AN INVESTIGATION OF RODENT AND INSECT CONTAMINATION OF CORN AND CORN MEAL

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I. INTRODUCTION

A. BACKGROUND AND OBJECTIVES OF THE PROGRAM

The problem of insect and rodent infestation of corn and wheat as a source of contamination of corn meal and flour has been recognized for several years. A report on a survey of wheat and flour has already been published.[1] As a result of a series of conferences between representatives of the milling industry and of government agencies on problems of insect and rodent contamination of flour and corn meal, the Food and Drug Administration prepared a plan for a survey of corn and corn meal. After discussion and review of the plan by representatives of industry and of the Grain Branch, and Bureau of Entomology and Plant Quarantine of the U. S. Department of Agriculture, the plan was issued on June 15, 1950. The primary objectives of this program were:

(1) To determine the relationship between insect infestation and rodent contamination of corn purchased for milling and the insect, rodent hair, and rodent pellet fragment count in the corn meal made from it.
(2) To evaluate the effectiveness of grain cleaning equipment and procedures in dealing with infestation and contamination.
(3) To determine over a twelve-month period the incidence and degree of insect infestation and rodent contamination in corn being milled and corn sold in grain markets in dry milling areas.

It was also anticipated that the program would provide information as to the accuracy of the methods available at the time the survey was begun for estimating the insect infestation in corn and for predicting the fragment count in the finished meal.[2,3] It was hoped that the use of these methods on the scale called for in the program would result in reducing the time required for examination, and might result in adaptations that would permit the use of the methods by non-technical personnel without extensive laboratory facilities.

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[1] *This Journal*, 36, 115 (1952).
[2] This report covers corn sampled from June 1950–June 1951.
[3] Cracking-flotation and fuchsin stain procedures described in the June 15, 1950 program. These methods are also described in this report.

B. Production and Utilization of Wheat and Corn

Data on production and utilization of corn were taken into consideration in planning the survey. Such data for 1950 are given below:[4]

Corn (1950): Total disappearance	3,164,288,000 bu.	
Dry process products	96,156,000 bu.	3.0%
Breakfast foods	11,000,000 bu.	0.3%
Custom ground, farm household use	15,156,000 bu.	0.5%
Corn meal, grits, flour, products for fermented malt liquors	70,000,000 bu.	2.2%
Wet process	133,191,000 bu.	4.2%
Alcohol, distilled spirits	45,191,000 bu.	1.4%
Seed	11,232,000 bu.	0.3%
Feed, other uses, and waste	2,771,287,000 bu.	87.6%
Exports[5]	107,231,000 bu.	3.4%
Corn (1950 crop): Production	3,057,803,000 bu.	
Used on farms, where grown	2,316,182,000 bu.	75.7%
Sold off the farms	741,621,000 bu.	24.3%

For comparison, the following data on wheat utilization are of interest:[4]

Wheat (1951): Total disappearance	1,158,249,000 bu.	
Domestic food use	479,832,000 bu.	41.4%
Military procurement	14,151,000 bu.	1.2%
Seed, industrial feed	191,603,000 bu.	16.5%
Exports (and shipments to territories)	472,663,000 bu.	40.8%

Of the 741,621,000 bushels of corn sold off the farms, 229,347,000 bushels, or 31 per cent was used for the production of breakfast foods, corn meal, grits, corn flour, wet process, and products for fermented malt liquors. If the exported grain is included here as food grain, the percentage becomes 51.1. Most of the total wheat supply is destined for food use.

The carry-over of old corn as of October 1, 1951, was 739,236,000 bushels. The corn harvested in the fall of 1950 amounted to 3,057,803,000 bushels.

In 1946, the last year for which complete figures are available, only 12 per cent of all corn produced was white corn. Since that time, the per cent of white has probably increased a little.[6] Of the corn graded by licensed inspectors in 1950, only 3.4 per cent[7] was white. The meal, grits, flakes, and hominy industries are the principal consumers of marketed white corn for which at present the demand exceeds the supply. This results, in some areas at least, in a price differential in favor of white corn as compared with the corresponding grade of yellow corn. The American Corn Millers' Federation has estimated that 80 per cent of the corn meal production is from white corn.[8] On the other hand yellow corn is also used

[4] Agricultural Statistics, 1952.
[5] Most of this was probably used for foreign relief and military food production.
[6] U.S.D.A. Bu. Agric. Econ. Bull. dated Dec. 26, 1946, and supplementary information supplied by that Bureau.
[7] Private communication from Grain Branch, P.M.A., U.S.D.A.
[8] Communication of Nov. 12, 1951.

in large quantities by manufacturers of starch, sirup, corn sugar, and related products (wet millers), and by distillers.

Many dry mills purchase corn on the basis of U.S.D.A. grade and moisture content. Corn of less than 17 per cent moisture content is preferred, which tends to limit milling corn largely to grades 1, 2, and 3, although lower grades may be bought if the down grading resulted from some factor other than moisture content, or if the mill has kiln drying equipment. Locally grown corn frequently goes direct from farm to mill, especially during the period immediately following the harvest. Such corn will not be officially graded, and of course does not move through the large grain markets. It may be shelled on the farm or may be delivered to the mill on the ear. Corn purchased by grade in the large grain markets is always shelled.

C. Summary of the Sample Collections and Analyses

1. *Mill phase.*—This phase was designed to provide the information called for by objectives 1 and 2 listed on page 5. Eight cooperating mills were visited twice a month during the months of May, June, July, August, September, and October, and once a month for the remainder of the twelve-month period. Mills were chosen with due regard to types of cleaning and milling, and size of mill. During each visit the sanitary condition of the mill was checked and samples were collected from:

a. Uncleaned corn
b. Same corn after cleaning
c. Corn meal and other edible products made from this corn

Each sample of corn was examined visually for rodent excreta pellets, insects, and insect-damaged kernels, and was examined by laboratory techniques for surface insects and for internal insect infestation. Each sample of corn meal and of other edible products was examined for rodent hairs, rodent pellet fragments, and insect fragments.

2. *Corn sampling phase.*—Corn was sampled both at mills and at terminal markets. Insofar as the mills selected for sampling were representative of the industry, the corn samples collected at mills show the type of corn actually being ground into corn meal. Since the amount of corn used for corn meal represents only about 2 per cent of the total corn consumption, samples were taken from carloads of corn in terminal markets to provide information on the extent of contamination in all of the market corn available to corn millers.

a. At mills.—The schedule called for collection of samples at monthly intervals from carloads of corn arriving at each of approximately 50 designated mills. If carload lots were not available for sampling, the same was taken from the storage or cleaning bin containing corn currently in use, or from the stream of uncleaned corn as it flowed to the cleaning equipment. These samples of corn were examined as under *Mill phase.*

b. In terminal markets.—Carloads of corn on railroad inspection tracks in terminal markets were sampled in accordance with the following schedule:

Terminal market	*No. of carloads to be sampled each month*
Chicago	20
Kansas City	5
Omaha	6
St. Louis	5
Peoria	6
Decatur (Ill.)	3
Indianapolis	5
Louisville	2
Birmingham	2
Total	54 (648 for 12 months)

These samples of corn also were examined as under *Mill phase.*

The mill phase resulted in the actual collection of 123 samples; corn sampling at the mills, 440 samples; and corn sampling at the terminal markets, 586 samples.

The inspection and analytical results were coded and punched on I.B.M. cards to permit machine sorting, tabulation, and partial calculation of the data. The use of punch card tabulation not only permitted the rapid and accurate handling of the data, but provided a flexible tool by which the information was re-arranged and compared.

The information called for by the report forms used in the survey is listed below:

By inspectors

Sample number
Phase of program:
  Mill
  Corn at mills
  Corn at terminals
Name of mill or R.R. terminal
Date of collection
Sample taken from:
  Car, barge, truck
  Bin
  Stream
Size of lot (in bushels)
Type of corn:
  Yellow
  White
  Mixed
State of origin (i.e., where grown, if available; otherwise, from which shipped) (States coded—i.e., Kan: 48; Neb: 47)
Type of last storage (i.e., farm, country elevator, terminal elevator, or direct from harvest)
On the ear or shelled (as received)
U.S.D.A. Grade

Weevily notation on U.S.D.A. Grade?
If corn was sample grade, was it so graded because of rodent pellets therein?
Date of last general fumigation (mill phase only)
Live insect infestation in lot?
Evidence of rodents in the lot?
Was lot reasonably homogeneous? (non-homogeneous to be eliminated)
Uncleaned corn—pellets per peck:
  Rat
  Mouse
Cleaned corn—pellets per peck:
  Rat
  Mouse
Type of edible products (i.e., whole meal, degerminated meal, grits, corn flour, etc.)

By analysts

Weight rodent pellets per peck uncleaned corn
Weight rodent pellets per peck cleaned corn
Visual examination of 200 grams uncleaned corn:
  Total number of kernels
  Number kernels showing insect tunneling
  Number kernels showing insect feeding punctures
  Number of kernels showing other insect damage
Visual examination of 200 grams cleaned corn:
  Total number of kernels
  Number kernels showing insect tunneling
  Number kernels showing insect feeding punctures
  Number kernels showing other insect damage
Number of storage insects recovered by sieving 1000 grams uncleaned corn
Number of storage insects recovered by sieving 1000 grams cleaned corn
Examination of 200 kernels by stain test—uncleaned corn:
  Number of kernels with weevil egg punctures
  Number of weevil egg punctures
  Number of kernels with weevil feeding punctures
  Number of weevil feeding punctures
  Number of kernels with insect cutting or tunneling
Examination of 200 kernels by stain test—cleaned corn:
  Number of kernels with weevil egg punctures
  Number of weevil egg punctures
  Number of kernels with weevil feeding punctures
  Number of weevil feeding punctures
  Number of kernels with insect cutting or tunneling
Examination of 100 grams by cracking-flotation test—uncleaned corn:
  Weevil and/or grain borer
  Flat grain beetle
  Grain moth
  Other
Examination of 100 grams by cracking-flotation test—cleaned corn:
  Weevil and/or grain borer
  Flat grain beetle
  Grain moth
  Other

Examination each type finished product:
(a) Number rodent pellet fragments
(b) Number rodent hairs
(c) Number whole insects or equivalent
(d) Number insect fragments

Samples were taken by Food and Drug Administration inspectors. Analyses were made in the Food and Drug Administration District laboratories. The Division of Microbiology in Washington served as the control laboratory and coordinated the program.

II. SAMPLING PROCEDURES AND ANALYTICAL METHODS

The instructions for sampling and analysis were essentially as follows:

A. Mill Survey

1. *Sampling*

a. Uncleaned corn—(1) *Samples for the laboratory.*—Sample falling grain dropping onto or off a conveyor just preceding the first cleaning unit. (Do not sample grain actually on a belt or conveyor of any type.) Take approximately one pint (fluid) of grain about every 10 minutes over a 25–30 minute period (4 samplings) to yield at least 2 quarts of grain. Take a full cross-section of the stream each time and repeat until 16 fluid ounces are obtained for each portion.

(2) *Sample to be examined by the inspector.*—In addition to the sample which will be submitted to the laboratory, examine 1 peck for rat or mouse pellets. Take the 1-peck sample from the stream over a 25–30 minute period (or longer if necessary), by taking four 2 quart portions over the same time interval used for collecting the 16 fluid ounce portions for the laboratory. Examine the 1-peck sample for rat and mouse pellets by spreading small portions at a time on a clean white paper; or, if preferred, in order to facilitate separation and counting of the mouse pellets, the sample may first be spread, a small amount at a time, on a No. 6 sieve. The mouse pellets will pass through the sieve, and the number in the "throughs" can be counted. The corn may then be spread on a paper and examined for rat pellets. Place the pellets in a vial labeled with the sample number and the designation "pellets from 1 peck sample." The weight of the pellets will be determined in the laboratory. Report the number of rat and mouse pellets obtained by the inspector.

b. Cleaned corn just prior to grinding.—Obtain sample from the same lot of corn sampled under "*a*," above, even though a tempering process may intervene. Sample the cleaned corn before it receives any break whatsoever, and before it is degerminated, if the mill has a degerminator ahead of the first rolls.

Repeat the procedure outlined above for uncleaned corn. Take 16 fluid ounce portions at ten minute intervals over a thirty minute period to obtain a 2 quart sample for the laboratory, and a 1 peck sample, as above, to be examined by the inspector on the spot for rat or mouse pellets.

c. Finished meal or other products.—Take a separate subsample from each of the streams of finished product for human consumption, including grits and/or corn floor if these products are made from the corn sampled under "*a*" and "*b*," above. Take an 8 fluid ounce portion from each finished stream, keeping each stream sample separate, at approximately ten minute intervals until 32 ounces (1 quart) has been collected. Determine the percentage of the total edible production represented by each finished product.

(Do not sample when the mill is changing from one lot of corn to another.

If the mill has been shut down, allow it to operate at least two hours before sampling finished products, and take this into account before beginning to sample the corn.)

Fumigate all samples, corn and edible products, by adding 10 ml of chloroform or carbon tetrachloride to a piece of absorbent cotton or blotter paper at the top of the container and immediately seal the container. Allow to stand upright so that the gaseous fumigant will penetrate downward. Tape the container cover on with Scotch tape and then either wrap in aluminum foil or store containers in metal container with tight fitting lid for twenty-four hours.

2. *Analytical*

Weigh the pellets submitted in vials and report to the nearest 0.01 g. Examine the uncleaned corn and the clean corn by the same procedures. Before undertaking any of the examinations called for below, render the entire sample homogeneous by use of Jones Sampler.

1. *Separation of aliquots of corn for examinations.*—Using the Jones Sampler, separate portions for the various examinations as follows: (a) Separate a 1000 gram portion for examination by sieving.

(b) After sieving the 1000 gram portion, separate *from it* a 100 gram portion for the cracking-flotation procedure.

(c) Recombine the remaining 900 grams of sieved corn with the original sample, and mix by several passes through the Jones Sampler.

(d) Starting with the recombined sample, separate a 200 gram portion for visual examination.

(e) Again recombine entire remainder, divide in half by passing through the sampler, and further reduce *each half*, by successive passes, to obtain two 100–150 kernel portions. Further reduce each of the two 100–150 kernel portions to exactly 100 kernels by quartering and adjusting further with a spatula, making every effort to eliminate any selection of kernels.

2. *Laboratory examinations of corn.*—(a) *Visual examination for insect damage.*—Using the 200 gram portion from (d) above, count the number of kernels in the portion. Spread the sample on a paper and examine each kernel with the naked eye. Report the number of grains showing (1) insect tunneling, (2) insect feeding punctures, (3) other evidence of insect damage, and report the total number of damaged kernels, counting each kernel only once. Calculate per cent of damage by count.[9]

(b) *Examination by sieving for surface insects.*—Use the 1000 gram portion. Transfer a small amount at a time to a U.S. No. 8 standard sieve, shaking and brushing with a stiff-bristled brush so that surface insects and larvae will be worked through the sieve as nearly quantitatively as possible. Receive the "throughs" on a clean sheet of white paper, and examine at 5–15× magnification for whole insects (adults, pupae, larvae) and cast skins, and report by type of insect.

(A part of the screened corn is used for the cracking and flotation procedure below.)

(c) *Cracking and flotation procedure.*—Using the 100 gram portion from the screened corn, grind it in a burr or Wiley mill, set so as to reduce the ground particles to not more than 2 mm maximum dimension while at the same time making a minimum of finely ground meal. Transfer the cracked corn to a 400 ml hooked-lip beaker and brush into the beaker any residue that may remain in the mill.

Add chloroform to within one-half inch of top, mix thoroughly, and allow to settle for at least ten minutes. Transfer the mixture to a filter paper in a Buchner funnel. Rinse the beaker with about 100 ml of carbon tetrachloride and add the

[9] In handling the data, the item "other insect damage," was found to be too variable and was not included in the calculated percentages.

carbon tetrachloride to the material in the funnel. Wash the material in the funnel with another 100 ml of carbon tetrachloride. Draw air through the material until the liquid has evaporated, then dry until no odor of chloroform or carbon tetrachloride remains. (The purpose of the procedure up to this point is to extract fat from the solid material.)

Transfer the residue to a 2-liter Wildman trap flask, add about 600–700 ml of 60% alcohol, and mix thoroughly. Wash down the sides of the flask with the alcohol and soak thirty minutes. Add approximately 35–50 ml of gasoline, mix thoroughly, and allow to stand five minutes. Fill with the alcohol, allow to stand thirty minutes, and trap off into a beaker. Rinse the neck of the flask with alcohol, then with water, catching rinsings in the same beaker. Add about 20–35 ml gasoline to the trap flask, mix down into the liquid, add sufficient 60% alcohol to bring the floating layer up into the neck of the flask, and after about thirty minutes again trap off into the same beaker. Rinse neck of flask as before. Filter through a 10XX bolting cloth and examine under the low-power microscope. Count only whole insects, heads, head capsules, pupae, larvae, and cast skins, but report by type of insect.[10]

(d) *Staining procedure.*—Using the two 100–150 gram portions, separated in the manner illustrated above, further reduce each portion to approximately 100 kernels by quartering and by the use of a spatula. Then adjust to exactly 100 kernels with the spatula, avoiding any tendency toward personal selection of the kernels. Examine each 100 kernel portion separately according to the comparable procedure for wheat given in *This Journal,* **35,** 121 (1952).

