis of milligrams of non-protein nitrogen released by the enzymes in 10 g of flour. The back-titration value for the five-hour digestion is corrected by subtracting from it the back titration volume for the 15 minute digestion. This difference represents the arbitrary proteolytic activity and is translated into mg N produced from the 5-g sample. This value is further corrected to show the non-protein nitrogen released from a 10-g sample of flour on a 14 per cent moisture basis.

RESULTS

The Use of Various Concentrations of Hemoglobin.—Bacto-hemoglobin of the "Difco" brand used for this work is prepared from defibrinated beef blood by washing the cells with a saline solution prior to desiccation. Other commercial hemoglobins are prepared from unwashed cells or from whole blood and are not suitable for this work. Samples from different lots of "Difco" hemoglobin vary only slightly in their availability to proteolytic action, as is indicated by the data in Table 1. The standard devia-

TABLE 1.—*Replicability of proteolytic activity determinations using different lots of Bacto-hemoglobin*

FLOUR	HEMOGLOBIN	PROTEOLYTIC ACTIVITY	STANDARD DEVIATION	MEAN
	Lot no.	*Mg nitrogen/10 g flour*		
Commercial patent	A	18.2		
" "	B	18.7		
" "	C	18.7		
" "	C	18.2		
" "	D	19.9	0.7	18.7
Malted wheat flour	A	50.9		
" " "	B	50.3		
" " "	C	50.3		
" " "	C	49.7		
" " "	D	50.9	0.5	50.5

tion is presented for both flours. Each sample was weighed on a moisture-free basis and treated like all the others in every respect.

The quantity of the auxiliary substrate influences the extent of proteolytic action up to a certain limit. The minimum amount on the moisture-free basis was determined to be 2.5 per cent. As will be seen in Figure 1, further additions of hemoglobin did not increase the non-protein nitrogen released.

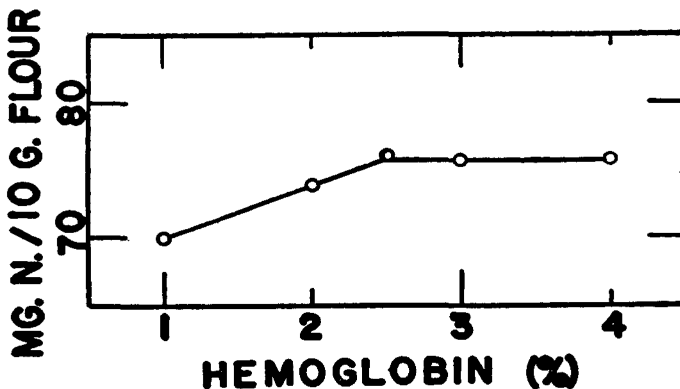

FIG. 1.—Proteolytic activity of malted wheat flour as influenced by the amount of Bacto-hemoglobin present.

Digestion Temperature.—The temperature of digestion has a pronounced effect on the amount of soluble nitrogen containing compounds produced. Previous workers have generally used temperatures from 37° to 45°C in determining proteolytic activity. The data in Figure 2 indicates that the greatest activity in wheat flour is attained at approximately

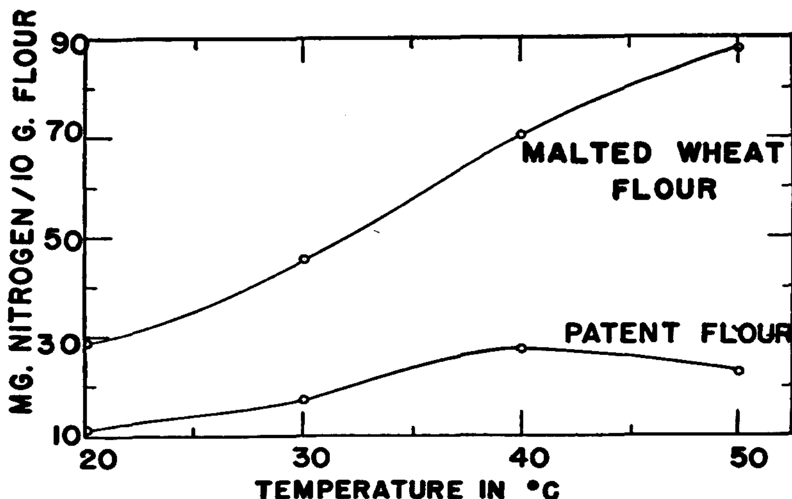

FIG. 2.—Proteolytic activity as influenced by digestion temperature.

40°C, but malted wheat flour appears to have an optimum beyond 50°C. A temperature of 40°C was used in this investigation for the standardized procedure.

Digestion Time.—The amount of proteolysis was determined for malted wheat flour and a commercial patent flour over periods varying from 5 minutes to 8 hours. These results are presented in Figures 3 and 4. No particular advantage is gained by allowing the reaction to progress beyond five hours; furthermore, this time period fits well into an experimental day.

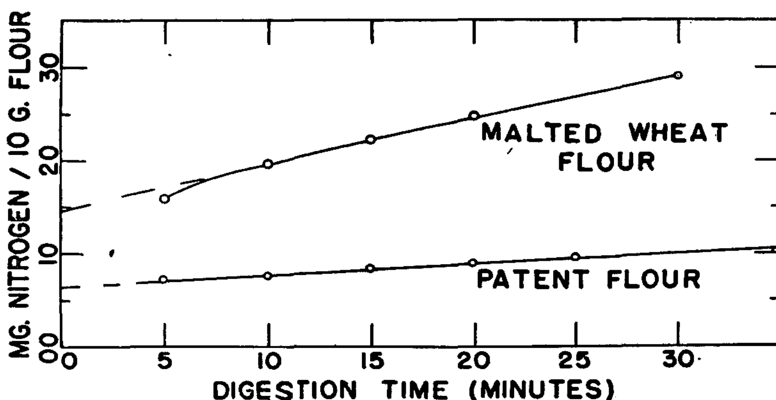

FIG. 3.—Proteolytic activity during the initial period of digestion.

The time required for the initial soluble unprecipitable nitrogen products to go into solution was arbitrarily selected from Figure 3 as fifteen minutes. The blank determination is then the titration value obtained from this 15 minute digestion and the empirical measure of proteolytic action is the difference between the value for the five hour digestion and the 15 minute digestion.

Influence of pH.—The effect of *p*H on the proteolytic activity of both

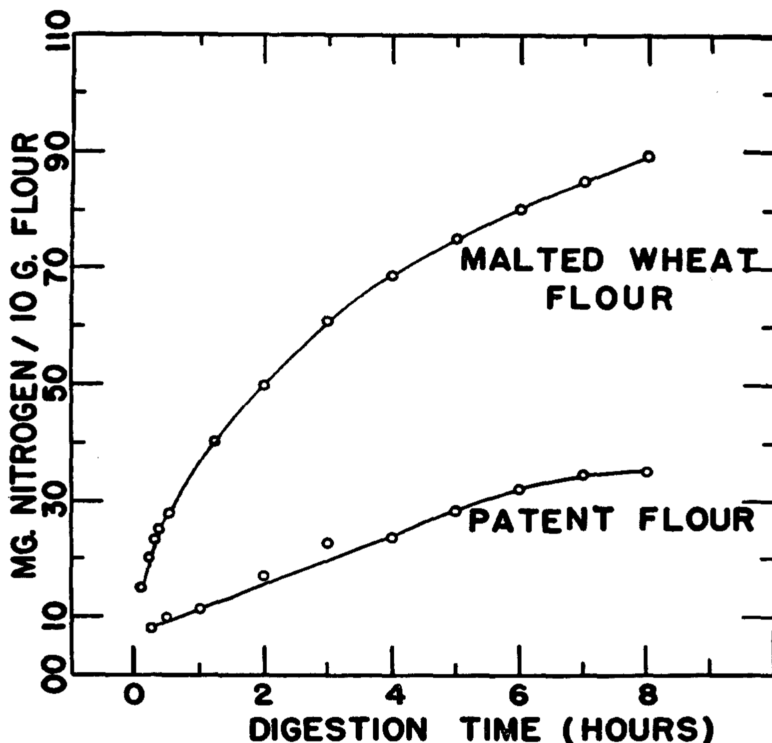

FIG. 4.—Proteolytic activity as influenced by long-time digestion.

malted wheat flour and commercial flour was determined by using a five-hour digestion period at 40°C with 2.5 per cent hemoglobin. The various *p*H values were maintained by using variations of the 0.1 *M* sodium acetate-acetic acid buffer. The *p*H readings were checked at the beginning and end of each digestion; no changes in *p*H were observed. In the case of both flours the optimum *p*H under the conditions studied was approximately 3.5. This low optimum value does not appear to be due to acid denaturation of the substrate, since an aliquot of hemoglobin treated with

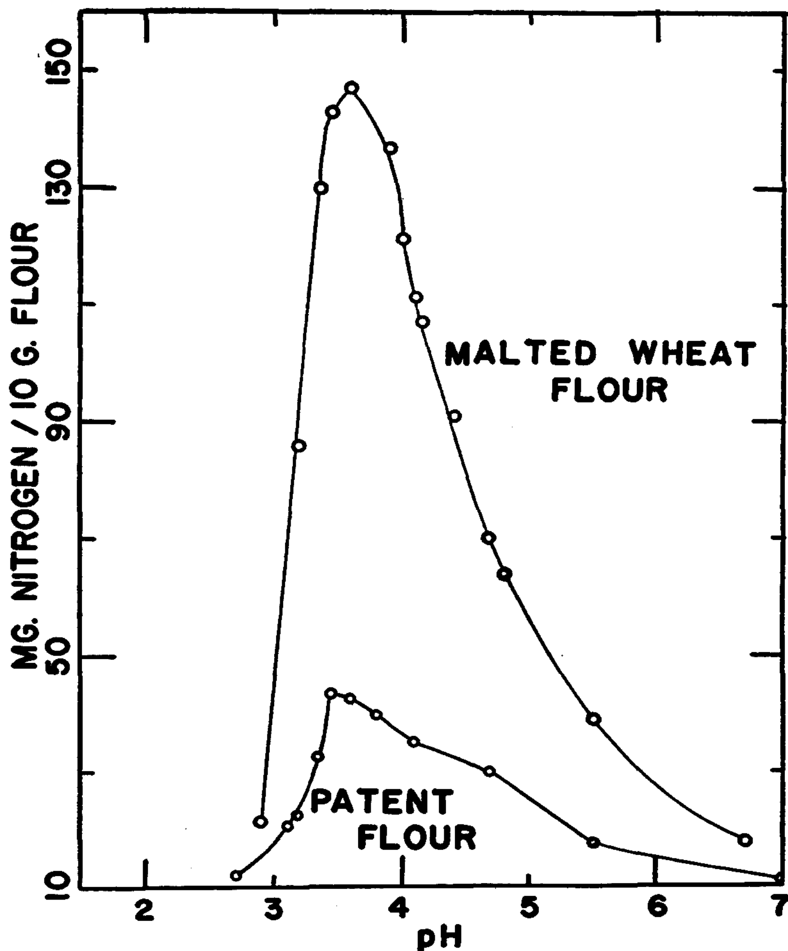

FIG. 5.—Proteolytic activity as influenced by *p*H.

strong acid and later adjusted to a *p*H of 4.7 and used as a substrate gave a duplicate result to an aliquot adjusted to a *p*H of 4.7 without acid treatment. The results of the *p*H study are shown in Figure 5.

Buffer solutions of different composition yield decidedly different results even at the same *p*H values, as indicated in Table 2. This may be due, as is indicated by previous workers, to an inhibitory effect of certain ions. The present work as well as data by other workers indicates that both the chloride and citrate ions have a detrimental effect on proteolytic activity.

Influence of Trichloroacetic Acid Concentration.—The concentration of

TABLE 2.—*Effect of different buffers on the proteolytic activity during a five-hour digestion period at 40°C*

BUFFER	pH	FLOUR	PROTEOLYTIC ACTIVITY
			*Mg nitrogen / 10 g flour**
0.1 *M* NaAc-0.1 *M* HAc	4.7	Over-malted wheat flour	183.6
0.12 *M* NaAc-0.08 *M* HAc=0.2 *M* NaCl	4.8	Over-malted wheat flour	154.8
0.24 *M* NaAc-0.16 *M* HAc=0.4 NaCl	4.8	Over-malted wheat flour	130.8
0.25 *M* Na Cit-0.25 *M* HCl	4.8	Over-malted wheat flour	129.6
0.1 *M* NaAc-0.1 *M* HAc	4.7	Malted wheat flour	76.2
0.2 *M* NaAc-0.2 *M* HAc	4.7	Malted wheat flour	76.2

* These values are for the five-hour digestion and are not corrected for the blank.

trichloroacetic acid used as the precipitating agent for the undigested protein nitrogen plays a distinct role in the results obtained. As is indicated by Figure 6, a minimum of 6 per cent by weight of acid is necessary to precipitate the maximum amount of protein nitrogen present.

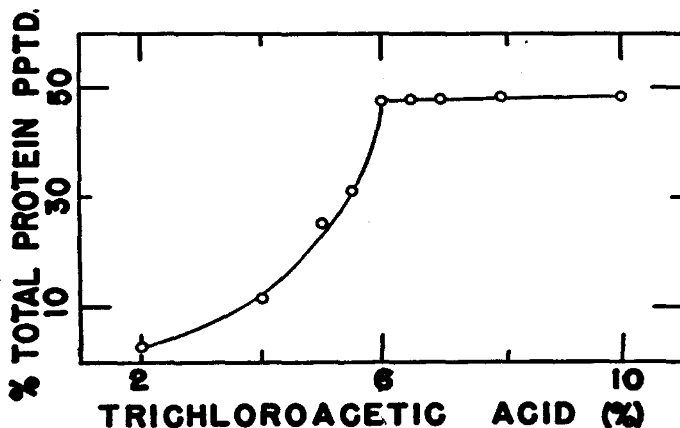

FIG. 6.—The relationship of the amount of total protein precipitated to the per cent (by weight) of trichloroacetic acid used.

Replication of Results.—Results obtained on a commercial flour and o1 a malt flour are tabulated in Table 3. Each result was obtained on a dif ferent day. The standard deviation is shown for each flour.

TABLE 3.—*Replicability of results obtained on separate days using the same experimental conditions*

FLOUR	TRIAL	PROTEOLYTIC ACTIVITY	STANDARD DEVIATION	MEAN
		Mg nitrogen/10 g flour		
Commercial patent	1	18.7		
" "	2	20.4		
" "	3	18.2		
" "	4	19.3		
" "	5	19.3		
" "	6	18.7		
" "	7	19.3		
" "	8	18.6		
" "	9	19.5	0.65	19.0
Malted wheat flour	1	50.3		
" " "	2	50.3		
" " "	3	49.2		
" " "	4	49.7		
" " "	5	49.7	0.47	49.8

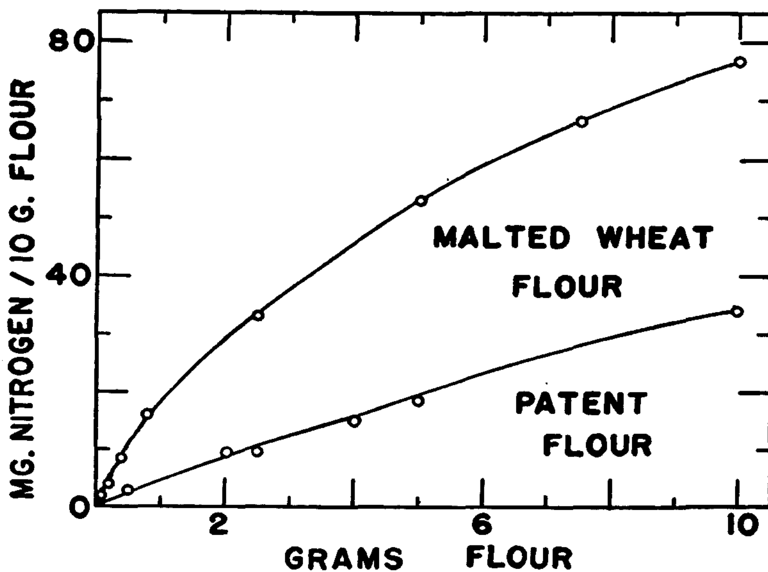

FIG. 7.—Proteolytic activity as influenced by enzyme concentration.

Proteolytic Activity as Influenced by Enzyme Concentration.—Figure 7 shows the relation of the amount of enzyme present to the amount of soluble nitrogen liberated during the usual five-hour digestion period. Since an excess of hemoglobin substrate is present, the addition of various amounts of flour with proportionate variation in protein content is of no consequence.

DISCUSSION

It is hoped that the studies reported here will lend confidence to the modified Ayre-Anderson method of determining proteolytic activity. An effort has been made to adapt the method to levels of activity varying from that of commercial bread flour to that of malted wheat flour.

Bacto-hemoglobin, unlike casein and gelatin, is a reproducible substrate. Different lots of hemoglobin are digested at the same rate by a given proteinase solution. Even when peptidase is present in addition to proteinase, Anson (2) states that the formation of products not precipitable by trichloroacetic acid is due as far as is known to proteinase action alone.

Previous workers (4, 8, 9) have found that the *p*H optimum for the proteolytic enzyme system in flour and wheat germ varied with the different proteins used as substrates. Howe and Glick (4) found the *p*H optimum to be 5.0 for the enzymes in whole wheat, germ, clear, and patent flours, with and without the addition of casein as an auxiliary substrate. In papers dealing with the proteolytic enzymes of sprouted wheat, Mounfield (8, 9) observed a *p*H optimum of 4.1 when edestin was used as an auxiliary substrate, 5.1 when gelatin was used, and 6.0 when an acetic acid dispersion of gluten was added. The present work indicates an optimum *p*H of 3.5 when Bacto-hemoglobin is used as the auxiliary substrate. Considering this variability of *p*H optimum and the possibility of hemoglobin denaturation at a *p*H of 3.5, it was decided to employ an arbitrary *p*H of 4.7 in the present investigation. This *p*H is within the normal operating range of enzymes in the natural state and can be maintained by a sodium acetate-acetic acid buffer system which has non-inhibitory ions. The buffer system proposed by Landis (5) contained chloride ions which are shown to be inhibitory to proteolytic action.

The greatest proteolytic activity occurred at a temperature of 40°C for patent flour, while a temperature of above 50°C provided the maximum activity for malted wheat flour. A standard arbitrary temperature of 40°C was adopted for this study as was suggested by Landis (5); other temperatures are, however, equally adaptable.

Relatively few operational changes in the method as modified by Landis (5) and Redfern (10) were made. The dry hemoglobin is added to individual reaction flasks instead of being added as a suspension of hemoglobin and buffer, in order to insure a more uniform and complete suspension of flour and substrate. A 125 ml Erlenmeyer flask was more adaptable to the reaction volume. The blank digestion time has been arbitrarily reduced from 25 minutes to 15 minutes on the basis of the curves in Figure 3.

In view of past criticism of this method it is necessary to stress certain precautions which this study has shown to be necessary in order to obtain satisfactory results. Most, if not all, of the difficulty in replication of results within a single laboratory and between collaborators is possibly due to differences in carrying out the Kjeldahl procedure. Prior to distillation of the digested mixture it is essential that all of the trichloroacetic acid which has volatilized and deposited on the neck of the flask during the digestion period be washed down into the solution where it can be neutralized by the sodium hydroxide. Even then a small portion of the trichloroacetic acid distills over into the receiver acid. This is compensated for, however, by subtracting the value for the 15-minute digestion from that for the five hour digestion since the same quantity of trichloroacetic acid is evolved in both cases.

Each Kjeldahl still should be carefully checked for leaks by distillation of a known quantity of nitrogen containing salt. Only those stills giving results which agree with the theoretical values should be used.

A consideration of Figure 7 indicates that the proteolytic activity for malted wheat flour is not directly proportional to the quantity of enzyme used in the experimental determination. Further, Figure 4 indicates that the proteolytic activity for this type of flour is not linearly related to the time used for digestion. The enzyme-substrate reaction is thus not a zero order reaction, in the case of malted wheat flour, and precludes the determination of specific proteolytic activity independent of time and quantity of enzyme. Similar curves are shown by Anson (2) for pepsin, trypsin, papain, and cathepsin. Figures 4 and 7 indicate essentially a zero order reaction for ordinary patent flour under the conditions of this experiment.

Relative proteolytic activities, however, are readily obtainable provided the conditions of the experiment are rigidly defined. Various patent flours produce curves which parallel closely the one shown in Figure 7. Thus relative proteolytic activity of a series of flours may be determined by comparing the mg of soluble nitrogen produced under specified conditions by a given quantity of these flours.

By using smaller quantities of substances having extremely high proteolytic activity, values may be determined for such substances as bran or germ fractions. Likewise, an accurate measurement of the activity of purified proteolytic enzymes is possible. In all cases it is necessary that the quantity of proteolytic enzymes is such that the amount of Bacto-hemoglobin present is not a limiting factor in the results obtained. For example, it is necessary to use no more than 25 mg of mold bran with the ordinary 2.5 per cent hemoglobin suspension in evaluating the proteolytic activity of mold bran preparations. A curve similar to that shown in Figure 1 should be obtained prior to evaluating the proteolytic activity of any substances other than flour or malted wheat flour.

It is frequently convenient, especially when the proteolytic activity is high, to use an extract of the material being analyzed rather that a quan-

tity of the solid material itself. In order to get complete suspension of the hemoglobin in such cases it is necessary to add approximately three grams of finely divided pumice or sand to the dry hemoglobin prior to the addition of liquid. In this case a more concentrated solution of trichloroacetic acid must be added at the end of the digestive period in order to limit the volume to 30 ml.

SUMMARY

The modified Ayre-Anderson method for the determination of proteolytic activity has been critically studied to determine the effects of various conditions on the results obtained. A patent flour and a malted wheat flour have been investigated.

A concentration of 2.5 per cent (dry weight basis) Bacto-hemoglobin was shown to be sufficient for levels of proteolytic activity as high as those found in malted wheat flour.

The activity of patent flour proteinase reached a maximum at 40°C. For malted wheat flour the optimum temperature was above 50°C.

The optimum *p*H for digestion was shown to be approximately 3.5 for both patent flour and malted wheat flour when using Bacto-hemoglobin as an additional substrate.

The use of at least 6 per cent (by weight) trichloroacetic acid as a precipitating agent of proteins is required, and certain precautions necessary in the Kjeldahl determinations of the soluble nitrogen components are indicated.

The level of precision obtained with substances having high or very low proteolytic activity appears to be satisfactory if the conditions herein described are maintained.