(e) *Whole, bolted or degerminated corn meal, or corn grits.*—Examine each subdivision separately. Examine for rodent pellet fragments, whole insects, insect fragments, and rodent hairs, as follows:

1. *Rodent pellet fragments.*—Weigh a 50 gram portion of the well-mixed sample into a 250 ml hooked lip beaker. Add chloroform to within ½ inch of top, mix thoroughly, and allow to settle at least thiry minutes. Several times during this period, stir layer that rises to top. Decant chloroform and floating corn tissue onto a filter paper in a Buchner funnel, taking care not to disturb the heavy residue in bottom of beaker. Before decanting, take care that floating layer has not become so compact as to render this operation difficult. Add a quantity of carbon tetrachloride equal to the quantity of chloroform and corn tissue left in beaker, allow to settle again, and decant as before. With a mixture of equal parts of chloroform and carbon tetrachloride, repeat this process until very little corn tissue remains in the beaker. The material in the Buchner funnel is used for Part 2 below. Be careful not to decant any rodent pellet fragments that may be present. Wash the residue in the beaker onto a 7-cm ruled filter paper, using a stream of chloroform or carbon tetrachloride, and examine microscopically at 30×. Count the rodent excreta fragments identified by the presence of rodent hairs. Do not count particles resembling excreta fragments unless hair is attached.

2. *Insect fragments and rodent hairs.*—Draw air through the material in the Buchner funnel until the liquid has evaporated. Air dry until no odor of chloroform remains and transfer the residue to a 1-liter Wildman trap flask. Add 100 ml of 60% alcohol and mix thoroughly. Wash down the sides of the flask with the alcohol until ca. 300 ml is added, and soak thirty minutes. Add about 35–50 ml of gasoline, mix thoroughly, and allow to stand five minutes. Fill with the alcohol, allow to stand thirty minutes and trap off into a beaker. Rinse the neck of the flask with alcohol, then with water, collecting the rinsings in same beaker. Add ca. 10 ml gasoline to the trap flask, mix down into the liquid, add sufficient 60% alcohol to bring the

[10] In this report, "insects" by the cracking-flotation test refers to whole insects or equivalents of whole insects, such as heads, head capsules, pupae, larvae, and cast skins.

floating gasoline layer up into the neck of the flask and, after ca thirty minutes, again trap off into the same beaker. Rinse neck of the flask as before. Filter, and examine microscopically at 30×. Count the insects, insect fragments, and rodent hairs. Identify insofar as possible the type of insect which contributed the fragments.

(f) *"Cream" meals, corn flour, and similar products consisting largely of finely ground material.*

1. *Rodent pellet fragments.*—Same as above for whole corn meal, etc.

2. *Insect fragments and rodent hairs.*—Proceed as directed above for whole corn meal, etc., except substitute saturated NaCl solution for 60% alcohol.

3. *Duplicate Samples*

As a check on the adequacy and reproducibility of the corn sampling technique, duplicate samples of uncleaned corn were taken in five of the mills involved in the mill survey portion of the program. These duplicate samples were taken, along with the regular mill survey samples, as additional peck and 2 quart aliquots. The results on these duplicate portions are shown in Table 1.

While the number of sets of duplicates is very small, these results give a measure of confidence in the sampling procedure.

TABLE 1.—*Results of analyses of duplicate corn samples taken simultaneously. Two different sampling periods two weeks apart from each of two mills, and one period from each of three mills*

MILL	SAMPLE	PORTION	ANALYTICAL FINDINGS ON UNCLEANED CORN						
			PELLETS PER PECK			KERNELS WITH INSECT TUNNELS	SIFTING PER 1000 G TOTAL INSECTS	TOTAL BY STAIN TEST	50 G CRACKING FLOTATION
			NO. RAT	NO. MOUSE	GRAMS, RAT AND MOUSE				
126	1	A	0	1	.06	21	2	11	20
		B	0	3	.21	15	1	11	16
	2	A	0	0	0	24	1	11	18
		B	1	0	.14	21	0	17	20
127	1	A	2	96	.79	0	1	0	0
		B	0	103	.82	0	0	0	0
	2	A	5	85	.98	0	1	4	2
		B	—[a]	—[a]	.53	2	1	0	2
417	1	A	0	2	.03	0	0	0	0
		B	0	0	0	0	0	0	0
813	1	A	10	75	1.06	0	0	0	0
		B	7	73	.95	0	0	1	0
129	1	A	4	14	.34	0	2	1	2
		B	1	19	.19	0	1	0	0

[a] No count was made.

B. Corn at Mills and Terminal Markets

In addition to the samples obtained at mills in connection with the mill program outlined above, collect samples of corn at monthly intervals at another group of mills, and from cars on the inspection tracks in some of the large grain markets.

1. *Sampling at mills.*—If the mill is receiving shelled corn in carload lots, and the corn is regarded by the mill as suitable and destined for manufacture into meal for human consumption, sample this corn. If several cars are available for sampling, make a random selection of the car or cars to be sampled by the following procedure:

Arbitrarily number the cars, e.g., from 1 to 10 if 10 cars are available. Place correspondingly numbered slips of paper in an appropriate container and mix. Draw a number (or numbers, if more than one car is to be sampled), and sample the car or cars corresponding to the number(s) drawn. Do not sample corn which will probably be used for animal feed. If no carloads (or boatloads) of corn are on hand, but the mill is grinding corn received within the 2 weeks preceding the inspector's visit, sample the corn in the storage bin from which corn is being drawn, or as it flows from the bin to the first cleaning equipment in the mill.

Collect one sample of not less than two quarts from each carload or bin and submit to the laboratory. Examine an additional sample of approximately one peak on the spot for rat or mouse pellets, as directed under "Mill Program" above. Collect this sample by taking additional probings with the trier evenly distributed over the lot, in the case of carloads, boatloads, or bins. (See below.)

The sampling procedure for carlots is based on the Department of Agriculture Grain Inspectors' Manual. A similar procedure may be used in sampling a bin or the hold of a boat by probing with a trier. If the sample is taken from grain flowing from the bin to the cleaning equipment, sample the falling grain, taking a small portion at a time and distributing the sampling over a thirty-minute period as in sampling under "Mill Program" above. Take the additional one-peck sample for on-the-spot examination in the same manner as the sample to be submitted to the laboratory. When sampling by probing with a trier use a double-tube compartment trier, 60 inches long, by probing in five or more places, well distributed in different parts of the car.

Spread the probings on a clean paper or sampling cloth and examine them, each in relation to the others, for uniformity of loading of the car. (This of course need not be done on all probings required to get the one-peck sample.)

2. *Sampling in the grain markets.*—In addition to the samples of corn to be taken at mills, take samples each month from cars of corn on the inspection tracks in several large grain market areas in accordance with the schedule mentioned earlier in the program summary.

In the larger cities there are several railroad yards with "inspection tracks" at which corn will be sampled by licensed grain inspectors. Each "inspection track" may serve several railroads, each bringing in grain from different geographical areas; or, cars brought in by one railroad may be inspected at any one of several yards. The samples called for by the above schedule should not all be obtained from cars brought in by any one railroad, if there are several, but should be distributed among the principal railroads and/or railroad yards, approximately in proportion (if practicable) to the amount of corn brought in by or received in each. Select the cars at random, using the procedure given under mill sampling.

In the case of corn received by barge, consider one hold as a unit, equivalent to a car, for sampling purposes. Follow the procedure as outlined for the collection of samples from carlots at mills, taking one sample of not less than 2 quarts to be submitted to the laboratory, and another sample of approximately 1 peck for on-

the-spot examination for rodent pellets. Sample without reference to color of the corn and without reference to official grade, except that corn which is musty, moldy, or otherwise unusable for human food should not be sampled.

Wherever practicable, obtain the official grade certificate, if any, or an abstract of it, on any lot or car of corn sampled. Attach this certificate to the form to be filled out for the sample.

Fumigate all samples before sending them to the laboratory.

III. CORN MEAL INVESTIGATIONS

A. Introduction

The purpose of this section is to compare the findings on corn and the corresponding findings on corn meal, and to discuss the relationship of one to the other. Corn meal and grits cannot be reduced to the common denominator of total edible fraction on a "straight-run" basis and compared from mill to mill as was done for flour.[11] Edible meal production from a mill making degerminated meal differs from burr or roller mill production of a whole or bolted meal in total yield and also from the standpoint of what happens to the contaminants. Hence, for purposes of this discussion the mills may be divided into two basic types: (1), mills grinding all, or essentially all, of the corn tissue by passing the kernels through one or more stone burrs or steel rolls. In this type of mill some coarse bran usually is sifted out and the resulting product is called bolted corn meal; (2), mills tempering their corn and then cracking out the germ and endosperm on a huller-degerminator, followed by reels which separate fine stock for animal feed, coarse stock for animal feed or for the lower end of the mill, and medium sized stock for recovery of endosperm in a manner comparable to patent flour milling. Of the eight mills on the meal investigations, five (numbers 126, 127, 129, 417, and 813) made undegerminated, bolted corn meal and three (numbers 509, 803, and 814) made degerminated meal, corn flour, and/or grits.

B. Undegerminated, Bolted Corn Meal

1. *Insect contamination of the corn and meal.*—A rapid evaluation of the relationship between findings on corn and insect fragments on corn meal may be obtained by calculating the correlation coefficients between values on each of the various tests on corn and the values on the corresponding samples of meal. Correlation coefficients, which are used as the basis of comparison in Table 2, range from 0.00 to 1.00. Perfect correlation would be indicated by 1.00; no correlation by 0.00. Correlation coefficients are shown in column 3 of Table 2 where it is seen that there is a closer relationship between the findings on the cleaned corn and the meal than between the uncleaned corn and the meal. This is true for all of the tests. The visual examination of 200 grams of cleaned corn gives fair correlation with fragment count of the bolted meal (.72), but is of

[11] *This Journal*, **35**, 124 (1952).

TABLE 2.—*Correlation of tests for insects in corn to insect fragments in bolted meal*

TEST	NO. SAMPLES EXAMINED	CORRELATION COEFFICIENT
Visual examination 200 grams uncleaned	73	.48
Visual examination 200 grams cleaned	57	.72
Sieving 1000 grams uncleaned	72	.36
Sieving 1000 grams cleaned	58	.58
Total damage by stain test 200 kernels uncleaned	72	.42
Total damage by stain test 200 kernels cleaned	57	.69
Cracking test 100 grams uncleaned	72	.67
Cracking test 100 grams cleaned	78	.87

TABLE 3.—*Comparison of results on the cracking-flotation of corn to insect fragments in the bolted meal*

CRACKING-FLOTATION 100 GRAMS CLEANED CORN—NO. OF INSECTS	NO. OF SAMPLES	INSECT FRAGMENT COUNT OF 100 G MEAL		RATIO AVERAGE INSECT FRAGMENT COUNT (50 G MEAL) TO CRACK-FLOTATION (100 G CORN)
		RANGE	AVERAGE	
0	13	1–29	6	
1	5	0–66	17	17.0
2	13	2–37	16	8.0
3	9	0–86	34	11.3
4	8	2–56	24	6.0
5	3	24–111	60	12.0
6	5	67–176	114	19.0
7	1		47	6.7
8	2	52–70	111	13.9
9	3	15–212	143	15.9
10	1		137	13.7
11	2	113–269	191	17.4
12	3	82–324	164	13.7
14	3	88–517	172	12.3
15	1		114	7.6
17	1		359	21.1
18	1		256	14.2
19	1		260	13.7
34	2	246–406	326	9.6
40	1		523	13.1
Total Number of Insects = 472 Average (all samples) 6.1	78		Total No. Ins. Frags. = 5913 76.1	Total Ins. Fragments ÷ Total Insects = 12.5

lesser value on the uncleaned corn (.48). The correlation coefficient between the visual examination of the uncleaned corn and the cracking-flotation test on the same corn is .70 (See Table 24, p. 29). The cracking-flotation test gives a better correlation than the stain test which was the other technical procedure under investigation. Since, of the procedures used, the cracking-flotation test gives the highest correlation to the meal and is a direct measure of internal insects, it has been used in this report as the basic measure of insect contamination in corn.

TABLE 4.—*Correlation of tests for rodent pellets to pellet fragments and rodent hairs in bolted corn meal*

	NO. OF TESTS	EXAMINATION OF MEAL			
		RODENT PELLET FRAGMENTS		RODENT HAIR FRAGMENTS	
		CORREL. COEFF. FOR ALL VALUES	CORREL. COEFF. OMITTING 1[a]	CORREL. COEFF. FOR ALL VALUES	CORREL. COEFF. OMITTING 1[a]
Number rat pellets per peck in uncleaned	71	.51	.25	.53	.35
Number mouse pellets per peck in uncleaned	71	.07	.33	.00	.12
Number rat pellets per peck in cleaned	71	.63	.49	.61	.43
Number mouse pellets per peck in cleaned	71	−.02	.02	.02	.13
Weight rat and mouse pellets in uncleaned	72	.30	.37	.25	.20
Weight rat and mouse pellets in cleaned	68	.39	.46	.45	.60

[a] One sample with 20 rodent pellet fragments and 58 rodent hair fragments in the meal, 9 rat pellets and 6 mouse pellets in the uncleaned corn, 4 rat pellets and 0 mouse pellets in the cleaned corn, 0.8 gram rat and mouse pellets in the uncleaned corn, and 0.1 gram rat and mouse pellets in the cleaned corn.

The relationship between insects in the corn and the fragment count of the finished meal is shown in Table 3. On the average, one insect in the cleaned corn gives 12.5 insect fragments in finished bolted meal.

2. *Rodent pellet fragments and rodent hairs in the meal.*—Rodent contamination in the corn was measured by picking the rat and mouse pellets from 1 peck of corn. Counts were made of the rat pellets, mouse pellets, and the weight of the combined rat and mouse pellets. Data on the relationship between these tests and pellet fragments and hairs in the meal are given in Tables 4 and 5. Note that one heavily contaminated sample has a pronounced influence on the correlation. All correlation coefficients are low. The highest figure, viz. 0.63, (including all values) was obtained by comparing rat pellets in the cleaned corn to rodent pellet fragments in

TABLE 5.—*Comparison of results on the pickout of rodent pellets from cleaned corn to rodent pellet fragments in the bolted meal*

PICKOUT OF RODENT PELLETS PER PECK CLEANED CORN—NO. OF PELLETS	NO. OF SAMPLES	RODENT PELLET FRAGMENT COUNT OF THE MEAL	
		RANGE	AVERAGE
0	51	0–4	.1
1	11	0–2	.3
2	3	0–4	1.3
3	3	0–6	2.0
4	1		20.0
5	2	0–2	1.0

the meal, and a correlation of 0.61 was obtained by comparing rat pellets in the cleaned corn to rodent hair fragments in the meal.

Mouse pellets are smaller than the corn kernels and may be sifted out. Many rat pellets are of approximately the same size and weight as corn and cannot be removed by this means. Although the contamination of corn meal with solid rodent droppings is largely a problem of rat pellets, mouse pellets in corn also present a real problem both as direct contributors of filth and as an index of contamination with urine. Hence, data on mouse pellets are included in this report.

The poor correlations shown here are in part influenced by the low numerical findings of rat pellets per peck in the mill samples (see Table 7).

C. DEGERMINATED PRODUCTS

Corn for degermination is moistened and passed through a degerminator which removes bran from the whole corn, breaks the corn into irregular pieces, and removes germ. Material from the degerminator is separated, on a size basis, into stock from which the meal, grits, etc., is made, and into feed stock of which no part is milled into human food. In addition to the action on the corn kernels, the degerminating process tends to remove insect material, rodent pellets, pellet fragments, and rodent hairs so that the relationship between contamination of the corn and the finished products would not be expected to be the same for degerminated meal as for whole or bolted meal. In the manufacture of bolted meal there is usually one end product for human consumption. In the manufacture of degerminated products, a mill may make any one or a number of products. The fragmentation of insects and rat pellets in cleaned corn used in the production of bolted meal, degerminated meal, and corn flour, are compared in Tables 6 and 7. Degerminated meal contains fewer insect fragments, per insect in the corn, than does bolted meal. The net effect of the degermination process is that of reducing the amount of extraneous material in the finished meal. Because of the marked influence one sample has on these data (see Table 4), and because approximately half the samples contained no pellets in the cleaned corn,

TABLE 6.—*Relationship between insects in the cleaned corn and insect fragments in the bolted meal, degerminated meal, and corn flour*

TYPE OF FINISHED PRODUCT	NUMBER OF SAMPLES	AV. NO. OF INSECTS BY CRACKING TEST 100 G CLEANED CORN	AV. NO. OF INSECT FRAGMENTS IN 50 G OF THE MEAL	RATIO AV. NO. INSECT FRAGMENTS IN 50 G MEAL TO AV. NO. INSECTS BY CRACKING 100 G CLEANED CORN
Bolted meal	78	6.1	76.1	12.5
Degerminated meal	38	3.6	6.2	1.7
Flour	25	3.2	9.5	3.0

TABLE 7.—*Comparison between rat pellets in the corn and rodent hairs and rodent pellet fragments in the bolted meal, degerminated meal, and corn flour*[a]

TYPE OF FINISHED PRODUCT	NUMBER OF SAMPLES	AV. NO. RAT PELLETS IN 1 PECK CLEANED CORN	AV. NO. RODENT PELLET FRAGMENTS IN 50 G MEAL	AV. NO. RODENT HAIR FRAGMENTS IN 50 G MEAL
Bolted meal	71	0.3	0.7	2.4
Degerminated meal	38	0.2	0.2	0.5
Flour	25	0.3	0.04	2.0

[a] Since many of the mouse pellets are sifted out, rat pellets appeared to be the better index of contamination by which the various types of products could be compared.