The method is adaptable to the determination of proteolytic activity in bran and germ fractions or purified proteolytic enzymes.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) AYRE, C. A., and ANDERSON, J. A., *Can. J. Research*, **17c**, 239 (1939).
(2) ANSON, M. L., *J. Gen. Physiol.*, **22**, 79 (1938).
(3) HILDEBRAND, F. C., *Cereal Chem.*, **17**, 635 (1940).
(4) HOWE, MARJORIE, and GLICK, D., *Ibid.*, **22**, 502 (1945).
(5) LANDIS, Q., *This Journal*, **25**, 631 (1942).
(6) LANDIS, Q., and FREY, C. N., *Cereal Chem.*, **15**, 91 (1938).
(7) *Methods of Analysis, A.O.A.C.*, Sixth Ed., 27 (1945).
(8) MOUNFIELD, J. D., *Biochem. J.*, **30**, 549 (1936).
(9) ———, *Ibid.*, **32**, 1675 (1938).
(10) REDFERN, S., *This Journal*, **28**, 496 (1945).
(11) ———, *Ibid.*, **29**, 258 (1946).

A MICROCHEMICAL SPOT TEST FOR MAMMALIAN URINE CONTAMINATION ON FABRICS

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Before the 6th edition of the A.O.A.C. Methods was received, this laboratory was requested to recommend a satisfactory method of confirming the evidence of fluorescence on flour bags by a microchemical type of test which should be specific for urine.

Attempts were made to develop a spot test type of reaction for well known constituents of urine, such as creatinine and urea. Direct chemical methods such as the familiar Biuret test for urea and the Weyl nitroprusside test (1), for creatinine, as applied directly to fabric, were unsuccessful. Evolution of ammonia from urea by urease and its detection by means of a sensitive manganese nitrate-silver nitrate impregnated paper (2) gave very satisfactory results and provided a rapid and sensitive test for urea. Upon receipt of the 6th edition of the A.O.A.C. Methods (3), it was considered desirable to make a comparison between the published methods and that developed in this laboratory as to dependability, sensitivity, and ease of manipulation. The new method is described in detail, followed by comparison with the A.O.A.C. procedures.

EXPERIMENTAL

REAGENTS

Urease Soln.—A 10% slurry of Jack Bean Meal in water is prepared and allowed to settle a few minutes; the supernatant liquid is used as urease soln. This slurry may be stored for as long as a week in a refrigerator without apparent loss of effectiveness.

Manganese Nitrate-Silver Nitrate Soln (2).—14 ml of 50% soln of $Mn(NO_3)_2$ are made up to 100 ml; add this soln to a soln of 4.0 g of $AgNO_3$ made up to 100 ml. Neutralize the combined solns with 0.1 *N* NaOH until a black precipitate first forms. Filter, and store filtrate in dark reagent bottle.

Filter Paper Impregnated with Manganese-Silver Soln.—Coarse filter paper is soaked in the neutralized $Mn(NO_3)_2$-$AgNO_3$ soln and dried quickly on the metal surface of a steam bath heated to approximately 100°C. (Samples of this paper stored for one week in a clean bottle in the dark developed brown stains. These did not interfere with the operation of the test. However, it is considered desirable to prepare a fresh supply, rather than to use paper stored longer than one week).

METHOD

Prior to applying the spot test, outline with a pencil on the fabric, a stain suspected of being due to urine, while observing its fluorescence under ultra-violet irradiation. If the stain is clearly visible under ordinary illumination, this step will not be necessary. Apply two to four drops of urease soln to the stained area of the cloth and allow to soak in for 5–10 seconds. Then place the cloth on the surface of a heated steam bath, and immediately place over it a piece of impregnated paper so that the paper is wetted by the damp area of the cloth. Maintain a firm contact by laying a piece of glass stirring rod across the moistened area and pressing firmly. If the stain was due to urine, a black spot appears on the filter paper in about thirty seconds.

Ammonia vapor in the laboratory should be avoided during the running of the test. The color development characteristic of ammonia is sufficiently rapid so that there is little likelihood of a false indication from this source. However, a sensitized paper placed beside the specimen under test serves to indicate the possibility of error.

Some heavily sized fabrics may cause difficulty with this test because of the inability of the urease solution to wet and penetrate the fabric. It has been determined that 0.1 per cent of a wetting agent such as Nacconol (National Aniline) added to the urease solution to aid penetration will neither give a false positive reaction in the absence of urine nor prevent the usual color development when urea is present.

Experiments showed that the procedure described above still gave a positive indication of the presence of urine on cloth powdered with grain products such as oatmeal, corn meal, fine wheat flour and "all purpose" flour. These grain products did not give a false positive reaction when urine was not present.

Sulfides (K_2S was used) can produce a black stain when direct contact with the paper is maintained. Sucrose and dextrose do not interfere, but molasses contains reducing substances which give a false positive reaction.

In the presence of either sulfides or strongly reducing organic materials, a false indication may be avoided by supporting the impregnated paper above the sample on two horizontal stirring rods placed an inch or so apart on the cloth. The test may be performed entirely satisfactorily in this manner, but the time required for development of a black stain due to urine is approximately doubled. Wetting the sensitized paper retards the test still further. No substance likely to be encountered in fabrics has been found to prevent a positive reaction when urine was present.

COMPARISON OF METHODS

The A.O.A.C. Methods were followed in order to form an estimate as to relative advantages of any one method.

The "Urease Test for Urea" (**42.98,** A.O.A.C., 6th Ed.) depends on the evolution of ammonia from urea by the action of urease as does the spot test here described. The ammonia is then detected by the formation of $(NH_4)_2PtCl_6$. This test was also found to be valid in the presence of grain materials likely to be encountered in flour sacking.

The "Xanthydrol Test for Urea" (**42.99,** A.O.A.C., 6th Ed.) depends on the formation, and microscopic examination, of xanthydrol urea. We used Eastman Xanthydrol #1559 for this test, and obtained a positive reaction, as evidenced by the characteristic star shaped crystals, even when urea was not present. No attempt was made to further purify the xanthydrol to determine whether the false positive test could be eliminated.

The method "Extraction of Urea and Crystallization of Urea Nitrate"

TABLE 1.—*Comparison of methods*

TEST	A.O.A.C. REF. 6TH ED. (1945)	MINIMUM CONC. OF UREA TO WHICH TEST IS POSITIVE	TIME REQUIRED FOR POSITIVE TEST AT MINIMUM CONCENTRATION
	sec	*per cent*	*min*
Urease Test for Urea	42.98	0.05	12
Xanthydrol Test for Urea	42.99	Gives positive reaction with clean cloth	—
Extraction of Urea and Crystallization of Urea Nitrate	42.100	Characteristic urea crystals but not any urea nitrate crystals	—
Microchemical Spot Test	—	0.01	0.5

(**42.100**, A.O.A.C., 6th Ed.) was found to be satisfactory, producing characteristic needle shaped crystals of urea by extraction with alcohol and acetone. However, it was found difficult to convert these crystals to a recognizable form of urea nitrate as described in the A.O.A.C. procedure.

Aqueous solutions of 1.0, 0.1, 0.05, 0.025, 0.01, and 0.005% urea were prepared. Samples of fabric were spotted with four drops of each the urea solutions. After drying, the spotted areas were tested by the A.O.A.C. procedures as well as by the proposed spot test. The results are recorded in Table 1. The time required by the new method for a positive reaction (and hence the likelihood of extraneous contamination) is less, and the sensitivity to small amounts of urea is greater, under the conditions of this test, than in the "Urease Test for Urea."

SUMMARY

A new spot test is described for the identification of urine stains on fabrics such as flour sacking. The method is rapid, simple, and dependable. Unusually low concentrations of urea produce a definite positive reaction. Excessive ammonia fumes in the laboratory must be avoided since the test depends on the evolution of ammonia by the action of urease on urea present as a constituent of the urine stain. The ammonia is detected by the black stain produced by its reaction with manganese-silver impregnated paper in close contact with the test specimen.

BIBLIOGRAPHY

(1) HAWK, P., and BERGEIM, O., "Practical Physiological Chemistry," 10th Ed., P. Blakiston's Son & Co. (1931), p. 721.
(2) FEIGL, F., "Qualitative Analysis by Spot Tests," 2nd Eng. Ed., Nordemann Publishing Co. Inc., (1939), pp. 154 and 353.
(3) *Methods of Analysis. A.O.A.C.*, 6th Ed., 1945, p. 800 (**42.97–42.100**).

ASCORBIC ACID IN GRAPEFRUIT JUICE, ORANGE JUICE, AND THEIR BLENDS: 1943

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In 1943 the Food and Drug Administration determined ascorbic acid retention during manufacturing and storage, in grapefruit juice, orange juice, and their blends, following the same general procedure used for tomatoes and tomato juice(1). Volatile oil, suspended matter, acidity, *p*H, soluble solids, consistency, and other ingredients or properties were also determined in the canned juices. Since that work was done, other investigators (2), (3), (4) have reported their findings on work conducted along similar lines.

Ascorbic acid retention during manufacturing was ascertained by collecting samples of juices in the factories at various stages of manufacture, taking them to the titrating table in the factory, and immediately determining their ascorbic acid content. Usually, during a day's visit to a factory, three series of four samples each were collected at the following sampling places: (1) after extraction; (2) after screening; (3) after pasteurization; (4) after closing can. Also, some samples were collected and titrated after deaeration. After the completion of a series, enough additional sealed cans of the juices were collected in succession from the production line to furnish samples for later chemical analysis including the determination of ascorbic acid retention. Retention was ascertained by determining ascorbic acid, usually in three duplicate cans, after each period of storage (3, 6, 9, and 12 months) at room temperatures, in Atlanta, in Houston, and in Los Angeles.

Ascorbic acid was determined by the 2,6-dichlorophenolindophenol titration method described by Hall (5). The samples were filtered through well-washed cheesecloth, discarding first portion of filtrate. Two portions, of 4 or 5 ml each, of the juices were pipetted into an equal volume of the metaphosphoric-acetic acid reagent and each portion was rapidly titrated with the dye. The dye was standardized daily with the purest ascorbic acid obtainable, usually Eastman catalogue No. 4640. Ascorbic acid purity was determined by titration with iodine standardized against National Bureau of Standards As_2O_3.

Table 1 deals with the scope of the work, and Tables 2, 3, and 4 contain such of the data as appear necessary to establish the range of factory and storage losses of the various citrus juices investigated. A flow diagram of

* The work was directed and participated in by the author of record, assisted by Dr. R. A. Osborn of the Food Division (W. B. White, Chief); and the following chemists and inspectors made very material contributions:

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Badger, C. H.	Holliday, D. J.	Rynerson, G. W.
Born, R. B.	Koelsche, C. L.	Sanders, J. W.
Durrenberger, W. R.	Lint, H. L.	Wilson, J. B.
Fowler, G. R.	Moses, W. R.	Winkler, W. O.

the manufacture of orange juice and grapefruit juice has been published (6), and might well be consulted in connection with this report.

Data on factory equipment and manufacturing processes were recorded but, because of the confidential nature of much of that information, are not here included. Source and variety of fruit were noted. The basic data

TABLE 1.—*Scope of investigation in 1943*
(Exclusive of data in Tables 5 and 6)

CITRUS AREAS	NO. OF FACTORIES VISITED	NO. OF SERIES[1]	NO. OF SAMPLES	NO. OF DETERMINATIONS IN FACTORY	NO. OF DETERMINATIONS ON STORED SAMPLES
	Sweetened grapefruit juice				
Florida	3	4	18	53	48
	Unsweetened grapefruit juice				
Florida	10	26	120	243	120
Texas	10	29	119	184	168
Calif., Ariz.	4	13	42	42	27
	Sweetened orange juice				
Florida	5	14	62	130	72
	Unsweetened orange juice				
Florida	3	7	31	68	36
Texas	1	3	11	16	16
Calif., Ariz.	8	23	83	83	65
	Sweetened blends				
Florida	3	7	33	93	60
Texas	1	3	10	13	19
Totals	48[2]	129	529	925	631

[1] A series consisted of from 3 to 5 (usually 4) individual samples taken at different steps in the process of manufacture of the juice. Usually 3 series were collected at each factory and assigned one number.
[2] 41 separate factories: Florida 18; Texas 10; Calif., Ariz. 13.

and compilations not included, as well as the factory data of a non-confidential nature, can be consulted in the files of the Food Division of the Food and Drug Administration.

The screened juices, instead of the extracted juices, are compared with the canned juices in Tables 2, 3, and 4, because the ascorbic acid was not determined in the freshly extracted juices in the samples of 49 of the series. However in 80 of the series, where ascorbic acid was determined in both the freshly extracted juices and the screened juices, the average of ascorbic acid in all of the freshly extracted juices was 45.2, and that in all of the screened juices was 45.1.

Apparent gains in ascorbic acid content during factory processing are

TABLE 2.—*Retention of ascorbic acid in canned grapefruit juice (27 factories)*

DATE 1943	CODE NO. OF FACTORY	ASCORBIC ACID FOUND IN SCREENED JUICE				PER CENT ASCORBIC ACID RETAINED IN CANNED GRAPEFRUIT JUICE AT TIME OF MANUFACTURE[1]				SERIES STORED	PER CENT ASCORBIC ACID RETAINED IN CANNED GRAPEFRUIT JUICE AFTER STORAGE—			
		SERIES I	SERIES II	SERIES III	AVER.	SERIES I	SERIES II	SERIES III	AVER.		3 MOS.	6 MOS.	9 MOS.	12 MOS.
		Mg per 100 ml				*per cent*	*per cent*	*per cent*	*per cent*	*No.*	*per cent*	*per cent*	*per cent*	*per cent*
						Florida grapefruit juice, unsweetened								
12/29[2]	4	40.9	—	—	40.9	96.8	—	—	96.8	I	96.0	87.9	80.6	73.5
2/24	1	36.6	—	—	36.6	96.4	—	—	96.4	I	94.6	85.6	81.3	75.4
3/3	2	41.0	45.3	42.0	42.8	98.0	90.9	90.0	93.0	I	91.8	82.1	78.1	73.4
3/15	3	43.9	43.4	43.4	43.6	98.6	91.2	98.6	96.1	I	93.1	82.7	80.1	73.7
3/17	7	39.8	39.6	39.6	39.7	99.0	100.0	97.5	98.8	I	93.4	82.7	81.5	75.6
4/13	9	40.6	39.5	38.3	39.5	95.6	97.7	101.0	98.1	II	93.5	82.6	75.9	74.9
4/19	11	43.1	46.8	40.5	43.5	95.6	87.8	101.0	94.8	III	93.2	85.1	77.8	76.3
4/22	15	41.1	40.2	40.5	40.6	100.0	99.0	98.3	99.1	II	97.0	86.2	80.4	76.1
4/26	10	39.9	36.9	39.2	38.7	91.7	97.6	96.4	95.2	II	97.5	85.0	78.9	75.0
6/10	16	43.3	40.7	41.9	42.0	99.1	96.6	96.9	97.5	II	90.1	87.0	82.2	77.1
						Florida grapefruit juice, sweetened								
12/30[2]	5	40.4	—	—	40.4	97.3	—	—	97.3	I	97.5	88.5	80.4	72.5
1/1	6	42.1	42.6	—	42.4	96.2	97.2	—	96.7	I	94.4	88.5	79.1	73.6
4/7	8	41.1	—	—	41.1	97.1	—	—	97.1	I	95.5	89.0	78.4	73.2
						Texas grapefruit juice, unsweetened								
1/6	24	39.8	37.8	38.9	38.8	94.7	97.9	95.1	95.9	I	95.2	84.9	77.2	71.6
1/18	20	42.5	38.4	40.4	40.4	95.5	104.2	105.2	101.6	I	99.3	89.7	83.0	75.4
1/19	28	38.5	39.6	40.5	39.5	114.3	98.2	98.3	103.6	III	93.2	84.7	77.4	70.9
1/20	26	38.1	34.9	37.9	37.0	96.3	102.6	97.9	98.9	III	91.9	82.5	75.2	69.3
2/15	21	45.5	45.7	45.4	45.5	97.1	97.8	97.4	97.4	III	92.5	80.5	74.7	68.8
2/16	22	38.7	39.2	39.7	39.2	100.0	97.2	97.5	98.2	III	91.7	81.7	74.9	69.3
2/17	25	40.6	43.5	43.3	42.5	98.0	98.2	95.2	97.1	III	92.7	82.0	74.8	68.2
2/18	27	37.8	39.3	40.8	39.3	96.0	96.2	95.1	95.8	III	92.8	81.4	74.7	68.8
2/19	23	39.4	41.0	—	40.2	94.9	95.1	—	95.0	II	97.4	84.6	78.5	72.3
3/29	29	39.3	37.7	36.1	37.7	95.4	98.1	98.1	97.2	I, II, III[3]	98.9	83.6	76.2	69.9
						California-Arizona grapefruit juice, unsweetened								
1/28	40	44.0	47.0	48.0	46.0	102.3	97.9	102.1	100.8	III	94.3	92.2	84.1	74.3
2/19	42[4]	44.5	44.2	44.0	44.5	109.4	105.2	103.6	104.6	II	95.9	88.2	69.5	66.0
4/15	43	42.0	44.7	38.7	41.8	93.6	85.2	93.0	90.6	II	95.5	89.0	.91.1	85.3
4/15	44	41.6	41.5	40.0	41.0	99.8	99.5	99.5	99.6	II	93.0	87.4		

SUMMARY TABLE 2

	ASCORBIC ACID FOUND IN SCREENED JUICE				PER CENT ASCORBIC ACID RETAINED IN CANNED GRAPEFRUIT JUICE AT TIME OF MANUFACTURE			PER CENT ASCORBIC ACID RETAINED IN CANNED GRAPEFRUIT JUICE AFTER STORAGE				
	NO. OF SERIES	MIN.	MAX.	AVER.	MIN.	MAX.	AVER.		3 MOS.	6 MOS.	9 MOS.	12 MOS.
		Mg per 100 ml			*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
Florida	30	36.6	46.8	41.1	87.8	101.0	96.6	Min.	90.1	80.5	69.5	66.0
Texas	29	34.9	45.7	40.0	94.7	114.3	98.2	Max.	99.3	92.2	91.1	85.3
Cal. Ariz.	13	38.7	48.0	43.5	85.2	109.4	99.3	Aver.	94.5	85.4	78.7	73.1
								No.	27	27	26	26
All	72	34.9	48.0	41.1	85.2	114.3	97.7					

[1] Data on juice before screening, after pasteurizing and at other intermediate steps in manufacturing are not included because of small "over-all" loss, and to save space.
[2] 1942.
[3] Cans from 3 series intermingled.
[4] A fourth series was run; screened juice, 45.3; per cent retained, 100.0.

TABLE 3.—*Retention of ascorbic acid in canned orange juice (17 factories)*

DATE 1943	CODE NO. OF FACTORY	ASCORBIC ACID FOUND IN SCREENED JUICE, SERIES I	II	III	AVER.	PER CENT ASCORBIC ACID RETAINED IN CANNED ORANGE JUICE AFTER MANUFACTURE[1], SERIES I	II	III	AVER.	SERIES STORED	PER CENT ASCORBIC ACID RETAINED IN CANNED ORANGE JUICE AFTER STORAGE, 3 MOS.	6 MOS.	9 MOS.	12 MOS.
		Mg per 100 ml				*per cent*	*per cent*	*per cent*	*per cent*	*No.*	*per cent*	*per cent*	*per cent*	*per cent*
						Florida orange juice, unsweetened								
2/24	1	47.6	—	—	47.6	97.5	—	—	97.5	I	94.0	86.6	80.8	76.1
3/18	13	66.8	67.8	57.2	63.9	100.0	100.0	101.2	100.4	III	92.9	82.0	78.1	74.8
5/22	17	45.9	44.1	46.2	45.4	99.8	100.2	98.3	99.4	II	90.5	85.1	80.5	75.6
						Florida orange juice, sweetened								
2/2	6	60.8	—	—	60.8	97.7	—	—	97.7	I	96.1	88.0	79.3	72.6
2/3	6	63.8	—	—	63.8	99.1	—	—	99.1	I	97.5	88.1	80.4	72.0
2/26	2	64.4	66.5	59.0	63.3	98.4	96.1	95.1	96.5	I	94.5	84.9	80.4	73.5
4/1	14	63.4	54.9	61.4	59.9	89.3	100.7	98.0	96.0	II	92.9	81.9	75.0	72.5
5/21	18	43.6	45.2	45.9	44.9	92.4	96.2	96.9	95.2	II	91.0	85.1	—	74.3
5/14	15	38.4	50.5	39.7	42.9	98.4	100.0	100.0	99.5	II	90.5	85.1	80.8	75.0
						Texas orange juice, unsweetened								
2/17	25	59.1	60.4	60.1	59.9	92.0	91.2	91.7	91.6	III	92.0	79.3	71.9	66.2
						California orange juice, unsweetened								
7/22	40	46.7	45.8	47.8	46.8	101.0	102.0	99.8	100.9	II	92.3	89.1	85.9	81.4
7/27	41	49.8	51.8	47.7	49.8	96.8	94.6	98.3	96.4	II	89.0	82.0	78.8	72.7
7/28	47	48.0	47.4	48.1	47.8	100.8	101.5	100.4	101.0	II	94.0	91.3	83.6	77.8
8/3	46	45.3	47.4	46.7	46.5	103.8	94.5	97.2	98.3	I	87.0	83.6	79.8	74.5
8/10	48	42.3	44.3	—	43.3	97.4	99.1	—	98.4	I	92.2	84.0	82.3	77.2
8/11	51	47.5	45.3	45.3	46.0	102.9	99.1	101.3	101.3	II	92.4	85.5	82.0	79.3
8/16	49	46.9	49.7	45.7	47.4	106.6	97.6	102.4	102.1	III	89.7	84.3	80.8	78.1
9/10	45	40.6	41.0	43.9	41.8	101.0	97.8	94.3	97.6	II	96.0	90.0	81.8	76.8

SUMMARY TABLE 3

	ASCORBIC ACID FOUND IN SCREENED JUICE, NO. OF SERIES	MIN.	MAX.	AVER.	PER CENT ASCORBIC ACID RETAINED IN CANNED ORANGE JUICE AT TIME OF MANUFACTURE, MIN.	MAX.	AVER.		PER CENT ASCORBIC ACID RETAINED IN CANNED ORANGE JUICE AFTER STORAGE, 3 MOS.	6 MOS.	9 MOS.	12 MOS.
		Mg per 100 ml			*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
Florida	21	38.4	67.8	54.0	89.3	101.2	97.9	Min.	87.0	79.3	71.9	66.2
Texas	3	59.1	60.4	59.9	91.2	92.0	91.6	Max.	97.5	91.3	85.9	81.4
California	23	40.6	51.8	46.3	94.3	106.6	99.6	Aver.	92.5	85.3	80.1	75.0
								No.	18	18	17	18
All	47	38.4	67.8	50.6	89.3	106.6	98.3					

[1] Data on juice before screening, after pasteurizing, and at other intermediate steps in manufacturing, are not included because of small "over-all" loss, and to save space.