TABLE 8.—*Summary of the relationship between insects in the cleaned corn and insect fragments in degerminated products (meal, grits and flour)*[a]

TYPE OF PRODUCT	NO. OF SAMPLES	CRACKING TEST RESULTS CLEANED CORN (INSECTS/100 GRAMS)		INSECT FRAGMENT TEST RESULTS ON COMMINUTED PRODUCT. (FRAGMENTS/50 GRAMS)[b]	
		RANGE	AVERAGE	RANGE	AVERAGE
Meal	38	0–18	3.6	0–27	6.2
Grits[c]	29	0–22	3.0	0– 9	1.6
Flour	25	0–22	3.2	0–40	9.5

[a] Other types of products were being milled in addition to those listed.
[b] The relationship between cracking test and fragments is dependent, among other things, on the various types of products being manufactured at the time. This was not constant.
[c] Several kinds of grits are grouped together.

TABLE 9.—*Distribution of insect fragments between degerminated corn meal and corn flour milled from the same corn*

MILL	NO. OF SAMPLES	INSECT FRAGMENTS IN FINISHED PRODUCT			
		DEGERMINATED MEAL		FLOUR	
		RANGE	AVERAGE	RANGE	AVERAGE
509	1		6		0
803	9	0–6	2.3	1–40	11.7
814	9	0–6	1.2	0–25	8.3
Combined	19	0–6	2	0–40	9.5

as well as other factors, the data will not warrant the calculation of a pellet-meal contamination relationship. However, the same tendencies followed by insects and insect fragments seem to be present with pellets, pellet fragments, and hairs.

In Table 8, a comparison is made of the distribution of insect fragments in corn meal, grits, and flour prepared from degerminated corn. The distribution of insect fragments in degerminated corn meal and flour produced by different mills is compared in Table 9.

D. Effect of Cleaning

The cleaning of corn has been investigated by many mill, machinery manufacturing, and laboratory groups. It is common knowledge that individual cleaning machines do remove some rodent and insect debris from corn. The effect of the degermination process is mentioned above.

1. *Removal of insects from corn.*—In Table 10 are tabulated the data on 117 samples of corn taken at eight mills before and after cleaning. There was an average reduction of about 25 per cent in the number of internal insects by mill cleaning for all mills. While there is some variation from mill to mill, it is apparent that mill cleaning is not very effective in reducing internal infestation.

Table 10.—*Effect of mill cleaning on internal insects in corn*

Mill	Cracking test (100 grams) averages		
	No. of samples	Uncleaned	Cleaned
126	17	20.4	13.6
127	16	6.6	5.4
129	8	7.0	5.8
417	18	1.9	2.1
509	18	6.2	4.7
803	19	5.7	3.9
813	11	1.9	1.9
814	10	3.1	1.3
All Mills	117	7.0	5.1[a]

[a] The probability that a difference as large as that observed between the average cleaned and the average uncleaned could have occurred by chance when there was no real effect of cleaning is less than 1 in 100.

In Table 11 is summarized the effect of cleaning as demonstrated by two other procedures: the visual examination of 200 grams for total damage (exclusive of minor surface nibbling) which is a measure of the amount of readily visible insect damage, and the sifting of 1000 grams which is a measure of the number of insects on the surface of the corn. The average reduction in the number of insect damaged kernels by the mill cleaning equipment is about of the same magnitude (20 per cent) as for internal insects and is statistically significant. The greater percentage

TABLE 11.—*Effect of cleaning out insect damaged grains and insects as measured by visual examination and sifting (all mills grouped together)*

METHOD OF EXAMINATION	NUMBER OF SAMPLES	NUMBER DAMAGED KERNELS OR INSECTS	
		UNCLEANED	CLEANED
Visual examination for kernels with insect tunneling or feeding punctures	97	6.2	4.9[a]
Sifting to remove surface insects	97	0.88	0.47

[a] See footnote to Table 10.

reduction of surface insects is not statistically significant, due probably to the small numbers originally present in the test portions and inconsistency in their removal.

2. *Removal of rodent pellets from corn.*—In Table 13 are tabulated the rat pellet data on 111 samples of corn taken at these same eight mills, and the same data are presented in Table 12 by the results on individual

TABLE 12.—*Effect of mill cleaning on rat pellets in corn. Summary of results on individual samples*

NUMBER OF RAT PELLETS PER PECK IN INDIVIDUAL SAMPLES		NUMBER OF SAMPLES
UNCLEANED	CLEANED	
0	0	63
1	0	7
2	0	8
3	0	8
4	0	2
5	0	3
6	0	1
8	0	1
9	0	2
18	0	1
0	1	2
1	1	1
2	1	1
3	1	2
4	1	1
5	1	1
7	1	1
10	1	1
1	2	2
9	2	2
9	4	4
14	4	4
5	5	5
Totals: 126	25	123

TABLE 13.—*Effect of mill cleaning on rat pellets in corn. Tabulated by mills*

MILL	NO. OF RAT PELLETS (PER PECK) AVERAGES[a]		
	NO. OF SAMPLES	UNCLEANED	CLEANED
126	17	0.9	0.4
127	11	1.2	0.5
129	8	0.6	0.3
417	18	1.4	0.1
509	18	2.4	0.1
803	18	2.6	0.2
813	11	1.8	0.2
814	10	2.2	0.6
Total and Averages	111	1.7	0.2[b]

[a] The maximum number of rat pellets in the cleaned corn was 5.
[b] See footnote to Table 10.

TABLE 14.—*Effect of mill cleaning on mouse pellets in corn. Tabulated by mills*

MILL	NO. OF MOUSE PELLETS (PER PECK) AVERAGES[a]			
	UNCLEANED		CLEANED	
	NO. OF SAMPLES	AV. NO. EACH MILL	NO. OF SAMPLES	AV. NO. EACH MILL
126	17	1.41	17	1.00
127	17	24.41	11	0.00
129	8	3.25	14	0.00
417	18	15.50	18	0.00
509	18	7.72	18	0.06
803	19	9.53	18	0.06
813	12	44.75	12	0.00
814	11	26.54	11	0.36
Total and Averages	120	15.77	119	0.19[b]

[a] The maximum number of mouse pellets in the cleaned corn was 5.
[b] See footnote to Table 10.

samples. These tables give the data in regard to rat pellets which are more difficult to separate from corn than are the smaller mouse pellets, (cf. Tables 13 and 14). In five out of eight of the mills there was a significant reduction in average numbers of rat pellets. However, all of them could not be removed.

IV. CORN SURVEY

A. Introduction

The investigation of levels of contamination of wheat delivered to flour mills, reported in *This Journal*, **35**, 115–158 (1952) was based upon the premise that most wheat is destined for milling into edible end products. Corn is largely used for animal feed and only a relatively small percentage is used for human consumption. Of the corn sold off farms, only about one-third goes for domestic food use. The problem of this survey, then, is one of sampling the corn on the market to determine, among other things, to what extent clean corn or corn with minimal contamination is available, assuming that it can be channeled to the food processing plants.

In this survey, the corn sampling phase was divided into two parts. Samples were obtained from railroad cars or trucks of corn being delivered to corn meal mills. The mills at which this sampling was conducted were selected by a statistical procedure from a list of all of the corn meal mills known to the authors, excepting those which had been determined by preliminary investigation to be custom grinders or small mills doing no interstate business. This part of the plan probably included some corn which moved directly from producer to mill and thus did not move through the cash grain markets. This part of the sampling was intended to reflect the character of the corn actually being used, whether or not it passed through the cash grain markets. In the second part of the plan,

TABLE 15.—*Sampling schedule by points at which samples were taken*

LOCATION BY STATE	NUMBER OF SAMPLES PER MONTH		
	AT MILLS	AT TERMINAL MARKETS	TOTALS
Virginia, West Virginia	2	0	2
Alabama, Mississippi, Arkansas	8	Birmingham 2	10
Texas, Oklahoma	5	0	5
Kentucky, Tennessee	7	Louisville 2	9
Ohio, Indiana, Illinois	8	Chicago 20, Peoria 6, Decatur, Ill. 3, Indianapolis 5	42
Wisconsin, Minnesota	2	0	2
Iowa, Missouri	5	St. Louis, 5, Kansas City 3 (approx.)	13
Kansas, Nebraska	2	Omaha 6, Kansas City 2 (approx.)	10
Pennsylvania	1	0	1
California	1	0	1
Totals	41	54	95
Actual no. samples collected[a] over 12 month period	440	586	1026

[a] Since lots were not always available for sampling, this figure is less than the number scheduled for collection.

TABLE 16.—*Number of samples taken at mill and terminal sampling points as related to type of corn (yellow and white) and location of the sampling points*

TYPE CORN[a]	AREA[b]	NUMBER OF SAMPLES TAKEN	
		AT MILLS	AT TERMINALS
Yellow	North	171	521
	Southeast	4	25
	Southwest	27	0
White	North	67	13
	Southeast	121	22
	Southwest	47	0

[a] Samples of "mixed" corn not included in this table.
[b] See Table 20 for description of areas.

TABLE 17.—*Per cent of samples in increasing order of number of internal insects (Blanks indicate no samples in particular category)*[a]

INTERNAL INSECTS BY CRACK TEST ON 100 G *per cent*	YELLOW		WHITE		YELLOW AND WHITE		MILL AND TERMINAL		ALL SAMPLES	
	MILL *per cent*	TERMINAL *per cent*	MILL *per cent*	TERMINAL *per cent*	MILL *per cent*	TERMINAL *per cent*	YELLOW *per cent*	WHITE *per cent*	NO.	CUMULATIVE PER CENT
0	66	66	23	37	42	63	66	25	561	55.2
1	9	11	14	9	12	11	10	13	113	66.3
2	9	7	14	14	12	7	8	14	95	75.6
3	3	2	8	11	6	3	2	8	40	79.5
4	3	4	11	11	8	5	4	11	59	85.3
5	2	1	6	6	4	1	1	5	24	87.7
6		3	4	3	2	3	2	4	25	90.2
7	2	0.4	4	9	3	0.5	0.8	4	17	91.9
8		1	3		2	1.5	0.8	4	16	93.5
9	1		1		1		0.3	1	4	93.9
10	0.5	1	3		2	1	0.8	2.2	12	95.1
11	0.5	0.5			0.3	0.5	0.5	0.4	5	95.6
12		1	3		2	1	0.5	3	11	96.7
13		0.1	1		0.3	0.2	0.1	0.6	3	97.0
14		0.1			0.2	0.2	0.1	0.4	2	97.2
15		0.1				0.2	0.1		1	97.3
16	1		1		1		0.3	0.7	4	97.7
17		0.5			0.2	0.4	0.3	0.4	4	98.1
18			1		0.1			0.4	1	98.2
19	0.5	0.1			0.1	0.2	0.3		2	98.4
22					0.1			1.4	1	98.5
23		0.1				0.2	0.1		1	98.6
24					0.1			0.4	1	98.7
25		0.1				0.2	0.1		1	98.8
26	1	0.1	1		0.6	0.2	0.2	0.7	5	99.3
32		0.1				0.2	0.1		1	99.4
38 or more	1.5	0.8	2		1	0.5	0.6	0.4	9	100
Totals (No. of samples)	202	546	235	35	437	581	748	270	1018	

[a] Samples of "mixed" corn not included in this table.

samples were taken in terminal markets from railroad cars on inspection tracks and from barges being unloaded into terminal elevators. This part of the sampling plan was designed to obtain a cross section of the market corn available in the cash grain markets. The distribution of sampling points under this part of the plan is given in Table 15. The sampling schedule called for a comparatively large number of samples to be collected in the corn belt and includes a great deal of corn which would not go into dry corn milling.

The number of samples of white corn and yellow corn was not predetermined but was guided by the type of corn available at the particular

TABLE 18.—*Per cent of samples in increasing order of weight of rodent pellets in grams per peck (average wt. rat pellet = .080 g.; average wt. mouse pellet = .008 g)*[a]

WEIGHT RODENT PELLETS GRAMS *per cent*	YELLOW		WHITE		YELLOW AND WHITE		MILL AND TERMINAL		ALL SAMPLES	
	MILL *per cent*	TERMINAL *per cent*	MILL *per cent*	TERMINAL *per cent*	MILL *per cent*	TERMINAL *per cent*	YELLOW *per cent*	WHITE *per cent*	NO.	CUMULATIVE PER CENT
0.0	19	8.4	52	26	37	10	12	48	214	22
0.1	12	10.6	11	8	11	10	11	11	112	33
0.2	11	9	6	12	9	9	10	6	88	42
0.3	7	8	3	5	5	8	8	4	71	49
0.4	7	9	5	6	5	9	8	4	73	56
0.5	5	4	3	3	4	4	4	3	43	60
0.6	4	5	2	6	3	5	5	3	43	64
0.7	4	5	3		3	5	5	2	41	68
0.8	6	4	1	5	4	4	4	1	36	72
0.9	3	4	1	9	2	4	4	2	35	75
1.0	1	4	3	6	1	4	3	3	30	78
1.1	4	2	0.5	5	3	2	3	1	24	80
1.2	2	3	0.5		1	3	2	1	21	82
1.3	3	2	1		2	2	2	1	19	84
1.4	1	1	2		1	1	1	2	16	86
1.5	1	2	1.7		1	2	1	1	13	87
1.6	1	1	0.3	3	1	1	1	1	13	88
1.7	1	2	1		1	2	2	1	14	89
1.8	2	2			0.9	2	2		14	90
1.9	0.3	1			0.2	1	1		5	90.5
2.0		1			0.2	1	1	1	9	91.4
2.1	0.5	1	1		0.5	0.7	1	1	6	92
2.2	1.2	1	0.2	3	0.7	1	1	1	9	92.9
2.3	0.8	1	0.4		0.5	1.2	1	0.3	10	93.9
2.4		0.4				0.1	0.3		2	94.1
2.5	0.5	1.1	0.4		1	1.0	1	0.3	8	94.9
2.6	0.5	1.1	0.4		0.3	1.0	1	0.3	8	95.7
2.7	0.5	0.4	0.4		0.5	0.5	0.3	0.3	4	96.1
2.8		0.2	0.2		0.2	0.2	0.1	0.3	2	96.3
2.9	1	1.1		3	0.5	1.2	1.0	0.5	9	97.2
3.0		0.2				0.1	0.1		1	97.3
3.1 or more	0.7	4.5			0.5	4	3.2		25	100
Totals (No. of samples)	202	546	235	35	437	581	748	270	1018	

[a] Samples of "mixed" corn not included in this table.

sampling point. The general geographical distribution of yellow and white corn samples obtained at mill and terminal markets is shown in Table 16.

B. General Levels and Variations in Contamination as Related to Geographical Factors and Type of Corn

Of the 1018 samples examined for both rodent pellets and internal insects, 38 of the samples contained neither rodent pellets nor internal insects. The average insect count per 100 grams by the cracking-flotation test was 2.6. The average number of rat and mouse pellets per peck was 78.5. The general distribution of the levels of internal insects and rodent pellets is given in Tables 17 and 18.

Table 19 shows that white corn is more heavily insect-infested than yellow corn, both at the mills and at the terminal markets. Corn at the mills is more heavily insect-infested than corn at the terminal markets.

TABLE 19.—*Variation in levels of insect infestation with sampling point and type of corn (cracking test per 100 grams—averages)*

	MILL		TERMINAL		BOTH[a]	
	YELLOW	WHITE	YELLOW	WHITE	YELLOW	WHITE
Av. cracking test	2.1	4.9	1.8	2.4	1.9	4.6
No. of samples	202	235	546	35	748	270

[a] The over-all incidence of insect infestation in white corn is significantly higher than that in yellow corn. The level of insect infestation at the mills is significantly higher than that at terminals only with respect to white corn. Samples of "mixed" corn are not included in this table.

For explanation of "significance," see footnote to Table 10, p. 20.

The reverse of this pattern with respect to rodent contamination is presented in Table 20. Rodent excreta contamination is greater in yellow than in white corn and in samples collected at terminals than at mills.

TABLE 20.—*Variation in levels of rodent pellet contamination with sampling point and type of corn (averages per peck)*

	MILL		TERMINAL		BOTH[a]	
	YELLOW	WHITE	YELLOW	WHITE	YELLOW	WHITE
No. of samples	202	235	546	35	748	270
Av. weight mouse and rat pellets (grams)	0.62	0.32	0.93	0.55	0.84	0.35
Av. No. rat pellets	3.2	1.4	5.1	1.4	4.6	1.4
Av. No. mouse pellets	71.4	30.1	94.0	62.6	89.4	34.3
Av. No. rat and mouse pellets	74.6	31.5	99.1	64.0	94.0	35.7

[a] Yellow corn showed significantly higher rodent pellet contamination than white corn at both mills and terminals. Contamination at terminals is significantly higher than at mills for both yellow and white. Samples of "mixed" corn are not included in this table.

For explanation of "significance," see footnote to Table 10, p. 20.