TABLE 4.—*Retention of ascorbic acid in canned sweetened blends of grapefruit juice and orange juice (4 factories)*

DATE 1943	CODE NO. OF FACTORY	ASCORBIC ACID FOUND IN SCREENED JUICE				PER CENT ASCORBIC ACID RETAINED IN CANNED BLENDS AT TIME OF MANUFACTURE[1]				SERIES STORED	PER CENT ASCORBIC ACID RETAINED IN CANNED BLENDS AFTER STORAGE			
		SERIES I	SERIES II	SERIES III	AVER.	SERIES I	SERIES II	SERIES III	AVER.		3 MOS.	6 MOS.	9 MOS.	12 MOS.
		Mg per 100 ml				*per cent*	*per cent*	*per cent*	*per cent*	*No.*	*per cent*	*per cent*	*per cent*	*per cent*
						Florida								
1/2	6	49.6	49.2	—	49.4	98.6	97.4	—	98.0	I	95.3	88.3	78.5	73.0
										II	96.9	88.7	79.1	72.0
1/6	8	51.3	—	—	51.3	97.5	—	—	97.5	I	93.8	87.4	78.2	70.8
2/24	1	45.3	—	—	45.3	95.8	—	—	95.8	I	93.3	85.7	79.0	73.5
5/20	1[2]	35.8	37.1	38.3	36.7	97.8	98.1	98.2	98.0	II	90.9	87.4	80.8	76.9
						Texas								
2/17	25	50.6	48.2	—	49.4	96.4	99.0	—	97.7	III	89.4	78.9	71.8	67.1

SUMMARY TABLE 4

	NO. OF SERIES	ASCORBIC ACID FOUND IN SCREENED JUICE			PER CENT ASCORBIC ACID RETAINED IN CANNED BLENDS AT TIME OF MANUFACTURE			PER CENT ASCORBIC ACID RETAINED IN CANNED BLENDS AFTER STORAGE				
		MIN.	MAX.	AVER.	MIN.	MAX.	AVER.		3 MOS.	6 MOS.	9 MOS.	12 MOS.
		Mg per 100 ml			*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*
Florida	7	35.8	51.3	43.8	95.8	98.6	97.6	Min.	89.4	78.9	71.8	67.1
Texas	2	48.2	50.6	49.4	96.4	99.0	97.7	Max.	96.9	88.7	80.8	76.9
								Aver.	93.3	86.1	77.9	72.2
								No.	6	6	6	6
All	9	35.8	51.3	45.0	95.8	99.0	97.6					

[1] Data on juice before screening, after pasteurizing, and at other intermediate steps in manufacturing, are not included because of small "over-all" loss and to save space.
[2] Fruit mixed 50–50 on belt instead of blending juices.

TABLE 5.—*Per cent oil, suspended matter, and insoluble solids in factory and warehouse samples of grapefruit juice, orange juice, and blends.*
Collected and examined 1942 and 1943

CODE MFR'S NO.	OIL	SUSPENDED MATTER BY CENTRIF.	INSOLUBLE SOLIDS
	per cent	*per cent*	*per cent*
Florida grapefruit juice unsweetened			
4	.065	4	.041
1	.015	6	.053
2	.005	5	
3	.012	4.5	
7	.012	6	
9	.018	3	
11	.008	8	
10	.005	5	
16	.001		
Florida grapefruit juice sweetened			
5	.002	5	.054
6	.005	7	.049
6	.010	8.5	
8	.015	6.5	
Texas grapefruit juice unsweetened			
29	.010	4	
24	.002	7	
20	.010	4	
28	.015	6	
26	.005	6.5	
21	.001	8	
22	.010	6	
25	.008	6	.064
27	.004	12.5	
23	.015	7	
California and Arizona grapefruit juice unsweetened			
40	.001	6	
40	.002	7	
40	.005	7	
41	.005	7	
40	.010	8	
42	.001	12	.159
43	.018	6	.026
43	.018	6	
44	.008	8	
Blends of Florida grapefruit and orange juices sweetened			
6	.015	9	
6	.035	9	
8	.020	8	
1	.018	4	
1	.015	6	
Blend of Texas grapefruit and orange juices, sweetened			
25	.035	9	
Florida orange juice unsweetened			
6	.033	14	
1	.010	6	
13	.045	10	
17	.020		
Florida orange juice sweetened			
6	.027	10.5	
12	.035	11	
14	.008	10.5	
18	.037	8	
15	.015	6	
Texas orange juice unsweetened			
25	.055		
California orange juice unsweetened			
45	.015	12	
46	.018	8	
40	.005	10	
40	.005	10	
46	.012	8.5	.148
46	.010	9.5	.133
47	.005	10	.211
47	.015	11	
48	.015	11	
48	.010	13	
49	.008	12	.173
49	.008	13	.156
40	.003	10	.149
40	.012	11	.156
47	.010	11	
48	.008	13	.199
49	.003	12	
49	.010	10	
50	.008	17	.268
50	.005	18	

Methods of analysis:
Oil—*This Journal*, XXVII, 1, 201 (1944).
Suspended matter—As described in United States Standards for Grades of Canned Concentrated Orange Juice U.S.D.A. Bulletin (1943).
Insoluble solids—*Methods of Analysis, A.O.A.C.*, p. 336, Fifth Ed. (1940).

recorded in Tables 2, 3, and 4. Such apparent gains are not unusual (5) in an investigation of this kind when the overall losses in the ascorbic acid content are small and the fruit being processed during the sampling operation varies with respect to source (such as stored packing house culls, freshly picked orchard run fruit, etc.), size, maturity, irrigation practice, and perhaps in other ways which may affect the ascorbic acid content. It was not possible to maintain exact identity of the juice at different stages in the manufacturing process, even though the times of sampling were planned to conform as nearly as possible to the rate of flow of the juice being processed. However, it is not believed that the variations due to this cause materially affected the overall averages of ascorbic acid retention recorded in the summaries in Tables 2, 3, and 4. Those averages check corresponding data reported in similar investigations (3) (4). We were not in a position to make an intensive study of each factor affecting the ascorbic acid content of the fruit being processed, nor was it considered that an extensive statistical study of the data was justified.

The percentages of ascorbic acid retained on storage, recorded in Tables 1, 2, 3, and 4, indicate that each of the citrus juices investigated retain about the same percentages of ascorbic acid on storage.

Sixty-eight factory and warehouse samples in sanitary cans (30 orange juice; 32 grapefruit juice; 6 blends) were collected in 1942 and 1943 and examined for ascorbic acid, volatile oil, soluble solids (by hydrometer, by refractometer, and by drying), insoluble solids by drying, suspended matter by centrifuging, acidity, *p*H, consistency, visible defects, flavor, dimensions of can, vacuum in inches, and fill of container. Soluble solids by drying and insoluble solids were not determined on all samples. For brevity, we are recording in Table 5 only the results on oil, suspended matter, and insoluble solids. The other data can be consulted in our files.

The data in Table 5 are largely self-explanatory. It is not claimed that they necessarily reflect trade practice. The average percentages of oil are: grapefruit juice 0.010%; orange juice 0.016%; blends 0.023%. The Cali-

TABLE 6.—*Texas grapefruit juice, 39 samples, early 1942*

	OIL	SUSPENDED MATTER	ASCORBIC ACID
	Per cent	*Per cent*	*Mg per 100 ml*
Unsweetened, 36 samples (19 factories)			
Minimum	0.002	3	32.0
Maximum	0.029	10	45.2
Average	0.014	6	37.8
Sweetened, 3 samples (1 factory)			
Minimum	0.007	7	31.6
Maximum	0.026	10	37.2
Average	0.015	8	35.0

fornia orange juices averaged 0.009% oil, and the Florida orange juices 0.026%.

Also, in January and February, 1942, 39 samples (36 unsweetened; 3 sweetened) of production line and warehouse samples of Texas grapefruit juice in sanitary cans were collected. These were analyzed in February and March of that year for soluble solids by refractometer and hydrometer, acidity, suspended matter, ascorbic acid, volatile oil, and *p*H. For brevity, only a part of these data is summarized in Table 6.

SUMMARY

(1) The average amounts of ascorbic acid in screened citrus juices and the average percentages retained during manufacture in 529 samples from 41 factories (925 determinations) were:

Kind	*Average ascorbic acid screened juice (Mg per 100 ml)*	*Per cent ascorbic acid retained*
Grapefruit juice	41.1	97.7
Orange juice	50.6	98.3
Blends	45.0	97.6

(2) The average percentages of ascorbic acid retained during storage at room temperatures, in 51 samples of citrus juices, were:

	Per cent ascorbic acid retained		
Months stored	*Grapefruit juice*	*Orange juice*	*Blends*
3	94.5	92.5	93.3
6	85.4	85.3	86.1
9	78.7	80.1	77.9
12	73.1	75.0	72.2

REFERENCES

(1) *This Journal*, **29**, 69 (1946).
(2) Ross, Edward, *Food Research*, **9**, 27 (1944).
(3) Moore, E. L. *et al.*, *The Canner* (I) **98**, 24 (1944); (II) **100**, 55 (1945); (III) **100**, 12 (1945).
(4) Wagner, J. R. *et al.*, *Food Research*, **10**, 469 (1945).
(5) *This Journal*, **27**, 102, 539 (1944).
(6) Moore, E. L. *et al.*, *Fruit Products J.*, **23**, 270 (1944).

DETERMINATION OF PHENOTHIAZINE IN MEDICINAL PREPARATIONS

By VINCENT E. STEWART (Food and Drug Laboratory, State of Florida, Department of Agriculture, Tallahassee, Florida)

Methods for the determination of phenothiazine in medicinal preparations were reviewed, and published methods were investigated, by Stewart (1).

A collaborative study of the colorimetric method, by oxidation with bromine, was reported by Stewart (2), and two of the collaborators, McClosky and Smith (3) studied the use of colored filters in this determination. The method was adopted as a tentative procedure in the *Methods of Analysis* (4). Payfer and Marshall (5) reported a gravimetric method for the determination of phenothiazine in which the phenothiazine is precipitated as $Pt(C_{12}H_9NS)_2Cl_4$ by means of chloroplatinic acid.

The gravimetric method of Payfer and Marshall has been studied with the purpose of determining whether it is suitable as an adjunct to the present A.O.A.C. tentative method or whether it could be used to replace this method. The method was found to be satisfactory as described; however, it was found possible to so modify the method as to simplify the procedure and improve the accuracy, by the use of larger volumes.

The method described by Payfer and Marshall requires the weighed sample to be extracted in a Soxhlet extractor with alcohol (95 per cent). Alcohol is a relatively poor solvent for phenothiazine, whereas acetone is a good solvent. The National Formulary (6) states that one gram of phenothiazine dissolves in about 75 ml. of alcohol or in 5 ml. of acetone. The reaction of phenothiazine with chloroplatinic acid proceeds as well in acetone solution as it does in alcohol solution. When using acetone as the solvent for phenothiazine, solution can be effected merely by stirring the finely ground sample with the solvent, thus eliminating the Soxhlet extraction. It is possible to eliminate the Soxhlet extraction when using alcohol as the solvent, provided that the sample is thoroughly shaken with the alcohol, *e.g.*, about ½ hour in a mechanical shaker; and that the amount of phenothiazine is well below the maximum amount which can be dissolved by the alcohol. However, the ready solubility of phenothiazine in acetone obviates these difficulties.