TABLE 21.—*Variation in average levels of contamination with area from which corn was shipped*

AREA	STATE OF ORIGIN	NO. OF SAMPLES	AVERAGE RODENT PELLET CONTAMINATION PER PECK			AVERAGE NO. OF INSECTS BY CRACKING TEST (100 GRAMS)
			RAT & MOUSE (GRAMS)	NO. RAT	NO. MOUSE	
Southeast: (South of the Ohio River and East of the Mississippi River)	Alabama	55	0.5	1.4	47.2	3.5
	Kentucky	15	0.7	2.7	53.3	4.3
	Tennessee	15	0.5	2.0	46.2	13.0
	Other	19	0.1	1.2	7.8	5.5
	Total Averages[a]	104	0.4	1.6	40.8	5.4
Southwest: (South of Parallel 37 and West of the Mississippi River)	Texas	20	0.0	0.8	1.2	8.5
	Other	16	0.3	0.5	29.7	2.3
	Total Averages[a]	36	0.1	0.7	13.9	5.8
North: North of the Ohio River and a line extending from the Ohio west at about Parallel 37 (Southern Borders of Missouri, Kansas, and Colorado)	Ohio	15	0.4	2.4	71.9	1.6
	Indiana	34	0.7	2.6	85.8	1.6
	Illinois	271	0.9	4.7	105.1	2.2
	Minnesota	25	0.5	2.6	59.2	0.1
	Iowa	106	1.3	7.4	123.8	0.9
	Missouri	42	0.3	1.8	24.1	3.6
	Nebraska	143	0.5	2.6	40.6	0.1
	Kansas	36	0.3	2.1	14.2	2.5
	Other	12	0.6	1.7	49.7	0.2
	Total Averages	684	0.8	4.1	80.6	1.7
Not known		199				
TOTAL		1023[b]				

[a] Weighted.
[b] Samples not having values for all tests are omitted.

TABLE 22.—*Variation in average levels of contamination with area in which the corn was sampled*

AREA	RAT PELLETS (AVERAGE NO. PER PECK)				INSECTS (NO. BY CRACKING TEST PER 100 GMS.)			
	CORN AT MILLS		CORN AT TERMINALS		CORN AT MILLS		CORN AT TERMINALS	
	AVERAGE	NO. OF SAMPLES	AVERAGE	NO. OF SAMPLES	AVERAGE	NO. OF SAMPLES	AVERAGE	NO. OF SAMPLES
Southwest[a]	1.9	77	—	0	3.8	77	—	0
North[a]	2.6	238	5.1	537	2.3	238	1.5	537
Southeast[a]	1.5	125	2.4	49	6.0	125	5.3	49

[a] For description of areas see Table 21.

The variation in insect and rodent contamination by the geographical area from which the corn was shipped is given in Table 21 and the same type of data (rat only) for the area in which the corn was sampled is given in Table 22. There is more insect contamination in corn from and in the Southern areas and more rodent pellet contamination in corn from and in the Northern areas.

C. Seasonal Fluctuations in Contamination

The corn crop ripens and matures during August, September, October, and November. In some instances, corn may remain in the field for several months after it has ripened.

From Table 23 it can be seen that rodent contamination was highest in July, August, and September. The effect of the new crop was apparent during October, November, and December, with the lowest level in the latter month. During the period January through June, there appears to be a slow build-up to the summer high. Insect contamination reached a high during September and October with a drop in December and, with fluctuations, a rather uniform level was maintained through June. Note that on a yearly basis, 10.2 per cent show no rodent pellets in the peck samples and 55.3 per cent show no insects in the 100 gram portions.

Table 23.—*Seasonal trends in rodent and insect contamination*

Month	No. of samples	Av. no. rat pellets	Av. no. mouse pellets	Av. rat & mouse pellets	Wt. rat & mouse pellets (grams)	Per cent with no pellets	Av. no. insects by crack. test per 100 g	Per cent with no insects
1950								
July	86	5.1	150.0	155.1	1.3	0.0	3.2	58.1
August	85	7.8	113.2	121.0	1.2	2.4	3.9	56.5
September	81	5.2	145.2	150.4	1.2	17.3	5.5	43.2
October	77	5.9	96.8	102.7	1.0	23.4	4.4	36.4
November	88	3.0	31.1	34.1	0.4	35.2	2.8	46.6
December	83	1.2	24.0	25.2	0.2	16.9	1.4	57.8
1951								
January	93	2.6	33.9	36.5	0.4	9.7	1.8	62.4
February	86	1.9	29.9	31.8	0.3	5.8	1.3	64.0
March	88	3.0	49.4	52.4	0.5	9.1	2.3	56.8
April	79	2.4	61.7	64.1	0.6	3.8	1.3	67.1
May	95	3.1	67.3	70.4	0.7	1.1	2.0	53.7
June	70	3.3	69.8	73.1	0.7	0.0	1.8	57.1
Total	1011[a]							
Weighted Averages		3.7	73.3	77.0	0.7	10.2	2.6	55.3

[a] June, 1950, samples not included.

D. Relationship Between the Level of Insect and Rodent Contamination and U.S.D.A. Grade

There is no direct reference to insect or rodent contamination in the first five U.S.D.A. grades which are based upon quality factors such as moisture, cracked corn, and foreign material. "Weevily" is a special grade and is applied only when a given level of live infestation is found. Rodent excreta in corn is a factor for grade only if it exceeds 0.2 per cent by weight, in which case the corn is considered to be "of distinctly low quality" and hence "sample" grade. The data on rodent pellets and internal insects by U.S.D.A. grade are compared in Table 24. Apparently the grade has no relationship to rodent or insect filth, and cannot be used for the selection of corn on the basis of relative amounts of contamination.

Table 24.—*Variations in levels of contamination with U.S.D.A. grade*

U.S.D.A. GRADE[a]	NUMBER OF SAMPLES	AVERAGE WT. OF RODENT PELLETS PER PECK	AVERAGE NO. INSECTS BY CRACKFLOAT PER 100 G
1	139	0.96	2.0
2	353	0.91	2.7
3	213	0.53	1.2
4	115	0.62	1.1
5	17	0.22	0.2
Sample	29	0.78	5.6
Not graded	128	0.36	6.4

[a] In some cases information on grade was not available.

V. RAPID SORTING PROCEDURES

Since the fuchsin stain test, visual examination of 200 grams, or sieving technique could be used as sorting procedures prior to the cracking-flotation test, comparisons were made between the results on all samples (mill samples, corn at mills, and corn at terminals) examined by each of these tests as compared to the cracking-flotation test (Table 25). The fuchsin stain test of 200 kernels and visual examination of 200 grams show fairly high positive correlations with internal infestation.

Table 26 summarizes the relationship between the degree of visual damage and the amount of internal insect infestation. Of the 499 samples showing no insect cutting or tunneling per 200 grams, only one sample (0.2 per cent) contained 15 or more internal insects per 100 grams by the cracking-flotation procedure. Of the 870 samples with not over 0.5% of the kernels showing cutting or tunneling, only two samples (0.2 per cent) contained 15 or more insects by the cracking test. It is apparent from this table that when the visual examination is relatively low, the amount of internal insect contamination usually will also be relatively low. The visual

TABLE 25.—*Correlation between cracking-flotation test on uncleaned corn and other tests for infestation of corn*

TEST COMPARED WITH CRACKING TEST	CORRELATION COEFFICIENT	NO. OF SAMPLES
Fuchsin stain 200 kernels (Total number kernels with insect damage)	.80	1143
Visual examination 200 grams (Total per cent kernels with insect tunneling or feeding punctures)	.70	1142
Sieving 1000 grams (Number of storage insects)	.42	1143

examination of corn thus can be used as a guide to the amount of internal insect contamination and may be worthy of trial as a rapid non-technical procedure.

TABLE 26.—*Relationship between the degree of internal insect infestation and the amount of visual insect damage*

PER CENT KERNELS WITH INSECT CUTTING, TUNNELING OR FEEDING PUNCTURES PER 200 GRAM SAMPLE	NUMBER OF SAMPLES					
	INTERNAL INSECTS BY CRACKING-FLOTATION TEST					
	0	1–9	10–14	15–50	OVER 50	TOTAL
0.0	350	144	4	1		499
0.1–0.5	166	188	16	1		371
0.6–1.0	35	44	5	11		95
1.1–1.5	5	11	5	4		25
1.6–2.0	3	3	2	3		11
2.1–2.5	2	1	1	2	1	7
2.6–3.0	2	3		1		6
3.1–3.5	1	1		1		3
3.8	1					1
4.7	1					1
5.7				1		1
6.0	1					1
6.1				1		1
7.4					1	1
17.0					1	1
25.6					1	1
37.8					1	1
						1026

VI. X-RAY EXAMINATION OF CORN

The possible application of radiographic techniques to an examination of corn for insect infestation and other characteristics was suggested after this survey had been started.[12,13,14] An article on this subject appears in *This Journal*, **36**, 3 (1953).

VII. SUMMARY AND CONCLUSIONS

1. Substantial proportions of both white and yellow corn being purchased by corn millers are contaminated with rodent pellets and internally feeding insects. As measured by the methods used, 3.7 per cent of all the corn samples contain no internal insects and no rodent pellets. Considering insects and pellets separately, 10 per cent of the samples showed no pellets per peck and 55 per cent showed no insects per 100 grams.

2. For all samples, there was an average reduction of about 25% in the number of internal insects by mill-cleaning procedures as shown by the cracking-flotation test before and after cleaning. It appears that a substantial internal infestation must already be present in order to obtain a significant reduction in internal insects, and even then the grain remains appreciably contaminated after cleaning.

Mill-cleaning does remove very substantial proportions of both rat and mouse pellets but in some cases, pellets were found in the cleaned corn going to the rolls or burrs.

3. The insect fragment count of bolted corn meal is closely related to the number of whole insects or equivalent (insect heads, capsules, and cast skins) inside the corn as measured by the cracking and flotation test for insects. The ratio of insect fragments in bolted meal to whole insects or equivalent in the corn from which it was made varies from mill to mill. For all mills the ratio of fragments in the bolted meal to insects in the cleaned corn was 12.5:1. The number of rodent pellet fragments and/or rodent hair fragments in bolted corn meal is related to the rodent pellet findings on the cleaned corn. Correlation in individual samples of pellet fragments and hair counts with the pickout count is low.

4. Degerminated corn products contain on the average fewer insect fragments per insect in the corn than does bolted corn meal. Of the degerminated products, the grits contain the smallest number of insect fragments per insect in the corn, degerminated meal contains a larger number, and corn flour the largest number. Correlation of insect fragment counts in degerminated products and insects in the corn is low. The degerminated products contain on the average fewer pellet fragments and hairs for a given number of pellets in the cleaned corn than does the bolted corn meal.

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[12] *J. Econ. Entomol.*, **43**, 933 (1950).
[13] *Nondestructive Testing*, **8**, (1950).
[14] *Food Technology*, **6**, 44 (1952).

5. The incidence of insect infestation in white corn is significantly higher than in yellow corn. The incidence of rodent pellet contamination in yellow corn is significantly higher than in white corn. The highest incidence of rodent excreta was found in the North and the highest incidence of insects was found in the South.

6. There is no direct relationship between U.S.D.A. grade and the amount of rodent or insect contamination.

7. Both insect and rodent contamination reach a peak toward the end of the crop year (October).

8. The rodent pellet pick-out on a peck sample is an informative test which requires no special training and no laboratory equipment. A visual examination of corn can be used as a guide to the amount of internal insect contamination.

VIII. ACKNOWLEDGMENT

To acknowledge completely all of the participants in this work would destroy the anonymity of the various mills. The authors, however, wish to list the inspectors and analysts in their organization who carried the great bulk of the load: J. C. Akers, V. F. Balaty, C. R. Baeuerlen, H. P. Bennett, N. A. Carson, W. S. Cox, G. M. Downard, W. R. Durrenberger, R. T. Elliott, E. W. Greve, W. C. Hill, D. J. Holliday, M. M. Jackson, G. E. Keppel, T. F. Loveridge, W. R. Moses, W. H. Munday, G. C. Reed, R. P. Reichardt, B. B. Shephard, R. D. Sherman, H. D. Silverberg, F. R. Smith, H. R. Southworth, E. S. Spivak, M. L. Strait, G. G. Thompson, H. C. Van Dame, W. W. Wallace, and M. G. Yakowitz. The mass of clerical work was handled by M. A. Pelstring. Statistical interpretations and punch card tabulations were by H. Edelson. The assistance of the American Corn Millers' Federation, Corn Industries Research Foundation, Millers' National Federation, Grain and Feed Dealers National Association, and of the U. S. Department of Agriculture is gratefully acknowledged.

USE OF A SIMPLE BLOWING DEVICE TO FACILITATE INSPECTION OF WHEAT FOR INTERNAL INFESTATION*

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(Kansas State College, Manhattan, Kansas)

Enforcement of sanitary practices in the commercial handling of wheat, particularly with reference to insect infestation, requires a method of inspection of wheat for internal or hidden insect infestation which is reliable, yet rapid enough for routine use. A number of inspection methods have been developed for this purpose, and a review has been published (1) of those proposed up to two years ago. A rigorous evaluation of several of these technics has been made recently (2).

The rapid inspection method suggested to the grain trade by the Food and Drug Administration (2), based on the results of an extensive study of the sources of insect fragments in mill products (3), involves gross examination of 100 grams of wheat for insect exit holes. More than three of such insect-emerged kernels in the sample indicate excessive internal or hidden infestation. This inspection method has been criticized as too time-consuming, and difficulty has been experienced in differentiating exit holes from deep insect feeding punctures.

An adjunct to the emergence hole test has been proposed by the Bureau of Entomology, United States Department of Agriculture (4), which involves the use of a solution of ferric nitrate as a flotation medium to cause the insect-emerged kernels to float to the surface. A device designed to facilitate the examination of grain samples for insect exit holes consists of a glass tray upon which the grain is placed and about which are arranged lights and mirrors so oriented that the inspector can view all surfaces of the kernels on the tray without manipulating them.§

Previous studies at Kansas State College on the problem of detection of internal infestation have led to the development of a fluorescent staining technic essentially specific for insect egg plugs (5), an X-ray inspection procedure (6–8) which is gaining widespread use in the milling industry and grain trade for inspection of grain in car-lot quantities, and an aural detection method using electronic technics (9) which shows considerable promise for evaluating the effectiveness of fumigants and for research studies on internally feeding insects.

The present communication deals with the discovery that a simple blowing device commonly used in seed testing laboratories[1] may be em-

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* Contribution No. 237, Department of Flour and Feed Milling Industries and No. 39, Department of Physics, Kansas Agricultural Experiment Station.
† Department of Flour and Feed Milling Industries.
‡ Department of Physics.
§ This device is manufactured by the Stedman Laboratories, Kansas City, Mo.
[1] In these experiments a machine manufactured by the Ames Powercount Co. of Brookings, S. D., was used.

ployed to produce a rapid and fairly quantitative segregation of wheat kernels containing exit holes, thus significantly reducing the quantity of material to be examined and hence the time required to complete the examination. Kernels containing only feeding punctures apparently tend to remain with whole or non insect-emerged kernels. The commercial apparatus used in this study is shown in Fig. 1.

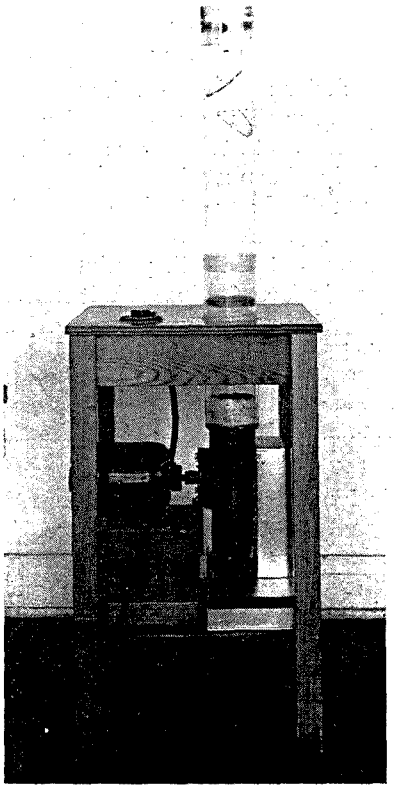

FIG. 1.—Seed blowing device used to separate insect-emerged kernels. Note separate vanes in column.

Since the blowing device is designed primarily to separate grain and seeds of different size and shape, it is necessary beforehand to make a rough separation of the infested sample into its component kernel sizes by sieving. Usually only two sieves are required, since for normal wheat the separations obtained with No. 8 and No. 9 mesh sieves are adequate. The fractions obtained in this manner are then blown separately.