Chloroplatinic acid solution can be prepared by the A.O.A.C. method which is used in preparing this reagent for the determination of potash by the Lindo-Gladding method. A solution containing the equivalent of 2.0 g platinum in 100 ml is satisfactory. Since an excess of hydrochloric acid in the reagent appears to have no effect on the determination, the hydrochloric acid need not be completely eliminated from the reagent.

Payfer and Marshall did not report any results obtained when the method was used with a pure grade of phenothiazine. In order to investi-

gate the reliability of the method the best grade of phenothiazine obtainable was repeatedly recrystallized from toluene, using activated charcoal, washed with petroleum ether, and dried in the oven at 100°C. The assay of this material was well within the limits of accuracy of the method.

METHOD

Powder the sample in a mortar if not already in the form of a powder. Weigh a portion of the sample equivalent to about 0.4 to 0.5 gram of phenothiazine in a 50 ml beaker. Stir the weighed portion thoroughly with acetone and pour the liquid through a retentive filter paper (*e.g.*, Whatman #5), collecting the filtrate in a 100 ml volumetric flask. Continue to wash the residue in the beaker, and the filter, with small portions of acetone until the volume of the filtrate is nearly 100 ml, then dilute to volume with acetone. Transfer, by means of a pipet, a 25 ml aliquot of the acetone solution to a 100 ml beaker and add 7.0 ml of the chloroplatinic acid solution (which contains the equivalent of 2.0 g Pt in 100 ml). Stir the soln thoroly, add 15 ml of water with stirring and allow to stand several minutes. Filter the green precipitate on a fritted glass crucible (Porosity F), or a Gooch crucible with a tightly packed asbestos mat. Wash the precipitate into the crucible with acetone and scrub the beaker with a rubber policeman. Wash the precipitate in the crucible with several small portions of water and finally with acetone. Dry the crucible to constant weight in the oven at 100°C.

$$\text{Wt. of precipitate} \times 0.5416 = \text{wt. of phenothiazine}$$

Each ml of the chloroplatinic acid solution is theoretically equivalent to about 41 mg of phenothiazine, but it is best to use a considerable excess of chloroplatinic acid solution in order to assure quantitative precipitation of phenothiazine. The filtrate should be yellowish-orange in color. The presence of a greenish coloration or a precipitate in the filtrate indicates that an insufficient amount of chloroplatinic acid, or of water, was added.

DISCUSSION

Several phenothiazine preparations, including both commercial preparations and mixtures of phenothiazine with various excipients prepared in the laboratory, were analyzed by the above method and by the colorimetric method described in *Methods of Analysis* (4). These samples were in most instances portions of samples which were analyzed by collaborators in the previous investigation of the colorimetric method (2). They included mixtures of phenothiazine with starch, lactose, and talc, two brands of commercial tablets, and two brands of commercial phenothiazine powder. The results obtained by the two methods agree very well. For example, a sample of medicinal grade phenothiazine, when analyzed by this method, gave the following assay: 99.8%, 99.9%, 99.9% (average 99.9%). The average result obtained by three different analysts, using the A.O.A.C. method, was 99.3%. A sample of commercial phenothiazine tablets, when analyzed by this method, gave the following assay: 73.5%, 73.6%, 73.1% (average, 73.4%). The average result obtained by three different analysts, using the A.O.A.C. method, was 73.6%.

The principle difficulties encountered with the gravimetric method

were: 1. The use of an insufficient excess of chloroplatinic acid. 2. The use of an insufficient amount of water in the reaction mixture. The precipitation is not quantitative unless the acetone is sufficiently diluted with water. The use of an insufficient amount of water results in the formation of additional precipitate in the filtrate. 3. The use of a filter lacking sufficient retentiveness. The main objection to the method is that it requires the use of chloroplatinic acid, an expensive reagent which must be recovered. This is not a serious objection since the platinum can be recovered from the precipitate by ignition. Excess platinum in the filtrate can be recovered by evaporation and ignition; however, because of the possibility of contamination from this source, the platinum should be purified by dissolving in aqua regia, precipitating ammonium chloroplatinate by the addition of ammonium chloride, and igniting the ammonium chloroplatinate.

The gravimetric method is a valuable adjunct to the colorimetric method described in *Methods of Analysis* (4). The choice of methods depends largely upon the individual preference of the analyst and the equipment which is available. In the event that interfering substances are encountered the analyst can check his results by the alternate method. Any highly colored substance may interfere to some extent with the colorimetric method, and substances which form insoluble complexes with chloroplatinic acid (*e.g.*, alkaloids) would be expected to interfere with the gravimetric method.

REFERENCES

(1) Stewart, V. E., *This Journal*, **27**, 343–6 (1944).
(2) ——, *Ibid.*, **28**, 693–6 (1945).
(3) McCloskey, W. T., and Smith, M. T., *Ibid.*, **28**, 696–9 (1945).
(4) *Methods of Analysis, A.O.A.C.*, 6th Ed., 1945, **39.175–7**.
(5) Payfer, R., and Marshall, C. V., *This Journal*, **28**, 429–30 (1945).
(6) *The National Formulary*, 8th Ed., 1946, page 388.

THE ANALYSIS OF A SUNBURN PREVENTIVE CREAM

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The Cosmetic Division of the U. S. Food and Drug Administration frequently has occasion to analyze various cosmetic creams. As sunburn preventive creams are becoming increasingly popular, it was decided to develop a system of analysis for one such cream to serve as a background for the investigation of other similar products.

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The formula of the cream chosen for this study is approximately that given in the "Draft of Proposed Quartermaster Corps Tentative Specification JCQD No. 1035, dated 29 June, 1945, for Cream, Sunburn Preventive," namely:

	% by weight
Glyceryl monostearate (self emulsifying)	13.0
Lanolin	4.7
Propylene glycol	4.7
Titanium dioxide	2.5
Iron oxides—q.s. color	
Methyl para hydroxybenzoate	0.15
Isobutyl para aminobenzoate	2.0
Isopropyl myristate-palmitate	23.4
Water—q.s. 100%	

To evaluate the analytical methods for the cream, it was first necessary to determine the composition of certain of the materials used in its preparation. This paper is therefore divided into two parts; one dealing with the analysis of these materials; and the other, with the analysis of the cream itself.

I. MATERIALS USED IN THE PREPARATION OF THE SUNBURN PREVENTIVE CREAM

1. Lanolin—U. S. P. Anhydrous Lanolin
2. Titanium dioxide—A technical product
3. Iron oxides—A technical product
4. Propylene glycol—A technical product was fractionated at atmospheric and reduced pressures. The collected fraction assayed 100% by the periodate method (1).
5. Methyl para hydroxybenzoate—A technical product melting at 126–127°C.
6. Isobutyl para aminobenzoate—A technical product melting at 63.2–63.8°C (literature (2) 65°C) gave no elevation of the melting point on two recrystallizations. This material was assayed and used without purification.

ANALYSIS

Wells (3) bromometric assay for aminobenzoic acid esters was found applicable to the raw material. Proceed as follows: Weigh about 0.15 gm of isobutyl para aminobenzoate on a watch glass and wash into a 500 ml iodine flask with 20 ml of (1+1) HCl. After solution of the material in the acid, dilute to approximately 200 ml with water, and add from a buret, with vigorous shaking, 0.1 *N* potassium bromate-bromide solution until a slight excess of bromine is evidenced by a light yellow color. Stopper the flask and allow to stand for five minutes. Add an excess of potassium iodide solution through the well of the flask, avoiding loss of bromine, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate using starch solution as an indicator. 1 ml 0.1 *N* potassium bromate-bromide =0.004831 gm of isobutyl para aminobenzoate. The average of three determinations on the product was 100 ±0.1%.

Identification.—The dibromo derivative prepared by Wells (3) is not very satisfactory because of its low melting point (74–75°C) and difficulty of recrystallization. The benzoyl derivative of isobutyl para aminobenzoate may be synthesized as follows:

Transfer 0.5 gm of isobutyl para aminobenzoate to a small glass stoppered flask and add 5 ml of water and 1 ml of benzoyl chloride. Then add 5 ml of 20% sodium hydroxide in small portions, shaking vigorously after each addition of alkali. Break up the coagulated precipitate with a glass rod and allow to stand for several minutes. Dilute the mixture with water, filter through a small Büchner funnel and wash precipitate thoroughly with water. Reprecipitate product from hot 75% alcohol and dry at 100°C. for 20 minutes. The compound crystallizes in fine white crystals. M.P. 127.8–128.2°C. (corr.). Theoretical nitrogen 4.71 per cent; found 4.65 per cent.

7. Isopropyl myristate-palmitate—The "Deltyl Extra" grade.

ANALYSIS

Saponify samples of about 1.8 gm by the procedure described under the analysis of the cream. Determine the unsaponifiable matter, the fatty acids, and the equivalent weight of the fatty acids. After the extraction of the fatty acids filter the remaining aqueous alcoholic solution into a distillation flask, distill, and collect the first 5–10 ml of liquid. Test the distillate for isopropyl alcohol by the method given in U.S.P. XII under "Spiritus Frumenti." The following results were obtained:

Unsaponifiable matter	0.16%
Fatty acids	84.5%
Equivalent weight of fatty acids	238
Test for isopropyl alcohol	positive

8. *Glyceryl monostearate (self emulsifying).*—The commercial self-emulsifying glyceryl monostearates are mixtures of a number of compounds. They contain free glycerine, mono-, di-, and tri-stearin as well as several per cent of the stearic acid soaps. The following analysis gives some of the constants of the material that was used.

ANALYSIS

Dissolve about 0.9 gm in 50 ml of chloroform and extract with three 20 ml portions of water acidified with a few drops of concentrated HCl. Reserve the chloroform solution. Combine the water extracts, wash with two 10 ml portions of chloroform and add washings to reserved chloroform solution. Filter the water extract, neutralize to methyl red, transfer to 100 ml volumetric flask, dilute to mark with water, and mix. Determine glycerine on a suitable aliquot by the periodate method (1). The result represents uncombined glycerine.

Filter the reserved chloroform solution through a cotton plug into a tared dish, evaporate the chloroform on the steam bath, dry in a 100°C. oven for 5–10 minutes, cool in a vacuum desiccator, and weigh as chloroform soluble material.

Dissolve the chloroform soluble material in benzene and saponify by the procedure described under the analysis of the cream. Determine the unsaponifiable matter, the fatty acids, the equivalent weight of the fatty acids, and the combined glycerine. The value for fatty acids represents both free and combined fatty acids. The following results were obtained:

	per cent
Uncombined glycerine	11.3
Chloroform soluble material	85.7
Unsaponifiable material	0.71
Fatty acids	76.0
(Equivalent weight of fatty acids	277)
Combined glycerine	12.7

II. ANALYSIS OF THE SUNBURN PREVENTIVE CREAM

A cream of the following composition was prepared and analyzed:

	% by weight
Glyceryl monostearate (self emulsifying)	14.04
Anhydrous lanolin	5.08
Propylene glycol	5.08
Titanium dioxide	2.70
Iron oxides	2.16
Methyl para hydroxybenzoate	0.16
Isobutyl para aminobenzoate	2.15
Isopropyl myristate-palmitate	25.22
Water	43.41
Total	100.00

METHODS OF ANALYSIS

Total ash.—Weigh about 0.6 gm. of the cream into a deep 75 ml porcelain crucible. Evaporate the volatile material under an infra red drying lamp, char and burn off most of the carbon with a Meker burner, ash in a muffle oven at 750°C., cool, and weigh as total ash. Reserve ash.