Evidence of the kind of separation achieved by this blowing technic

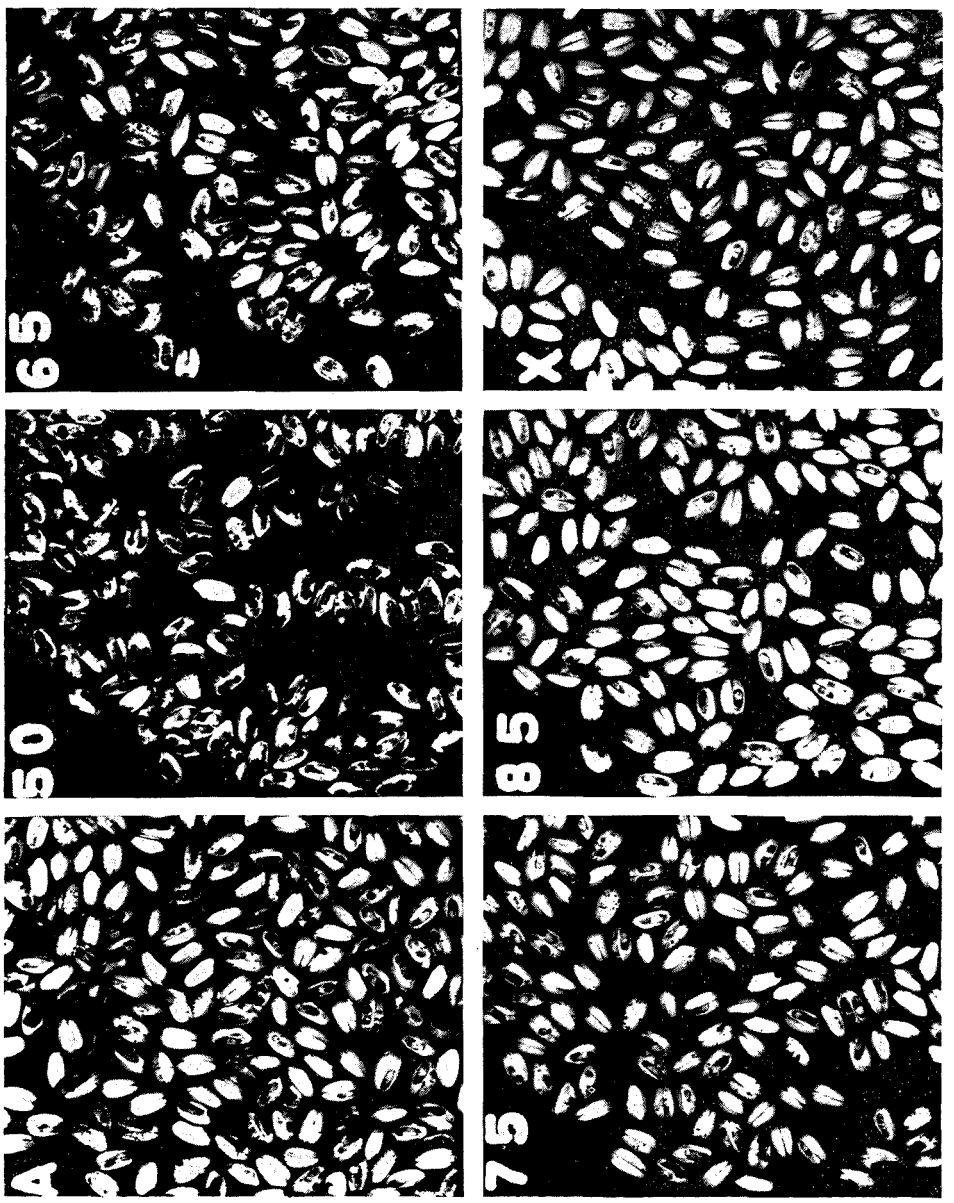

FIG. 2.—Radiographs showing separation of insect-emerged kernels achieved by blowing. Picture A is the original sample. Pictures marked 50, 65, 75, 85, and X are the fractions obtained by progressive increases of intensity of blowing, using an arbitrary scale of designation.

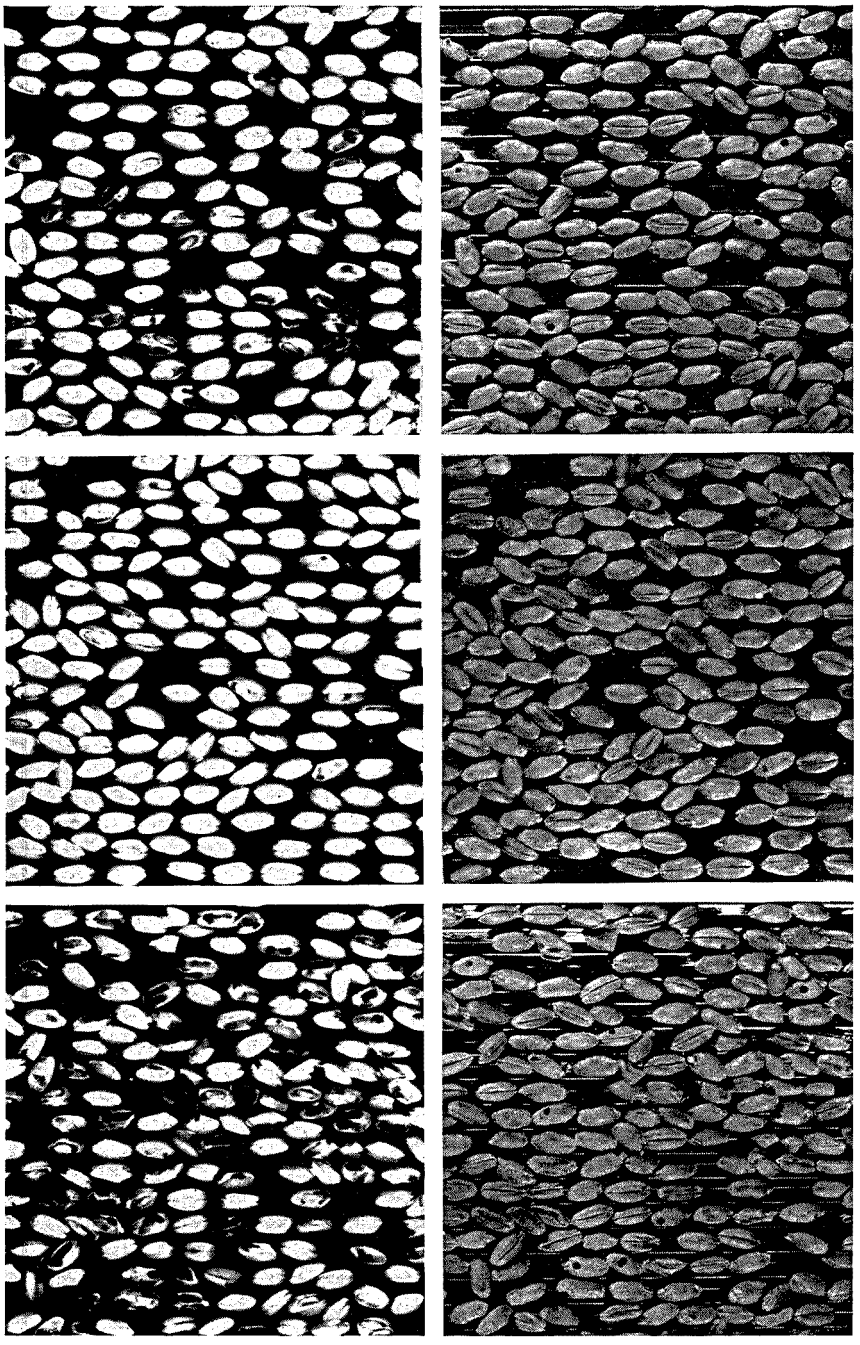

FIG. 3.—Radiographs and ordinary photographs of a one-step separation of insect-emerged kernels from a sample of infested wheat. The left vertical row of radiographs (top to bottom) shows the original sample, the residue after blowing, and the material removed by blowing respectively. The photographs on the right are of the precise field shown in the radiographs immediately to the left.

appears in Figs. 2 and 3. The series of radiographs in Fig. 2 includes an X-ray picture of the original sample of infested grain (A), in which various stages of infestation due to rice weevil, including numerous insect-emerged kernels, are shown. The subsequent pictures of this series are of fractions recovered from the original sample by progressively increasing the intensity of blowing. It is to be noted that virtually all the insect-emerged kernels are removed in the first two fractions, marked 50 and 65. This operation, while not precise, does produce an excellent separation of insect-emerged kernels from those in which no emergence has occurred.

The pictures in Fig. 3 provide an indication of the separation achieved with a single setting of the blower on a sample of mixed wheat containing various degrees of internal infestation. The vertical row of pictures on the right in Fig. 3 are ordinary photographs of the identical portions of the samples shown in the radiographs immediately to the left. In the left row, the radiograph at the top shows a portion of the original sample, and the radiograph at the bottom is representative of the material removed, which in this case constituted 22 per cent of the original 100 gram sample. This fraction contained nearly all the insect-emerged kernels present in the original sample. The X-ray picture in the center is of the residue remaining after blowing, which constituted 78 per cent of the original sample.

The sieving and blowing of grain samples in the simple manner outlined achieves a concentration of the insect-emerged kernels into a fraction of the original sample, in which detection of the kernels with exit holes is relatively quick and easy. An apparatus designed specifically for this purpose would probably produce an even better separation. The efficiency with which a concentration of insect-emerged kernels was achieved from the samples used in this study, which contained infestation far in excess of that normally found in commercial grain, indicates that the insect-emerged kernels in commercial samples could be removed in a much smaller fraction. It would appear, therefore, that this technic of concentrating the defective kernels speeds up the exit hole inspection procedure in commercial samples by a factor of ten or better.

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MOISTURE DETERMINATION OF FOODS BY HYDROGEN NUCLEI MAGNETIC RESONANCE*

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The rapid and accurate determination of the moisture content of biological materials is still a problem of major importance. Particularly in the food industry, improved methods are sought for materials containing as much as 90 to 95 per cent moisture, as in fresh vegetables, or as little as 2 to 4 per cent, as in dried egg solids. Of especial value would be methods that could be used to follow changes in moisture continuously during dehydration or other processing operations, and which would require at most only minor changes in chemical or physical properties of the product.

Methods commonly used for the determination of the moisture content of foods fall into three general clases: (*a*) analytical chemical methods, such as distillation and oxidimetric dichromate, oven, and Karl Fischer; (*b*) physical chemical methods, which are based largely on moisture-equilibrium (vapor-pressure) measurements; and (*c*) physical methods, which depend upon the electromagnetic properties of the sample. As indicated by the title, the present paper deals with a method of the latter class.

About six years ago, "nuclear magnetic resonance absorption" or "nuclear induction" was demonstrated in liquids and solids simultaneously at Harvard University (3) and Stanford University (1). Subsequent experiments on various hygroscopic materials have shown that the nuclear magnetic absorption of hydrogen nuclei can be utilized for the measurement of water content (4). In the present paper, additional results are reported for food.

THEORETICAL CONSIDERATIONS

Nuclear magnetic absorption can be described as consisting of electromagnetic effects accompanying the reorientation of nuclear moments. For the case under discussion, the hydrogen nuclei in water and in tissues behave like small spinning magnets. When these nuclei are placed in a mag-

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netic field of strength H, they precess at a frequency f. If the precessing nuclei are exposed to an additional external radio-frequency magnetic field, correctly oriented, of frequency f, they will reorient or "flip." This forced reorientation absorbs energy from the radio-frequency magnetic field. The amount of energy absorbed is proportional to the number of hydrogen nuclei in the radio-frequency field. The absorption actually occurs over a narrow band of frequencies centered about the frequency, f, given by the formula:

$$f = 2\mu H/h$$

where μ is the magnetic moment of the hydrogen nucleus, and h is Planck's constant. For a magnetic field, H, of 6300 gauss the frequency f is about 27 megacycles.

In a previous paper (4) it was shown that the peak intensity of the proton absorption line can be used as a measure of the water content of vegetable materials. The apparatus used in the previous work has been replaced by a more sensitive recording radio-frequency (r-f) spectrometer (2). The spectrometer, however, does not furnish a record of the absorption directly. Under the usual conditions of operation the spectrometer record is a good representation of the first derivative of the absorption curve. The absorption curve can be obtained from the spectrometer record by graphical integration. Figure 1 shows a typical recording together with the proton absorption obtained by graphical integration of the spectrometer record.

The two quantities readily obtained from the recorded absorption line derivative are the line width, δf, and the peak-to-peak amplitude, D. For the present purpose the quantity D has been chosen arbitrarily as a simple measure of the absorption. Only for absorption lines of the same shape is D rigorously proportional to the peak absorption. Since constancy of line shape is probably only approximately true for the lines to be discussed here, the above limitation should not be overlooked.

It is necessary, of course, to consider the absorption due to hydrogen nuclei in both the aqueous and non-aqueous components of the material. It has been found for proteins (5) and for a number of other solid constituents normally found in biological materials, that the proton absorption line width, δf, due to the protons in these solids, is many times larger than for water. Thus, although the absorption for all protons is centered closely on the same frequency, the absorption due to the protons in the solid constituents is essentially constant over the narrow frequency region occupied by the absorption line due to protons in water. In view of this fact, the record provided by the spectrometer is due almost entirely to the protons in water, which follows from the fact that the recorded derivative is zero over frequency regions where the absorption is constant. In other words, the contribution of the protons in the solid material to the ampli-

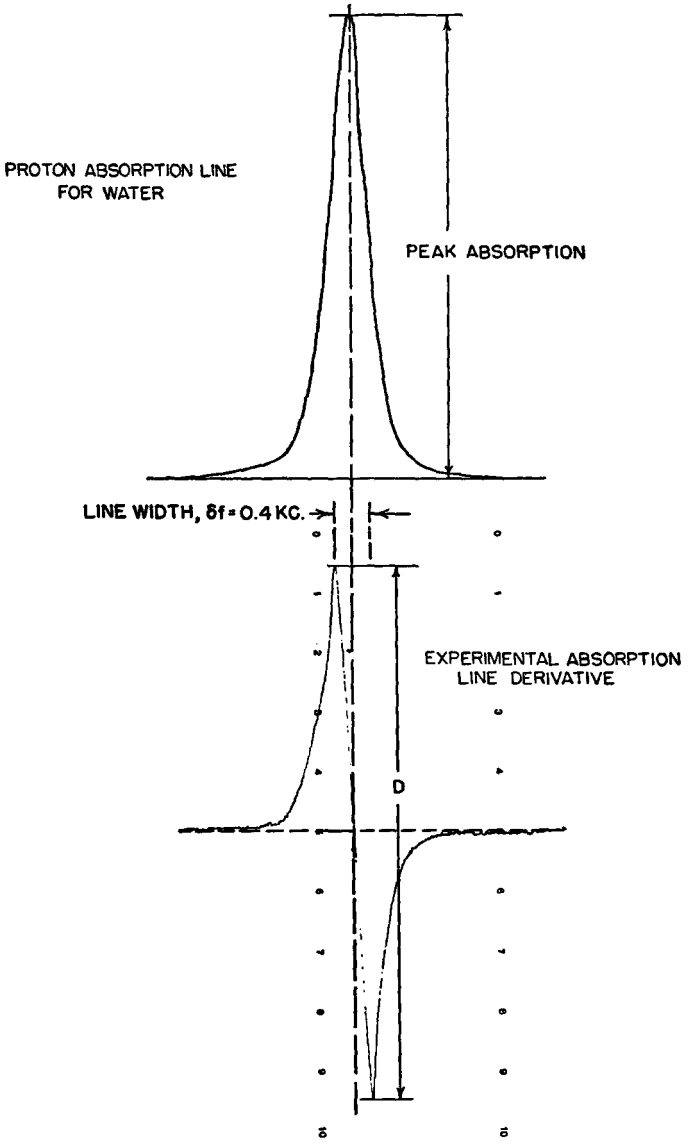

FIG. 1.—The proton magnetic resonance absorption line and its derivative for water. The derivative of the absorption line was determined experimentally; the absorption line was obtained by graphical integration.

tude of the peaks of the derivative curve is negligible, compared to the contribution by the protons in water.

From these considerations it is clear that the problem of determining the water content consists of measuring the absorption by the water protons and distinguishing it from that due to protons contained in the non-aqueous constituents. Although experience has shown that this is possible for many materials, it is likely that systems will be encountered where the line widths of the absorption for non-aqueous components will vary with the water content, or the material will contain other liquid components besides water. In such instances it may be impractical to determine unambiguously the absorption which should be attributed to protons in the water.

From the experimental evidence obtained to date, it appears that moisture content of biological materials can be determined by two methods. One method utilizes either the peak absorption or the quantity D; the other method utilizes the line width of the absorption, δf.

EXPERIMENTAL

Test specimens were placed in standard Pyrex test tubes. For starch and pectin, the specimen consisted of about 1 gram in a tube of 1 cm O. D.; for potato and apple, the specimens weighed about 15 grams and were contained in tubes of 2.5 cm O. D. The tubes were placed in the *r-f* coil of the spectrometer (2) and the derivative of the proton absorption was recorded. The time required for a single determination was about five minutes. The measurements were made at room temperature, $26° \pm 1°$C. At the end of the spectrometer run, the moisture content of the samples was determined by vacuum oven drying procedures.

RESULTS AND DISCUSSION

Figure 2 shows a plot of D versus the water content for starch and for pectin, two materials typical of many of the water sorbing constituents normally found in foods. The vacuum oven method used for determining moisture in starch consisted of heating for five hours at 100°C.; and for pectin, heating for sixteen hours at 70°C. Curves of the type shown in Fig. 2 serve as a calibration of the apparatus for the determination of moisture by the magnetic resonance method. The useful range of the curves shown in Fig. 2 appears to be from about 7 to 20 per cent water, and over this range the curves are almost linear. In this range, experience has shown that the moisture content of fine powders such as starch can be determined to within about ± 0.2 per cent after the calibration curves are established. The precision depends to a large extent on reproducibility of the packing of the test specimens. Thus for materials composed of larger particles which do not pack as reproducibly as starch, the precision of the moisture determinations is lower.