Titanium dioxide.—To the crucible containing the total ash add 4 gm of Na_2SO_4 and 8 ml of H_2SO_4, cover the crucible with a watch glass and heat on a hot plate until solution is complete. Cool and transfer to 100 ml volumetric flask containing 25 ml of cold water, cool, dilute to mark with water and mix. Determine the titanium colorimetrically (4) with H_2O_2 in a suitable aliquot.

In this particular study the following procedure was used: To a 50 ml ¾" diameter test tube were added: a 2 ml aliquot of the soln, 7 ml of solvent (20 ml H_2SO_4 and 10 gm Na_2SO_4 diluted to 250 ml with water), and 1 ml of 3% H_2O_2. After mixing the soln, its transmission was measured by means of an Aminco Type "F" Photometer with No. 42 filter, and compared with the transmission of standard solns of titanium prepared by treating titanium dioxide in the same manner as the ash of the cream.

Iron oxides.—An approximation of the iron oxides can be obtained by subtracting the titanium dioxide from the total ash. The result for iron oxides will be in error by the amount of ash that is not iron oxides or titanium dioxide. This, however, should not be greater than 0.1 or 0.2 per cent.

Propylene glycol and free glycerine.—Weigh about 1.8 gm of the cream into a 50 ml beaker, add 10 ml of a (1+1) mixture of absolute alcohol and chloroform; heat with stirring until mixture boils, and decant through a Gooch crucible using suction. Re-extract the residue in the beaker with a 10 ml and then a 5 ml portion of hot (1+1) absolute alcohol-chloroform solution, and finally with 15 ml of hot chloroform, decanting each time through the Gooch crucible. Discard the residue. Transfer the extract to a separatory funnel with the aid of 25 ml of chloroform, and extract with a 40 ml aliquot of water acidified with one or two drops of HCl. Continue the extraction with a 10 ml and then a 5 ml aliquot of water. (Reserve the chloroform solution for the next determination.) Neutralize the combined aqueous extracts to

methyl red making the final adjustment with 0.02 *N* NaOH, filter through a cotton plug into a 100 ml volumetric flask, dilute to mark with water and mix. Transfer a 45 ml* aliquot into a 100 ml volumetric flask, add 50 ml of 0.04 *N* KIO_4 (0.02 *M*), dilute to mark with water, mix, and allow to stand for one hour. Pipet 20 ml of the oxidized soln into a flask, dilute to 50 ml with water, add about 1 gm $NaHCO_3$ and 0.2 gm KI, and titrate the liberated iodine with standard 0.04 *N* arsenite soln using starch soln as an indicator. Standardize 10 ml of the 0.04 *N* KIO_4 with the standard 0.04 *N* arsenite soln by the same procedure. From the difference in the two titrations calculate the amount of periodate required to oxidize the 45 ml aliquot. Transfer another 45 ml aliquot into a glass stoppered flask, add the calculated amount of periodate plus 1–2 ml in excess, stopper the flask, allow to stand for one hour, add another drop of methyl red indicator and titrate to a clear yellow end point with 0.02 *N* NaOH.

1 ml 0.02 *N* NaOH = 1.84 mg glycerine
1 ml 0.04 *N* KIO_4 = 0.92 mg glycerine
1 ml 0.04 *N* KIO_4 = 1.52 mg propylene glycol.

Make the following calculations:

a. Estimate the amount of glycerine from the alkali titration.

b. Calculate the amount of periodate required to oxidize this amount of glycerine.

c. Subtract the periodate required to oxidize the glycerine from the total amount of periodate that was required to oxidize the 45 ml aliquot and compute the remainder as propylene glycol.

The determination of glycerine by the alkali titration is a modification of the method advocated by Shupe (1). Shupe recommends running a blank on the KIO_4 solution for any acidity that may be present. It was observed that the KIO_4 solution used was acid to methyl red, and that the addition of 0.02 *N* $NaOH_4$ caused a slow unsatisfactory end point change that never became a clear yellow. On the other hand, KIO_3 solution is acid to methyl red, but gives a sharp change to a clear yellow on the addition of 0.02 *N* NaOH. That the unsatisfactory end point with KIO_4 was probably due to the KIO_4 itself was demonstrated in the following manner: To a large excess of propylene glycol in aqueous solution were added a few drops of methyl red indicator and enough 0.02 *N* NaOH to make the solution a clear yellow. When a solution of KIO_4 was added, the resulting solution turned red, However, as the periodate became reduced to iodate by the excess propylene glycol, the red color faded and the solution reverted to a yellow color. In the determination of glycerine by the alkali titration, the use of only a slight excess of periodate thus serves a twofold purpose: it eliminates the periodate blank and improves the end point.

Isobutyl para aminobenzoate.—Pass the reserved chloroform soln through four separatory funnels containing, respectively, 30, 20, and 10 ml of (1+1) HCl and 10 ml of water. (Reserve chloroform soln for the next determination.) Combine the (1+1) HCl and water extracts, dilute to about 200 ml with water, and filter through a cotton plug into a 500 ml iodine flask. Add 10 ml of 0.1 *N* potassium bromate-bromide soln, stopper the flask, shake well, and allow to stand for several minutes.

* If 50 ml of periodate are insufficient to oxidize the 45 ml aliquot, a smaller aliquot should be used.

Drain excess potassium iodide soln into the flask and titrate the liberated iodine with 0.1 *N* sodium thiosulfate using starch soln as an indicator. One ml 0.1 *N* potassium bromate-bromide soln =0.004831 gm of isobutyl para aminobenzoate.

(The extraction of isobutyl para aminobenzoate from chloroform was tested by dissolving the material in chloroform and following the above procedure. Three samples varying in weight from 45 to 142 mg gave an average recovery of 100 ±0.2%. When the sample size was increased to 250 mg., troublesome emulsions were encountered.)

Anhydrous lanolin, glyceryl monostearate, methyl para hydroxybenzoate, and isopropyl myristate-palmitate.—Filter the reserved chloroform soln through a cotton plug into a tared dish, evaporate the chloroform on the steam bath, dry in a 100°C. oven for 5–10 minutes, cool in a vacuum desiccator, and weigh as anhydrous lanolin, glyceryl monostearate, methyl para hydroxybenzoate, and isopropyl myristate-palmitate. Reserve residues for the lanolin determination.

Anhydrous lanolin.—Dissolve the above residue in 50 ml of benzene, transfer to a flask, add 25 ml of absolute alcohol and 1 gm of KOH, and reflux for 2 hours. Transfer the saponified material to a separatory funnel, add 50 ml of hot water, shake well, and draw off the aqueous layer. Extract the aqueous soln with two more 20 ml portions of hot benzene. (Reserve the aqueous soln for the fatty acid determination.) Combine the benzene extracts, wash with several 10 ml portions of 30% alcohol, and add washings to reserved aqueous soln. Filter the washed benzene extract through a cotton plug into a tared dish, evaporate benzene on steam bath, dry in a 100°C. oven for 5–10 minutes, cool in a vacuum desiccator, and weigh. This weight multiplied by the factor 1.96 approximates the anhydrous lanolin. (On a number of samples of anhydrous lanolin, it was observed that the unsaponifiable portion was about 51% of the sample.) The result for lanolin will be high if any of the other materials contain unsaponifiable matter.

The weighed material can be checked qualitatively for lanolin by the Liebermann-Burchard color test. Dissolve a portion of the material in 10 ml of chloroform and add 4 ml of acetic anhydride and several drops of H_2SO_4. A green color is a positive test for lanolin.

Fatty acids.—Acidify the reserved aqueous soln with HCl and extract with three 20 ml portions of chloroform. (Reserve the acid aqueous soln for the combined glycerine determination.) Wash the combined chloroform extracts with several 10 ml portions of water and add washings to reserved aqueous soln. Filter the washed chloroform extract through a cotton plug into a tared beaker, evaporate chloroform on a steam bath, dry in 100°C. oven for 5–10 minutes, cool in vacuum desiccator and weigh as fatty acids. Reserve fatty acids.

Equivalent weight of fatty acids.—Dissolve the fatty acids in ethyl alcohol (neutralized to phenolphthalein with alkali), and titrate to the phenolphthalein end point with 0.1 *N* NaOH. Calculate the equivalent weight of the fatty acids.

Combined glycerine.—Filter the reserved aqueous soln through a cotton plug, evaporate to about 25 ml on the steam bath, carefully neutralize to methyl red indicator with alkali and analyze for glycerine by the periodate method (1).

Water.—Water can be most accurately estimated indirectly. Add the percentages of "Total ash," "Propylene glycol and free glycerine," "Isobutyl para aminobenzoate," and "Anhydrous lanolin, glyceryl monostearate, methyl para hydroxybenzoate, and isopropyl myristate-palmitate." Subtract this total from 100% and the remainder is the percentage of water.

(Water can be determined directly by xylene distillation. However, the propylene glycol will also distill with the water. The substitution of a lower boiling liquid such as heptane does not help. Accordingly the volume corresponding to the amount of the previously determined propylene glycol must be subtracted from the total volume. Experiment showed that the volumes of propylene glycol and water

are additive when mixed in the ratio of 1:11 by weight. The percentage recovery of water was about 96%.)

Qualitative test for methyl para hydroxybenzoate.—To about 2 gm of cream in a 100 ml beaker, add 3 ml of absolute alcohol and 2 drops of concentrated HCl, and heat to boiling on a hot plate. Remove beaker from the hot plate, cool, add 50 ml of ether with stirring, filter through Gooch crucible with suction, and transfer filtrate to separatory funnel with 25 ml of ether. Discard residue. Extract ether soln with four 25 ml portions of (1+1) HCl. Discard (1+1) HCl extract and wash ether soln with two 5 ml portions of water. Transfer ether soln to beaker, evaporate ether on steam bath, treat residue with 20 ml boiling methanol, stir vigorously, cool, and filter. Add 10 ml of water, and one drop of HCl to the methanol soln and extract with three 20 ml portions of petroleum ether. Discard the petroleum ether extracts. Evaporate methanol soln to a few ml on the steam bath, dilute with 20 ml boiling water, cool, transfer to separatory funnel, and extract with three 15 ml portions of ether. (This additional extraction removes any remaining HCl which would inhibit the development of color in subsequent steps.) Combine the ether extracts and wash with 5 ml portions of water until washings are neutral to litmus paper. Evaporate ether on steam oath, take up residue in 20 ml boiling water, transfer to 50 ml test tube, cool, add 2 ml of Millon's reagent* and immerse in a boiling water bath for 5 minutes. A rose color indicates the presence of methyl para hydroxybenzoate. (Isobutyl para amiaobenzoate also gives a positive test with Millon's reagent and must be completely removed by the HCl extraction.) Efforts to modify the above procedure to obtain quantitative recoveries were unsuccessful. A recovery of 50% was the best that could be obtained.

The method separating fatty materials from methyl para hydroxybenzoate by the use of petroleum ether and about 60% methanol should be generally applicable to a number of cosmetic preparations.

RESULTS OF ANALYSIS

	Found per cent	*Theoretical per cent*
Ash 750°C.	4.98	
Titanium dioxide	2.71	2.70
Iron oxides (by difference)	2.27	2.16
Free glycerine	1.54	1.59
Propylene glycol	5.17	5.08
Isobutyl para aminobenzoate	2.17	2.15
Anhydrous lanolin Glyceryl monostearate Isopropyl myristate-palmitate Methyl para hydroxybenzoate	42.0	42.91
Unsaponifiable matter	2.71	
Anhydrous lanolin	5.31	5.08
Fatty acids	34.2	34.7
(Equivalent weight of fatty acids	252.3)	
Combined glycerine	1.74	1.78
Water (by difference)	44.14	43.41
Methyl para hydroxybenzoate	present	0.16

* Millon's reagent: One part by weight of mercury is dissolved in twice its weight of concentrated nitric acid with gentle warming. The resulting solution is diluted with twice its volume of water.