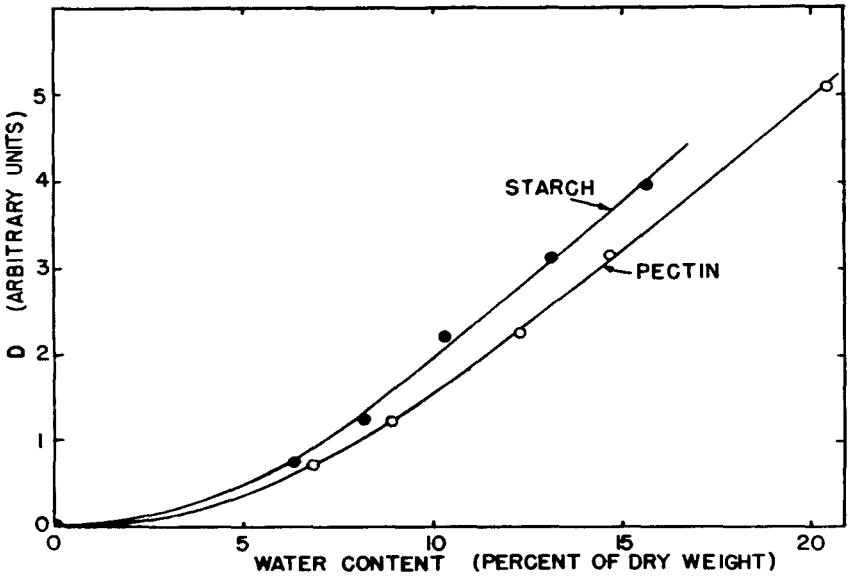

FIG. 2.—Plot of D as a function of the water content for starch and pectin. The modulation of the magnetic field used to record the absorption line derivative was held constant at 1 gauss for these measurements.

A number of experiments have also been performed for materials containing more than 20 per cent water.

Figure 3 summarizes the results obtained for potato containing from 20 to 85 per cent water. The curve compares the results obtained from measurements of D by the proton resonance method with the results of the vacuum oven method which consists of heating for forty hours at 70°C. The general agreement between the two methods is good. Individual differences between the results of the two methods, however, range up to about 5 per cent. Various tests show that about 1 per cent error is attributable to the proton resonance apparatus. The balance of the error is presumed to be due to lack of uniformity in the test specimens. The standard deviation of the results for the raw potato is about ± 2 per cent. A similar result was obtained in tests on apples.

The non-linearity which is apparent at lower moisture contents in the curves shown in Fig. 2 is a result of an interaction between the sorbed water and the sorbent. The interaction causes a change in the character of the proton absorption of the sorbed water which varies with the amount of water sorbed. As a result, D is not a linear function of the water content.

The nature of the changes in the character of the proton absorption which occur at low moisture contents is shown in Fig. 4. The curve shown

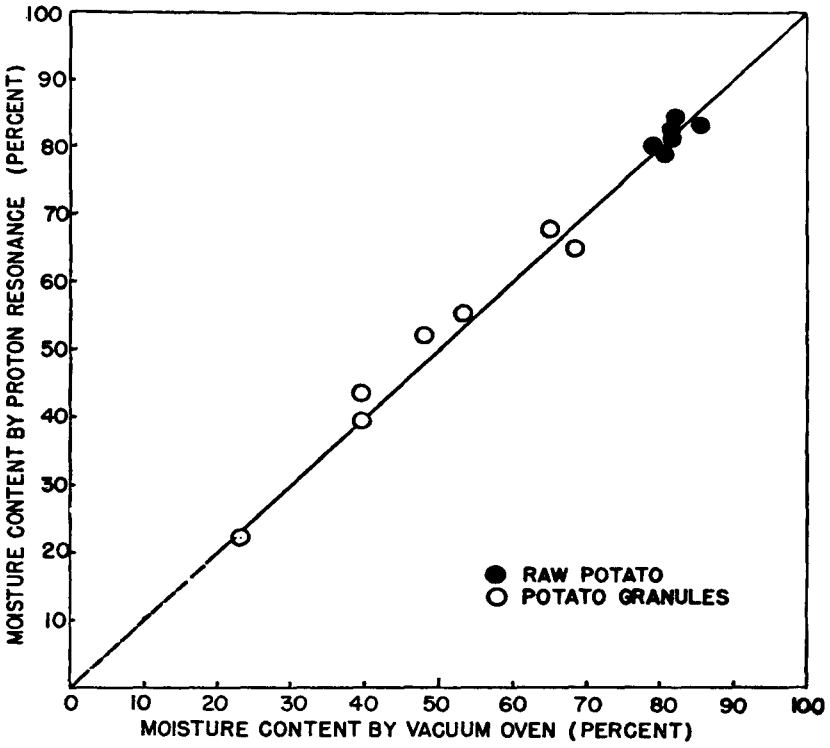

FIG. 3.—Comparison of moisture determinations for potato by proton resonance method and vacuum oven method.

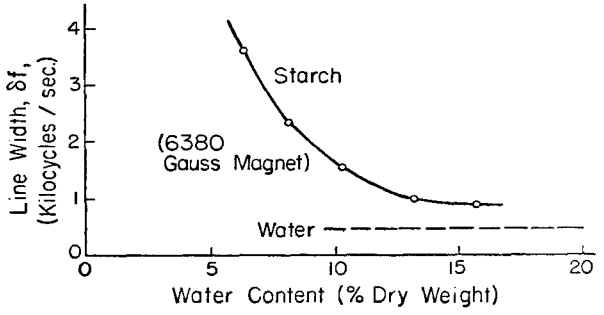

FIG. 4.—Plot of line width, δf, as a function of water content for starch. The modulation of the magnetic field used to record the absorption line derivative was set by successive trials to a value less than the line width.

is a plot of the line width of the proton absorption for water sorbed on starch. For starch containing about 15 per cent water, the line width of the proton absorption is about two times that of water alone. As the water content of the starch is decreased, the line width of the absorption increases rapidly.

The variation in line width (δf) with water content, shown in Fig. 4, may also be used as a convenient measure of the water content at least over the limited range of moisture where significant changes in line width occur. The measurement of δf rather than D has certain advantages since the quantity used and the packing of the test sample are not critical. In addition, the sensitivity of the spectrometer does not have to be as closely controlled as it does when the intensity of the proton resonance is used. The data in Fig. 4 for starch could be used in the range from about 12 per cent to about 5 per cent water or possibly less. Further experiments are required to determine the practical limits of this procedure.

CONCLUSION

The results presented show that the magnetic resonance absorption due to hydrogen nuclei can be used to measure the water content of food materials. The principal limitation on the precision of the method appears to be the lack of reproducibility of the packing or non-uniformity of test specimens. It appears possible that this limitation may be substantially reduced by appropriate changes in the experimental conditions. Such means are currently being investigated.

ACKNOWLEDGMENT

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RAPID METHOD FOR THE ESTIMATION OF WATER-INSOLUBLE FATTY ACIDS (WIA) IN CREAM AND BUTTER*

By FRED HILLIG (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

The official method for the determination of WIA in cream and butter (1, 8) does not meet the needs of convenience for a rapid "sorting" method, nor does the modification of the original method (7) which cuts one and one-half hours from the time necessary to make the determination. Thus the development of a more generally applicable method for the rapid estimation of WIA has been studied by several investigators.

Claydon (2) proposed a method for estimating the quality of cream by comparing, with a permanent standard, the blue color developed by adding crystal violet solution to a cream sample previously treated with a fixed quantity of NaOH.

Greenberg, Nelson, and Wood (3) modified the Knaysi and Guthrie method for the estimation of butter fat acidity to facilitate its use in the field testing of cream for WIA. The method is based on the color produced when xylol saturated with basic neutral red is added to the butter oil.

Roberts, Epple, and Horrall (4) suggested the *alpha*-naphtholphthalein (ANP) color method for measuring fat hydrolysis. In this method the color produced by the addition of the indicator to the isolated fat is measured by comparison with standards. Armstrong and Harper (5, 6) studied this method to adapt the test to routine plant use. They found that a medium green color indicates a WIA content of 100–225 mg per 100 g of fat; light green color, 200–325 mg; orange color, 300–425 mg; and a yellow color, 400–900 mg.

All of the above methods are based on the color produced by the addition of an indicator to the cream or to the isolated fat.

The procedure presented herein adheres to the original principle of isolation and titration, rather than depending upon a colorimetric reaction applied in a heterogeneous system.

By definition WIA are insoluble in water but they are soluble in fat. The quickest procedure for obtaining fat from a cream is by churning. In order to apply a titration procedure to the butter, a method for washing the butter free of water-soluble acids is also necessary. These two requirements were met, first, by churning the cream, cooled to 10°C., in a 125 ml standard-taper, glass-stoppered Erlenmeyer flask, and second, by making use of a special strainer to facilitate washing the butter free of the water-soluble acids. In preliminary experiments, the washed butter was

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dissolved in ether and titrated with standard NaOH, using phenolphthalein as indicator. It soon became apparent, owing to the difficulty in securing a definite end point in the titration, that it would be preferable to isolate the ether-fat solution, necessitating titration with sodium ethylate.† With this procedure, sharp end points were obtained. From the titration and the average mean molecular weight of WIA in cream and butter of 270 (7), the quantity of WIA in the sample can be estimated.

The method follows:

METHOD

PREPARATION OF SAMPLE

Cream.—Weigh 20 g into a 125 ml glass-stoppered Erlenmeyer flask, standard taper #22. Add 25 ml ice cold H_2O, cool to 10°C., and shake until butterfat separates in granular form. Discard if granular fat conglomerates into one lump.

Butter.—Weigh 10 g into 125 ml glass stoppered Erlenmyer flask, standard taper #22, warm to melt butter, and cool until butter is of thick, creamy consistency. Add 50 ml of ice cold H_2O, shake, cool to 10°C., and shake for about 5 sec.

DETERMINATION

Insert filter sieve (see Figures 1, 2, and 3) and pour off the serum layer. Add 50 ml of ice cold H_2O, insert glass stopper, and shake for about 5 sec. Pour off the liquid through inserted filter sieve. Wash 3 addnl times. Dissolve washed butter in 25 ml of ethyl ether, pour into small separatory funnel, wash the Erlenmeyer with a few ml of ether and add to the separatory funnel. Allow to settle for a few minutes and drain off the water-curd layer. Draw the ether-fat solution into a 125 ml Erlenmeyer flask, wash the separatory funnel with a few ml of ether, add to the Erlenmeyer, and titrate with 0.05 *N* sodium ethylate,† using phenolphthalein as indicator. Compute WIA in mg per 100 g of fat.

1 ml of 0.05 *N* sodium ethylate equals 13.5 mg of WIA.

The method was applied to a number of samples of cream and butter. WIA were also determined by the official method (1, 8). The results are given in Tables 1 and 2.

The method was subjected to a collaborative study. The results obtained, compared to those found by the official method, are presented in Tables 3, 4, and 5.

Duplicate determinations by all collaborators were satisfactory. Agreement between collaborators was also satisfactory and the results by the "rapid" method closely approximated those obtained by the official method.

Statistical analysis of the data indicated that in the case of butter there was no significant difference between the two methods. In the case of cream, there was a tendency for the rapid method to give higher results in the region of 1000 mg WIA per 100 g of fat and above. This bias, however,

† The titrant can be prepared as in *Official Methods of Analysis*, 7th Ed., Association of Official Agricultural Chemists, Washington, D. C., 1950, p. 283, 16.29, except that 95% ethyl alcohol may be substituted for absolute alcohol. Sodium or potassium methylate, prepared from colorless methyl alcohol, may also be used.

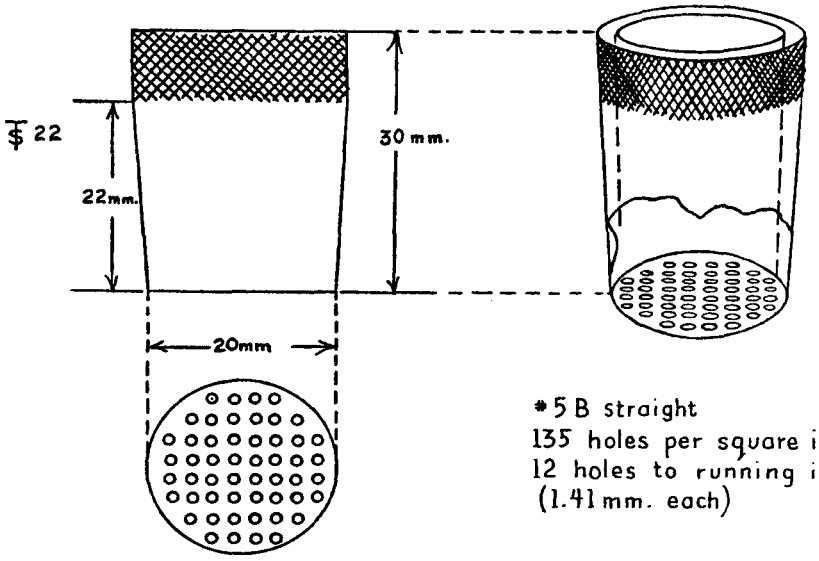

FIG. 1

FIG. 2

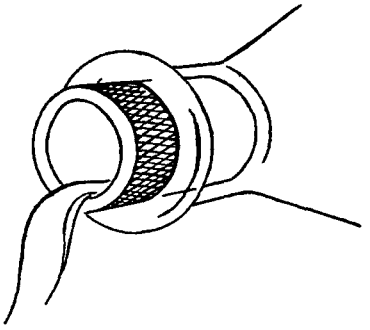

FIG. 3

FIGS. 1, 2, and 3.—Filter sieve.

is not important, since the region of interest is usually considerably below this value. An analysis of variance of the collaborative data also indicated no statistically significant difference between the methods.

SUMMARY

A "rapid" method is proposed for the estimation of WIA in cream and butter. The isolated fat, washed free of water-soluble acids and dissolved in ether, is titrated with standard sodium ethylate. Based on the titration and the average mean molecular weight of WIA in cream and butter of

TABLE 1.—*Comparison of proposed "rapid" method and official method applied to cream*

SAMPLE NO.	RAPID METHOD, MG/100 G FAT	OFFICIAL METHOD, MG/100 G FAT
1	1208	1129
2	1520	1317
3	1538	1428
4	2208	2134
5	1656	1267
6	333	309
7	405	469
8	585	503
9	1448	1432
10	1054	983
11	284	273
12	135	158
13	411	429
14	600	559
15	886	714
16	250	264
17	429	347
18	293	267
19	197	173
20	219	188
21	176	159
22	201	208

TABLE 2.—*Comparison of proposed "rapid" method and official method applied to butter*

SAMPLE NO.	RAPID METHOD, MG/100 G FAT	OFFICIAL METHOD, MG/100 G FAT
1	325	333
2	429	444
3	297	220
4	174	205
5	131	149
6	237	287
7	405	275
8	360	348
9	322	328
10	345	338
11	319	318
12	462	469
13	586	535
14	351	344
15	294	368
16	232	275
17	344	339
18	423	396

TABLE 3.—*Collaborative estimation of WIA in cream using the proposed "rapid" method; mg/100 g fat (Cincinnati)*

SAMPLE NUMBER	OFFICIAL METHOD (HILLIG)	RAPID METHOD			
		(COLL. A)	(COLL. B)	(COLL. C)	(COLL. D)
1	262	250	236	262	281
	271	250	250	279	274
2	450		418	448	438
	433	418	408	413	423
3	255	240	235	236	240
	262	236	229	240	236
4	239	270	273	262	246
	243	262	266	250	266
5	412	340	353	349	373
	418	324	363	349	388
6	224	217	217	231	
	209	221	210	227	
7	209	193	197	193	193
	185	185	204	189	204
8	350	316	365	304	304
	329	316	357	341	324
9	319	363	363	305	378
	304	343	343	328	324
10	956	1048	1065	1073	
	1007	1073	1069	1103	1065

TABLE 4.—*Collaborative estimation of WIA cream using the proposed "rapid" method; mg/100 g fat (St. Louis)*

SAMPLE NUMBER	OFFICIAL METHOD (HILLIG)	RAPID METHOD			
		(COLL. A)	(COLL. E)	(COLL. F)	(COLL. G)
1	335	365	379	365	375
	328	368	379	361	379
2	445	473	480	484	495
	452	484	473	488	491
3	277	236	236	236	274
	359	244	244	244	270
4		255	270	274	
	284	259	266	285	
5	228	215	215	215	
	229	215	223	215	
6	170	177	169	158	
	173	166	177	177	
7	Lost	262	266	298	
		266	278	298	

TABLE 5.—*Collaborative estimation of WIA in cream using the proposed "rapid" method; mg/100 g fat (Kansas City)*

SAMPLE NUMBER	OFFICIAL METHOD (HILLIG)	RAPID METHOD		
		(COLL. A)	(COLL. H)	(COLL. I)
1	174	148	158	149
	179	164	158	170
2	309	318	315	332
	287	315	318	312
3	619	655	604	596
	637	659	619	600
4	546	546	607	534
	557	552	558	546
5	319	335	349	
	311	335	340	326
6	—	208	208	220
		220	208	224
7	261	315	303	276
	268	291	303	291
8	400	396	389	405
	388	386	380	396
9	510	550	516	550
	519	550	526	526

270 (7), the quantity of WIA in a sample can be estimated. Approximately fifteen minutes is required to complete a determination.

ACKNOWLEDGMENT

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COMPARISON OF RESULTS OF ANALYSES FOR POTASSIUM IN JAM AND JELLY BY THE CHLOROPLATINATE AND FLAME PHOTOMETER METHODS

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The increasing use of the flame photometer (1–3) for the analysis of the alkali metals prompted the authors to investigate the possibility of using this instrument to effect a saving of time in the determination of potassium in fruit spreads. Preliminary trials were so encouraging that a broad survey of the method was immediately undertaken on the numerous samples of jam and jelly received in this laboratory for acceptance testing. In the subsequent study of the determination of potassium by flame photometry, the official A. O. A. C. chloroplatinate procedure (4) was used as a basis for comparison.

The procedure for the determination of potassium in fruit spreads by the flame photometer, as developed by the authors, is given below. It is to be noted that a dilute sample solution is atomized through the instrument directly. This eliminates the necessity for ashing the sample, and thereby effects a great saving of time. As is evident from the procedure outlined, the time required for obtaining the flame photometric readings on which the potassium calculations are based is less than ten minutes per sample. It is thus possible to obtain readings for potassium for as many as 50 samples of fruit spreads in an eight-hour day, provided that the sample solutions have been previously prepared.