In computing the theoretical value for the total percentage of anhydrous lanolin, glyceryl monostearate, isopropyl myristate-palmitate, and methyl para hydroxybenzoate, the percentage of uncombined glycerine in the glyceryl monostearate (1.59%) was not included.

The theoretical value for the fatty acids was estimated from the analysis of the glyceryl monostearate and the isopropyl myristate-palmitate ingredients, and from the fact that previous work had indicated that lanolin contains approximately 51 per cent of fatty acids. The contribution of the hydroxybenzoic acid from its methyl ester was also included in the estimate.

Conclusion.—A detailed analysis has been developed for a "Sunburn Preventive Cream." It is believed that this analysis will serve as a general outline for development of procedures for analysis of similar products.

REFERENCES CITED

(1) SHUPE, I. S., *This Journal*, **26**, 249 (1943).
(2) GRÜTTEFIEN, W., U.S. Pat. 958,110, May 17, 1910.
(3) WELLS, E. H., *This Journal*, **25**, 537 (1942).
(4) KOLTHOFF, I. M., and SANDELL, E. B., "Textbook of Quantitative Inorganic Analysis," Revised Edition (1943), page 742.

NOTE

A Note on the Adulteration of Shredded Coconut with Papaya Stem Tissue

By WILLIAM V. EISENBERG (Washington, D. C.) and ROBERT E. O'NEILL (Atlanta, Georgia), U. S. Food and Drug Administration

Our attention was recently called to the adulteration of desiccated shredded coconut with papaya stalk tissues (*Carica papaya* L.). The sample of imported shredded coconut that was subjected to routine microscopic examination showed the presence of tissues that were foreign to the cellular content of shredded coconut. The tissues in question consisted of thin-walled rounded parenchymal cells containing occasional rosette crystalline aggregates of calcium oxalate and xylem reticulate vessels (Figs. 1 and 2). The calcium oxalate rosettes were usually 45 to 65 microns in diameter and the vessels were up to 300 microns for the larger forms.

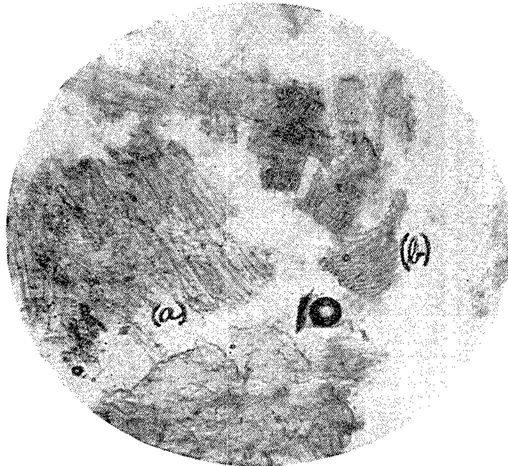

FIG. 1.—Shredded coconut adulterated with papaya stem tissues: (a) coconut endosperm cells; (b) reticulate vessel from papaya stem xylem. ×58.

None of these elements are found in shredded coconut, which consists almost wholly of the isodiametric and radially elongated cells of the endosperm, with oily contents. Fragments of the spermoderm or outer brown skin of the coconut meat, consisting of large elongated cells with porous walls in the outer layer and small nonporous cells in the inner layers, are infrequently observed. The presence of the vascular vessel elements, and cells with calcium oxalate rosettes, could therefore be attributed only to the addition of some adulterant.

Microscopic comparison of the questionable tissue with the fleshy xylem and pith tissues from the stem of *Carica papaya* showed the two to be identical (Fig. 3). The particles of questionable material were fleshy and white, and to the unaided eye seemed quite similar to the fragments of shredded coconut. However, under the Greenough type microscope at a magnification of about 30×, the questionable particles exhibited a yellowish cast due to the vascular elements which contrasted with the

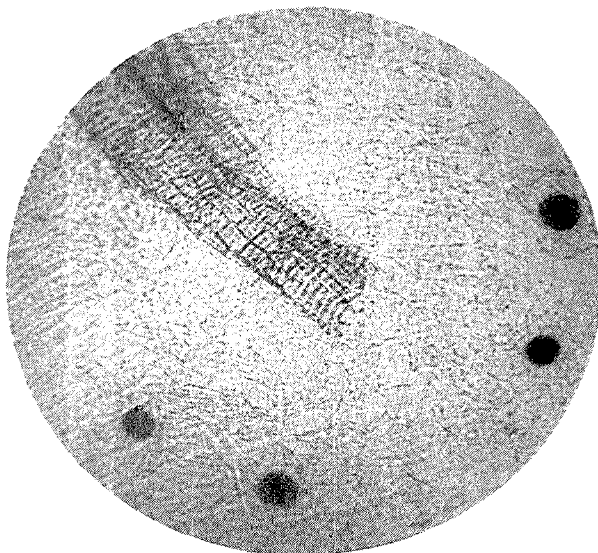

FIG. 2.—Papaya stem tissue isolated from sample of adulterated shredded coconut: Reticulate vessels, xylem parenchyma, and crystal cells each containing a rosette of calcium oxalate are shown. ×75.

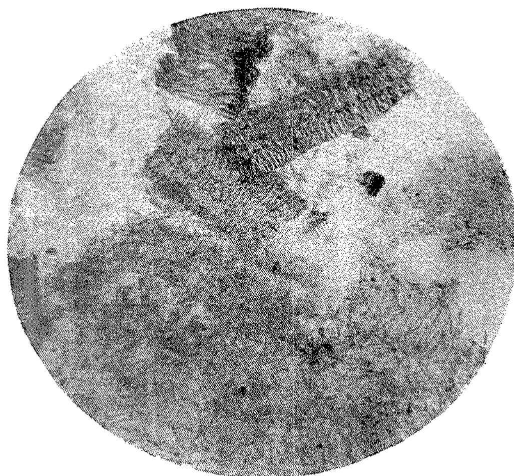

FIG. 3.—Papaya stem xylem tissues shredded from authentic material: Reticulate vessels and xylem parenchyma are shown. ×58.

snow white appearance of the coconut. By immersing the material in 60 per cent alcohol in a flat dish and searching under the Greenough microscope, the questionable pieces can easily be removed for further examination with the compound microscope.

The presence of crystal cells containing rosettes of calcium oxalate, and of reticulate vessel elements, serves to distinguish readily coconut endosperm tissue from papaya stem tissue. Crystal cells are characteristic of the papaya plant generally, being also found in the fruit tissues. In contrast, the coconut is devoid of calcium oxalate rosettes in any part of the fruit, or of the developing embryo. Vessel elements are found in parts of the coconut fruit other than the fleshy endosperm. However, those from the developing cotyledon in sprouted coconuts are of the spiral type, while the straw-like fibrous mesocarp of the coconut fruit which contains reticulated vessels is of such texture and appearance that it cannot be confused with the fleshy white endosperm.

BOOK REVIEWS

The Control of Insects in Flour Mills. By J. A. Freeman and E. E. Turtle. Published by H. M. Stationery Office, London, 1947, 76 pages, index, full page plates and text figures. Price $2.35.

The bulletin covers material similar to that in many publications in this country but has several unique features that make it a useful addition to its field, and perhaps of special interest in cases where control measures must be undertaken on a small scale by relatively inexperienced operators. Attention is given to details of sanitation, often overlooked by entomologists but which are finding increased attention from regulatory officials, such as bag cleaning, mill hygiene, modern self cleaning design, cleaning and sweeping, elimination of unnecessary accumulations of grain residues and trash. The section on "life histories and habits" includes an introduction of general entomological information, and material on the parasitic wasps.

Aside from being written on a public appeal level, and the sanitation and grain pest parasite sections, there is little to recommend this pamphlet at its price over the standard works already available more cheaply in this country.

Kenton L. Harris

Two Blades of Grass—A History of Scientific Developments in the U. S. Department of Agriculture. By T. Swann Harding. University of Oklahoma Press, Norman, Okla. (1947) 6×8½", 352 pp. 24 illus. Price $3.50.

This is a history in the best and most modern sense of the term. When the author, in his introduction, calls it a popular presentation, he does not mean that it is highly dramatized or over-simplified, after the manner of those books which so nauseate the scientifically trained reader; it is nothing of the sort.

The author has had a practically life-long connection with the Department of Agriculture, first as a scientist in his own right in the old Bureau of Chemistry and later in the Bureau of Animal Industry, second as editor of scientific publications, and finally as editor of the departmental house organ, "U.S.D.A.," with which many of our readers are familiar. In the latter capacity he has actually visited virtually all of those departmental agencies, both in Washington and in the field, about which he writes; he has discussed, at length and with real understanding and sympathy, the various projects with those who were actually doing the work; he has delved deeply into the historical background of the Department from the feeble beginnings in the Patent Office in 1836; he has read the important scientific papers and

bureau reports of the earlier years; he is thoroughly familiar with the present complex organization of the Department, with its gradual evolution, and with the way its work has been, and is, interwoven with that of other governmental agencies, State and Federal. It is no wonder, therefore, that he has produced a book which everyone with either a professional or a cultural interest in Agriculture will wish to own. With his easy, informal style and his gift of selection, condensation, and synthesis, he has managed to pack more useful, yes, and inspiring, information into one volume, than in this reviewer's opinion, can be found between the covers of any other single book on the subject. It is a pleasure to call attention to the index, which was prepared by an Agricultural indexer who knew her business. One can locate in it, at a glance, all of the important names and projects down through the years. Such an index, to this reviewer, adds enormously to the value of a book which is literally crammed with useful information, and with references to important source material not readily accessible even to the specialist seeking information outside of his own familiar field.

After five brief preliminary chapters which deal with early origins and high spots, and which give vignettes of some of the earlier scientists with and for whom the author worked, the succeeding chapters deal with cooperative relations with the State Experiment Stations, with animal industry, human nutrition and homemaking, soils and their conservation, the dairy industry, agricultural engineering, weather, roads, wild life, food and drug administration, and the scientific publications of the Department.

In each chapter the author weaves together a number of threads so successfully that, in the compass of from a dozen to 3 dozen pages, he has managed to produce a firm tapestry. We have a brief account of the important researches, a vivid vignette of the worker himself; a resume of the social and monetary implications of the work, and of its interconnection with other studies in or out of agriculture; and numerous interesting sidelights which make for unity and perspective.

Let the scientific reader not be misled by these novel-like chapter titles: these are merely "come-ons" to the non-scientific reader!

The faults of the book are decidedly minor and might not even strike another reviewer as faults. The illustrations are excellent and well-chosen, but they could have been placed in closer relationship to the text; and there are many places where the lowly comma might have been used to advantage in the interest of smooth and rapid reading.

The book has a brief but warmly commendatory foreword by Secretary Clinton P. Anderson. Its dedication by the author is worthy of quotation:

"Dedicated to those sincere, kindly, self-effacing, unavaricious humanitarians of highest integrity, the scientists who made the Department of Agriculture great as a research institution, and to my colleagues in information work in the various agencies of the Department, who so cheerfully and generously helped me to gather together the material comprising this book."

W. B. White

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