A Perkin-Elmer flame photometer (Model 52-C) was used in this investigation.

PROCEDURE

REAGENTS

(a) *K stock soln.*—5.2059 g KCl (70 milliequivalents K) per l.
(b) *Li stock soln.*—2.0% Li (184.38 g $Li_2SO_4 \cdot H_2O$) per l.

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* Present address: Armour Research Foundation, Chicago 16, Illinois.

(c) *Standards.*—Each diluted to one l with double distd H_2O:
(1) 0.28 milliequivalent K/l in 0.08% Li (4.00 ml **a**, 40.0 ml **b**).
(2) 0.24 milliequivalent K/l in 0.08% Li (3.43 ml **a**, 40.0 ml **b**).
(3) 0.20 milliequivalent K/l in 0.08% Li (2.86 ml **a**, 40.0 ml **b**).
(4) 0.10 milliequivalent K/l in 0.08% Li (1.43 ml **a**, 40.0 ml **b**).
(5) 0.00 milliequivalent K/l in 0.08% Li (40.0 ml **b**).

SAMPLE PREPARATION

Prepare sample soln as directed in *Methods of Analysis* (5). Transfer 3 ml of this soln to a 100 ml volumetric flask. Add 4 ml of reagent **b** and bring to vol. with H_2O. This constitutes the prepared sample soln.

FLAME PHOTOMETRY

Prepare the flame photometer for use in accordance with the manufacturer's instructions. When the instrument is properly warmed up and the dial settings are at the starting positions, pass double distd H_2O into the atomizer funnel to condition the atomizer and chamber to operating temperature. Pass 2–5 ml of standard into the atomizer funnel to rinse it out. Check for maximum response to the potassium wavelength (ca 767 mμ), by adjusting the wavelength selector while the standard is being atomized and set the selector to maximum deflection. (The method is designed to nullify variations in results due to fluctuations in atomizer rate of flow, gas and air pressure, and disturbances in the electronic circuits. This is accomplished by submitting both the unknown and the standard to the same conditions.) Prepare the standard curve from readings obtained with solns **c**-1, **c**-2, **c**-3, and **c**-4. Plot the dial readings against milliequivalents of potassium, or milligrams of K_2O per 100 grams, if the graph is to be used for K_2O detns directly. The plotted values are the ratios of the intensity of the internal standard and potassium standard vs. the internal standard and potassium unknown. The sample analysis is performed against standard **c**-3 which is alternately atomized with the prepared sample soln. The procedure is repeated with both sample and standard until the difference between sample and standard readings for three consecutive pairs of readings varies less than 2 units on the internal standard dial. The internal standard dial reading of the sample is subtracted from the reading obtained with standard **c**-3 and the corresponding value for potassium is obtained from the standard curve.

EXPERIMENTAL

Samples of fruit spreads were analyzed for potassium content by the above flame photometric procedure and comparative results were obtained by the official chloroplatinate method (4).

The results (averages of replicate runs) are given in Table 1 for jam and in Table 2 for jelly. The average deviation for all samples of jam analyzed to date by both methods was 4.0 mg K_2O per 100 g, taking the result obtained by the chloroplatinate method as the true value. Similarly, the average deviation for the jelly samples was 4.0 mg K_2O per 100 g. It is of interest to note that all of the results for grape jam were higher with the flame photometer than by the chloroplatinate method. An investigation into the effects of interfering substances (6, 7) would probably elucidate this and other trends apparent in the results. The following tables include only those findings where sufficient data were available to demonstrate a significant correlation.

TABLE 1.—*Jam*

SAMPLE	FRUIT	Mg K_2O/100 g A.O.A.C. 20.20 (b)	Mg K_2O/100 g FLAME PHOTOMETER	Mg K_2O/100 g DEVIATION
A	Cherry	100.5	103.0	2.5
B	Cherry	97.5	100.0	2.5
C	Cherry	107.5	111.0	3.5
D	Cherry	96.6	96.0	−0.6
E	Cherry	93.0	94.0	1.0
F	Cherry	100.0	103.0	3.0
G	Cherry	115.1	120.0	4.9
A	Grape	152.2	154.0	1.8
B	Grape	74.9	88.0	13.1
C	Grape	99.7	102.0	2.3
D	Grape	141.0	143.0	2.0
E	Grape	128.6	137.0	8.4
F	Grape	150.7	155.0	4.3
G	Grape	136.0	145.0	9.0
A	Peach	72.7	70.0	−2.7
B	Peach	101.2	103.0	1.8
C	Peach	108.4	113.0	4.6
D	Peach	102.5	105.0	2.5
E	Peach	107.5	114.0	6.5
F	Peach	79.4	86.0	6.6
G	Peach	100.2	100.0	−0.2
H	Peach	104.8	105.0	0.2
A	Pineapple	62.5	65.0	2.5
B	Pineapple	81.0	82.0	1.0
C	Pineapple	67.9	67.0	−0.9
A	Strawberry	117.5	117.0	−0.5
B	Strawberry	90.0	90.0	0.0
C	Strawberry	100.8	100.0	−0.8
D	Strawberry	109.2	103.0	−6.2
E	Strawberry	102.2	103.0	0.8
F	Strawberry	102.2	102.0	−0.2
G	Strawberry	123.1	137.0	13.9
H	Strawberry	113.0	106.0	−7.0
I	Strawberry	124.7	127.0	2.3
J	Strawberry	104.6	113.0	8.4
K	Strawberry	100.3	100.0	−0.3
L	Strawberry	97.1	110.0	12.9
M	Strawberry	104.0	114.0	10.0
N	Strawberry	108.7	110.0	1.3
O	Strawberry	113.4	127.0	13.6

TABLE 2.—*Jelly*

SAMPLE	FRUIT	Mg K_2O/100 g A.O.A.C. 20.20 (b)	Mg K_2O/100 g FLAME PHOTOMETER	Mg K_2O/100 g DEVIATION
A	Grape	101.0	99.0	−2.0
B	Grape	86.0	84.3	−1.7
C	Grape	77.3	79.0	1.7
D	Grape	94.8	95.0	0.2
E	Grape	69.3	76.0	6.7
F	Grape	69.1	84.0	14.9
G	Grape	74.0	80.0	6.0
H	Grape	65.2	73.0	7.8
I	Grape	91.0	94.0	3.0

DISCUSSION

The determinations listed demonstrate that the flame photometer is a useful tool for a preliminary observation of the potassium content in fruit spreads. Nevertheless, due to an occasional lack of good agreement, it is wise to proceed conservatively in the use of this method for regulatory purposes. It is suggested that this method be used in acceptance testing and other control work for approval of samples which, on analysis, obviously show a sufficient potassium content. When results with the flame photometer indicate borderline, doubtful, or deficient quantities of potassium, the results should be verified by the official procedure until closer studies in connection with reproducibility are available for analysis of variance between the flame photometer and chloroplatinate methods.

Although the tabulated data show highly significant correlation, the limited scope precludes definite conclusions with regard to studies on reproducibility. However, comparison of duplicate determinations of potassium for the jam samples showed an estimated average standard deviation of 3.36 mg of K_2O per 100 g for the flame photometer, in contrast to an estimated average standard deviation of 1.01 mg K_2O per 100 g for the comparative data obtained by the A.O.A.C. method. The greater of these standard deviations amounts to less than two pounds of fruit per 55 pounds of sugar in the final calculation for fruit content. Regarding jelly, the flame photometer method compares favorably with the chloroplatinate method, for the standard deviation derived from a considerably smaller number of tests yielded an estimated standard deviation considerably lower than that obtained for the corresponding chloroplatinate determinations. Because of the limited amount of data, no estimates of reproducibility with respect to individual operators was possible. The estimated reproducibility values obtained covered the operations of the laboratory as a whole. It is suggested that the reproducibility values given herein be regarded as indications of possible trends, pending development of sufficient data through collaborative work to establish definite re-

producibility factors. Further studies should extend the scope of these test methods.

SUMMARY

Flame photometry was found to be readily adaptable to the determination of potassium in jams and jellies. The procedure offers a great saving of time over conventional methods. Since ashing is unnecessary, and sample solutions are passed through the instrument directly, basic data for potassium are obtained in about ten minutes per determination. Comparative results for potassium in fruit spreads obtained by flame photometry and by the official chloroplatinate procedure are presented. The applicability of the method for use in screening tests in laboratories receiving numerous samples of fruit spreads for analysis is apparent.

ACKNOWLEDGMENT

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COMPARISON OF RESULTS OF ANALYSES OF PHOSPHORUS IN FRUIT SPREADS BY OFFICIAL VOLUMETRIC AND RAPID COLORIMETRIC PROCEDURES

By JOHN E. DESPAUL and CHARLES H. COLEMAN (Subsistence Testing Laboratory, Chicago Quartermaster Inspection Service Field Office, Chicago Quartermaster Depot, U. S. Army, Chicago 9, Illinois)

A rapid colorimetric procedure for the determination of phosphorus in fruit spreads was developed to expedite the analysis of numerous samples of jam and jelly evaluated in this laboratory. The method is based upon the familiar molybdenum blue reaction used in the current official method, par. **20.48** (1), and utilized in a wide variety of analytical procedures (2–4). The use of perchloric acid for the digestion of many samples was considered hazardous. Furthermore, the use of a water bath at the temperature of boiling water for prolonged periods of time was thought to be impractical. Consequently, the method was investigated with a view toward simplification of the determination of phosphorus in fruit spreads.

A search of the literature and experimentation revealed that the method of Fiske and Subbarow (5) as modified by Snell (6) provided the desired reaction. Since the complete analysis of fruit spreads requires a determination of the ash content, it was not necessary to digest the sample with acid and the same ash could be used for the phosphorus determination. The reducing agent in the reaction selected is 1-amino-2-naphthol-4-sulfonic acid. This eliminates the need of the boiling water bath required in the official procedure, since the blue color is fully developed with this reagent at 37°C. Furthermore, the preparation of the reagents is easier and the number of steps involved in the determination are fewer and simpler than in the official method. In consequence, a considerable saving of time and of required technical skill is effected.

The molybdenum blue reaction of Fiske and Subbarow, as developed by the authors for the colorimetric determination of phosphorus in fruit spreads, is given below. A photoelectric colorimeter was used for the measurement of the colors.

PROCEDURE

REAGENTS

(a) *Ammonium molybdate solution.*—2.5% $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 5 *N* H_2SO_4.

(b) *1-Amino-2-naphthol-4-sulfonic acid.*—Dissolve 1 g $NH_2C_{10}H_5(OH)SO_3H$ in 390 ml of 15% sodium *m*-bisulfite, add 10 ml 20% Na_2SO_3 to clear, and filter. Prepare fresh every two weeks.

(c) *Standard phosphate solution.*—Dissolve 0.4394 g KH_2PO_4 in H_2O. Add 5 ml of 1 *N* H_2SO_4 and 3 drops 0.1 *N* $KMnO_4$. Dil. to one l. Ten ml equals 0.0010 g P. Soln keeps indefinitely.

(d) *Nitric acid.*—1+9.

(e) *Sulfuric acid.*—1+99.

(f) *Sodium hydroxide.*—10%.

SAMPLE SOLUTION

Determine the ash as directed under **20.12** (1), and dissolve it by the addn of 5 ml of HNO_3 (1+9). Heat gently. Filter through No. 31 Whatman 9.0 cm paper into 100 ml volumetric flask. Wash several times with small portions of hot H_2O (90–100°). The total vol. may be ca 75 ml. Add one drop of 1% phenolphthalein soln. Neutralize with 10% NaOH to just pink. Make colorless with H_2SO_4 (1+99). Fill to the mark with H_2O and mix well. This constitutes the prepared sample soln.

COLORIMETRY

Pipet 10 ml of standard phosphate soln into a 100 ml volumetric flask, and make up to vol. with H_2O. (This is the dild standard; its concn is 10 mmg/ml of P.) Transfer 25 ml aliquot of the dild standard to a 100 ml volumetric flask and dil. to 50 ml with H_2O. The concn of P in this flask when brought to vol. will be 2.5 mmg per ml. Into 3 other 100 ml volumetric flasks pipet 15, 25, and 40 ml aliquots of the prepared sample soln. This will provide 15/100, 25/100, and 40/100 dilns of sample solns. Dil. each to 50 ml with H_2O. Add 20 ml of soln **a** to the standard and sample dilns respectively. Swirl, and let stand 15 min. Add 10 ml of soln **b**, and immediately dil. to the mark with H_2O. Mix by inverting 4 times. Place in a 37° H_2O bath for 30 min. and mix at 10 min. intervals. At the end of the 30 min. period, transfer to matched cells for reading in the photoelectric colorimeter. Adjust the instrument to show 37% transmittancy at 650 mμ for the standard. Obtain comparative readings for the three sample dilns and use the reading closest to 37% transmittancy for the unknown, discarding the other two values. (The transmittancy for the sample should be within a few percentage units of the standard. If it is not, a sample aliquot giving a reading near the standard should be taken. This technic for comparing sample with standard is preferable to the preparation of a standard curve, due to slight changes in the curve from day to day).

SAMPLE CALCULATION OF RESULTS

$$\text{Milligrams } P_2O_5 \text{ per 100 grams} = \frac{0.457 \times 0.573 \times 100 \times 100}{0.432 \times 15 \times 25} = 16.2$$

where 0.457 and 0.432 are the optical densities of the sample and standard respectively; 0.573 is the quantity of P_2O_5 in milligrams represented by 25 ml of the standard; 100×100 are the dilution volume in milliliters and the factor for reporting the results per 100 grams respectively; 15 is the sample weight in grams; and 25 is the volume in milliliters of the prepared sample solution.

EXPERIMENTAL

Samples of fruit spreads received for a period of ninety days were analyzed for phosphorus content by the molybdenum blue procedure described. Each value obtained by the new method was verified by the official ammonium phosphomolybdate volumetric procedure given in par. **20.47** (1). All results reported are the averages of duplicate or triplicate checks. In Tables 1 and 2, the comparative results are given for jam and jelly respectively.

DISCUSSION

The results obtained by the official A.O.A.C. method were treated as true values; i. e., no consideration was given to their reproducibility ex-

TABLE 1.—*Comparative results for phosphorus in jam by the official method and simplified colorimetric procedures*

SAMPLE	FRUIT COMPONENT	Mg P_2O_5/100 g A.O.A.C. 20.47 (1)	Mg P_2O_5/100 g COLORIMETRIC	Mg P_2O_5/100 g DEVIATION
A	Cherry	19.4	16.7	−2.7
B	Cherry	19.0	15.3	−3.7
C	Cherry	13.2	15.6	2.4
D	Cherry	24.0	25.1	1.1
E	Cherry	22.5	24.3	1.8
F	Cherry	20.9	22.1	1.2
G	Cherry	24.3	23.0	−1.3
H	Cherry	18.6	19.0	0.4
I	Cherry	23.1	21.6	−1.5
J	Cherry	21.1	22.7	1.6
K	Cherry	22.3	20.3	−2.0
L	Cherry	12.4	15.8	3.4
M	Cherry	22.2	20.8	−1.4
N	Cherry	40.9	38.9	−2.0
A	Grape	12.8	11.3	−1.5
B	Grape	20.2	16.6	−3.6
C	Grape	15.7	14.7	−1.0
D	Grape	20.2	17.3	−2.9
E	Grape	18.6	15.6	−3.0
F	Grape	16.1	14.0	−2.1
G	Grape	15.9	14.9	−1.0
H	Grape	16.9	13.5	−3.4
I	Grape	18.6	15.3	−3.3
J	Grape	16.1	13.1	−3.0
K	Grape	18.9	18.6	−0.3
A	Peach	16.5	14.1	−2.4
B	Peach	13.7	12.9	−0.8
C	Peach	22.3	23.4	1.1
D	Peach	18.6	16.4	−2.2
E	Peach	18.6	19.5	0.9
F	Peach	34.3	32.1	−2.2
G	Peach	25.6	24.3	−1.3
H	Peach	25.1	22.3	−2.8
I	Peach	25.0	21.6	−3.4
J	Peach	28.7	26.2	−2.5
A	Pineapple	24.0	25.2	1.2
B	Pineapple	7.0	8.7	1.7
C	Pineapple	8.3	10.2	1.9
D	Pineapple	8.2	8.9	0.7
E	Pineapple	10.7	8.8	−1.9
F	Pineapple	11.8	11.4	−0.4
G	Pineapple	22.9	22.4	−0.5
H	Pineapple	6.2	6.3	0.1

TABLE 1—*Continued*

SAMPLE	FRUIT COMPONENT	Mg P_2O_5/100 G A.O.A.C. 20.47 (1)	Mg P_2O_5/100 G COLORIMETRIC	Mg P_2O_5/100 G DEVIATION
A	Plum	15.7	14.0	−1.7
B	Plum	18.2	14.4	−3.8
C	Plum	20.6	17.7	−2.9
D	Plum	19.0	17.4	−1.6
E	Plum	19.0	15.6	−3.4
F	Plum	22.0	19.8	−2.2
G	Plum	22.8	21.3	−1.5
H	Plum	20.2	19.2	−1.0
I	Plum	21.0	18.2	−2.8
J	Plum	16.9	14.6	−2.3
K	Plum	22.4	21.6	−0.8
A	Strawberry	23.5	22.2	−1.3
B	Strawberry	23.3	19.6	−3.7
C	Strawberry	24.8	22.8	−2.0
D	Strawberry	17.2	19.5	2.3
E	Strawberry	16.7	15.4	−1.3
F	Strawberry	20.9	20.2	−0.7
G	Strawberry	25.2	21.9	−3.3
H	Strawberry	21.1	21.9	0.8
I	Strawberry	26.9	24.6	−2.3
J	Strawberry	21.9	21.7	−0.2
K	Strawberry	22.7	21.7	−1.0
L	Strawberry	21.6	20.6	−1.0

cept through the averaging of duplicate and triplicate determinations. While such results are not necessarily accurate criteria for judging the accuracy of a new test method, they furnish an indication of value not otherwise obtainable with limited data.

For statistical analysis, it was necessary to treat each kind of jam and jelly separately. However, it is of interest to note that the sum total of all

TABLE 2.—*Comparative results for phosphorus in jelly by the official method and simplified colorimetric procedure*

SAMPLE	FRUIT COMPONENT	Mg P_2O_5/100 G A.O.A.C. 20.47 (1)	Mg P_2O_5/100 G COLORIMETRIC	Mg P_2O_5/100 G DEVIATION
A	Grape	14.9	15.0	0.1
B	Grape	14.5	14.9	0.4
C	Grape	14.5	13.4	−1.1
D	Grape	13.6	11.8	−1.8
E	Grape	17.7	17.2	−0.5
F	Grape	18.6	17.3	−1.3
G	Grape	13.2	13.7	0.5
H	Grape	24.8	22.3	−2.5

observations to date considered collectively gives an average deviation of 1.7 for 79 samples of jam and 1.5 for 34 samples of jelly. The estimated collective standard deviation is 1.5. Part of this data has been excluded from Tables 1 and 2 because of the limited number of observations made for certain types of jam and jelly. Each separate category of jam and jelly listed demonstrates a correlation which is highly significant and, exclusive of grape and plum jam, shows highly significant correlation collectively. It will be noted that all findings for grape and plum jam exhibit minus deviations only, although this deviation is no greater than is exhibited by other jam. Exploring the possibility that a correction constant for grape and plum jam might yield more precise results than are obtainable for other jams, it was found that there is a 99 per cent probability that a correction factor does exist and that it lies in the range of minus 1.16 through minus 3.40 for grape jam, and in the range of minus 1.25 through minus 3.11 for plum jam. The estimated standard deviation for grape jam is 1.12 and for plum jam is 0.925. This can be compared to a rough estimate of the collective standard deviation of 1.5. The minus deviations of plum and grape jam are so similar that the same correction constant could probably be used for both. On the basis of the limited data available, it appears that this constant should be 2.24.

SUMMARY

An improved technique is given for the determination of phosphorus in fruit spreads. The method is based on the molybdenum blue reaction and involves the use of 1-amino-2-naphthol-4-sulfonic acid as a reducing agent. Use of perchloric acid and a 100° water bath are obviated. The procedure is abbreviated and considerably simplified over other colorimetric methods for phosphorus.

The reliability of the method is partially demonstrated by comparison of the results obtained. Comparative results obtained by the official ammonium phosphomolybdate volumetric method were taken as the true value for phosphorus.

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THE LAW OF LAMBERT-BEER, AND COLOR DETERMINATION IN RAW CANE SUGARS AFTER CELITE FILTRATION*

By F. W. ZERBAN and LOUIS SATTLER (New York Sugar Trade Laboratory, New York 4, N. Y.)

Peters and Phelps (1) have stated that the Lambert-Beer law is not always valid under the conditions which prevail in the sugar industry. They found the law to apply only upon the condition that stable transparency was produced in the solution by rigorous clarification with asbestos. Deitz (2) later called attention to the fact that technical sugar products which contain turbidity, or light-scattering particles, do not follow Beer's law, but that the reciprocal of the negative logarithm of the transmittancy for unit thickness and concentration of the solution increases linearly with the concentration. He proposed that in this case the former "absorbancy index," termed "specific absorptive index" by Peters and Phelps, be termed "attenuation index." When the scattering particles are removed, Beer's law is followed, and the constant index is still called "absorbancy index." In a previous publication (3), the authors had found that the filtering agents generally used for color determination in sugar products, namely asbestos and Celite, and also silica gel, do not remove turbidity completely from technical sugar solutions, and that at the same time a small amount of the larger molecules of coloring matter which presumably have the same physical dimensions as the remaining light-scattering particles are also not removed. It was therefore necessary to investigate whether the method by which the color was determined in the collaborative work on this subject, reported at the 1953 meeting of this Association (4), actually produces filtrates that follow Beer's Law.

For this purpose, solutions of three raw sugars, one each from Cuba, Puerto Rico, and Hawaii, were prepared and filtered with Celite analytical filter aid, as described in the 1953 report of the Associate Referee. The transmittancies of the solutions were measured with the Beckman Model B spectrophotometer. Then each of the filtered solutions was diluted with colorless sucrose sirup of about the same Brix as the filtered sugar solutions, so as to contain about 80, 60, 40, and 20 per cent of the coloring matter of the original solution. The method of Peters and Phelps (1) was

* Presented at the Sixty-seventh annual meeting of the Association of Official Agricultural Chemists, Oct. 12, 13, and 14, 1953, at Washington, D. C.

used, with dilution by weight with the colorless sucrose solution, and the transmittancies of these diluted solutions were again determined in cells of appropriate thickness. Thicknesses were 0.106, 0.246, 0.509, 1.001, 2.006, and 3.007 cm, and were selected so that the transmittancy of each solution fell within the proper range.

The colorless sucrose sirup was prepared by the preferred method of the U. S. National Committee of the I.C.U.M.S.A., as follows:

"Weigh 360 g of confectioners white sugar into a Pyrex beaker, add 356 ml of hot distd H_2O (90–95°C.), and stir until thoroly dissolved. Defecate with 3.6 ml of dil. H_3PO_4 (44 g of 75% H_3PO_4 per liter) and enough $Ca(OH)_2$ to adjust *p*H to 8.0 ± 0.2. Stir to complete the defecation. Place in hot H_2O bath (70–80°C.). Add 6 g (ca 1.5% of dissolved solids) of Darco S-51 and leave in bath for one hour with frequent stirring. Add one heaping tablespoon of kieselguhr and filter through a double thickness of filter paper. Adjust the density of the soln, and the *p*H to 7.0 ± 0.2. Polish by passing the soln through a 'fine' sintered glass filter."

In this work a medium granulated sugar was used and the kieselguhr employed was the analytical grade of Celite. The transmittancy of a 10 cm layer of decolorized sirup was 100.0 per cent at both 505 and 560 mμ, against distilled water.

The final colorless sugar sirup had a Brix of 54.14 by refractometer, and the final filtrates of the raw sugar solutions had a Brix in the same neighborhood: Cuban 54.14, Puerto Rican 54.45, Hawaiian 54.81. All solutions were adjusted to *p*H 7.0 ± 0.2.

The concentration of colored dry substance in grams per milliliter by refractometer, in the different solutions prepared, is shown in Table 1.

TABLE 1.—*Concentration of colored dry substance in the solutions*

COLORED SOLUTION, PARTS	COLORLESS SIRUP PARTS	GRAMS PER MILLILITER		
		CUBAN SUGAR	PUERTO RICAN SUGAR	HAWAIIAN SUGAR
100	0	0.7220	0.7277	0.7345
80	20	0.5780	0.5822	0.5859
60	40	0.4334	0.4364	0.4408
40	60	0.2881	0.2910	0.2926
20	80	0.1441	0.1445	0.1476

The transmittancies of these solutions were read as stated above, and the attenuation indices were calculated at two wavelengths: at 560 mμ as given by Peters and Phelps, and also at 505 mμ, the average mean wavelength of the integrated area below the attenuation curves (5). The values are shown in Table 2, together with the percentage deviations from the averages.

The results show that the percentage deviation from the means averages only 0.78 per cent, exceeds 3 per cent in only one case, and exceeds

TABLE 2.—*Attenuation indices at different concentrations of colored dry substance, and percentage deviations from the calculated mean*

CONCENTRATION AT DIFFERENT DILUTIONS, PER CENT	ATTENUATION INDICES					
	CUBAN SUGAR	DEVIATION FROM MEAN, PER CENT	P.R. SUGAR	DEVIATION FROM MEAN, PER CENT	HAWAIIAN SUGAR	DEVIATION FROM MEAN, PER CENT
Measurements at 560 mμ						
100	1.2783	+0.39	0.4144	+0.27	0.6715	+2.33
80	1.2736	+0.02	0.4176	+1.04	0.6476	−1.31
60	1.2673	−0.48	0.4195	+1.50	0.6609	+0.72
40	1.2726	−0.06	0.4148	+0.36	0.6629	+1.02
20	1.2752	+0.14	0.4004	−3.12	0.6380	−2.77
Av.	1.2734	±0.22	0.4133	±1.26	0.6562	±1.63
Measurements at 505 mμ						
100	2.2156	+0.29	0.6812	−0.60	1.0645	+1.42
80	—	—	0.6847	−0.09	1.0359	−1.31
60	2.2008	−0.42	0.6889	+0.53	1.0561	+0.62
40	2.2065	−0.14	0.6867	+0.20	1.0529	+0.31
20	2.2149	+0.26	0.6849	−0.06	1.0385	−1.06
Av.	2.2095	±0.22	0.6853	±0.30	1.0496	±0.94

2 per cent in only two other cases. It is found that the deviation is not greater than was previously reported for the attenuation indices of raw sugars (4). It may be concluded that Beer's Law holds within the limit of error of transmittancy measurements for solutions obtained from filtration with Celite analytical filter aid by the method described in the report submitted to the Association, and that the negative logarithm of the transmittancy, for unit thickness and concentration, is equivalent in practice to the absorptivity index.

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CARBOHYDRATE COMPOSITION OF HYDROL

By EDNA M. MONTGOMERY and F. B. WEAKLEY (Northern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Peoria 5, Illinois, and Corn Industries Research Foundation, New York 17, New York)

"Hydrol" is a carbohydrate-rich, molasses-like sirup obtained as a by-product of the manufacture of the industrial corn sugars (the dextroses) by the hydrolysis of cornstarch in the presence of aqueous mineral acid. Hydrol is the final mother liquor remaining after the separation of the crystalline sugar from the refined corn sugar sirups. Produced at an annual rate of about 100,000 tons (1), hydrol is of considerable commercial importance. It is used chiefly as an ensilage supplement and in stock feeds. The composition of hydrol is not yet definitely established; published information regarding the kinds and amount of carbohydrates present is relatively meager.

The use of hydrol as well as the selection and development of methods for its analytical evaluation are dependent on adequate knowledge of its composition. The objective of the present work, therefore, was to analyze hydrol in enough detail to obtain concrete evidence of both the amounts and the types of carbohydrates in the sirup. The work was developed as a fundamental exploration including three phases: (*a*) a chromatographic fractionation, by means of a carbon column, of the carbohydrates in hydrol according to molecular size, and the quantitative recovery of the respective fractions; (*b*) structural investigation of some of the components; and (*c*) use of a direct method for the determination of the total carbohydrate content of hydrol.

SAMPLES ANALYZED

Two samples of hydrol, A and B, were used in these studies. The procedure used for the analysis of sample A was designed to insure quantitative data on the amounts of mono-, di- and higher oligosaccharide fractions present. A more detailed analysis was made of sample B to provide additional information on the composition of the disaccharide fraction.

Sample A was furnished in 1952 by the Clinton Foods, Incorporated, of Clinton, Iowa. Routine analyses on this sample were made in seven laboratories under the direction of a Committee on reducing power standards headed by W. R. Fetzer of Clinton Foods, Incorporated. The average results,[1] expressed as per cent, are: moisture, 26.04; ash, 8.80; protein, 0.199; total solids, 73.96; total sugars,[2] 65.0; reducing sugars, 55.14; dextrose equivalent (reducing power as dextrose, dry basis), 74.55. Additional

[1] Private communication from W. R. Fetzer.
[2] Calculated as sirup free of water, protein, and ash [100 − (26.04 + 0.20 + 8.80)].

analyses obtained by the authors, expressed as per cent, include dextrose equivalent, 74.62; total carbohydrate evaluated by the anthrone (2) method, 67.2, calculated as dextrose; 5-hydroxymethylfurfural, 0.31.

Sample B was furnished in 1948 by the Corn Products Refining Company of Argo, Illinois. Analyses obtained by the authors on this sample, expressed as per cent, are: ash, 9.65; total solids, 74.5; dextrose equivalent (reducing power as dextrose, dry basis), 75.5. These values are within the ranges for the respective determinations found by Corn Products Refining Company[3] on routine production samples. In addition, in these samples the Company found approximately 0.20 per cent of protein and 2 per cent of 5-hydroxymethylfurfural.

Chromatographic analyses were made on ash and protein-free sirups; each sample of the hydrol was weighed, de-proteinized by addition of basic lead acetate, and then de-ionized. In order to avoid loss of sugar by occlusion, the sirup was first diluted to 5 per cent concentration and the *p*H adjusted to 6.0. The precipitated proteins were removed by filtration and shaken with distilled water to remove all sugars; the washings and filtrate were then concentrated to a smaller volume and passed through columns of Amberlite resins IR-100 and IR-4B[4] to remove lead, iron, sodium, etc., and acid ions.

METHODS OF ANALYSIS AND IDENTIFICATION OF SUGARS

The determination of the yields of oligosaccharide fractions was made with the aid of the refractive index.[5] The spectrophotometric anthrone method (2) was used to confirm the carbohydrate character of some of the fractions and to measure directly the total carbohydrate content of sample A. Reducing powers were determined by A.O.A.C. standard procedures, either by the Lane-Eynon (3) or Munson-Walker unified methods (4).

Dextrose was shown in the analysis of sample A to be the only reducing substance present in the first or aqueous fraction from the carbon column by paper partition chromatography (5), using 2 per cent solutions applied one to five times at the starting point on the paper. Whatman No. 1 paper was used with a solvent mixture of *n*-butanol, pyridine, and water (6:4:3). The chromatograms, developed in eighteen to twenty-four hours, were air-dried and sprayed with ammoniacal silver nitrate. Similar chromatograms showed no dextrose in the disaccharide fraction.

In the analysis of sample B, dextrose was identified and measured quantitatively by crystallization as the α-dextrose monohydrate and the probable loss in the crystallization step was minimized by the large size (69 grams) of hydrol sample used for the isolation. The sugars identified in the disaccharide fraction were isolated as crystalline octaacetyl deriva-

[3] Private communication from Corn Products Refining Company, Argo, Illinois.
[4] The mention of specific commercial products throughout this paper is for convenience and does not imply that they are endorsed or recommended by the Department of Agriculture over others of similar nature not mentioned.
[5] National Bureau of Standards Circular C440 ,p. 652.

tives. Each was crystallized to high purity as shown by specific rotation and mixed melting points with authentic substances. Some of the more weakly adsorbed disaccharides were removed from the column in the aqueous effluent (dextrose fraction) in the analysis of sample B. These disaccharides were recovered as the residual sirup after the isolation of the readily crystallized α-dextrose monohydrate; the essentially disaccharide character of the sirup was determined by paper partition chromatography.

Throughout the chromatographic analyses, extreme efforts were made to recover all of the carbohydrate material; in order to minimize irrevocable losses on the carbon, the work was carried out after the columns had been in use for several weeks. The over-all accuracy of the methods is probably not greater than ± 1 per cent but since some losses, however small, must have occurred, the results reported represent minimum rather than maximum or average values for the amounts of the saccharides in the hydrols.

TYPES OF CHROMATOGRAPHIC APPARATUS

Two types of carbon columns were used. Both are adapted from previously published methods. The type (6) used in the analysis of sample A is shown in Fig. 1. The chromatographic tube of Pyrex double-tough glass pipe[6] is 3 inches in diameter and 48 inches long, drawn out at the lower end to a diameter of 8 mm. The tube is packed to about one-third of its capacity with 680 g of intimate mixture of equal parts by weight of Darco G60 Carbon[7] and Pittsburgh S.G. carbon,[8] conveniently introduced as a water slurry. The column rests on a plug of glass wool; a thin layer of glass wool on the top of the column prevents splashing when liquid is introduced. The top of the tube is slightly enlarged and fitted with a cast iron flange. The top of the flange is overlaid with a rubber gasket on which rests a stainless steel plate. Brass tubes, $\frac{3}{8}$ inch (0.91 cm) in diameter, led through the plate, furnish a safety outlet and a T-shaped inlet for nitrogen gas and liquids.

When the automatic fraction collector was used, the column was usually operated under a pressure of 3 to 8 pounds. The turntable[9], 32 inches (80 cm) in diameter, is supplied with interlocking gears so that speeds varying from 7.5 to 130 hours for one complete revolution are possible. Eighty 50 ml calibrated tubes are used at one time. Uniform collection fractions are obtained by use of glass adapters with spoon-shaped sidearms, fitted into the collection tubes (Fig. 1). Where higher flow rates are desired, the effluent flow can be increased to 2–4 liters per hour by the use of 30 to 50 pounds of pressure; the effluent is received directly into flasks. Lowering of the flow rate as a result of too close packing of the carbons after long periods of daily use under high pressure was avoided by allowing the carbons to expand in the solvent without pressure and with the tube outlet closed when the column was not in use. For safety, the chromatographic tube is placed in a wooden box with a removable Lucite window on one side.

The column used in the analysis of sample B was described in 1949 by Montgomery, Weakley, and Hilbert (7). The method used in the refractionation of the disaccharide fraction was adapted from the work of Claesson (8).

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[6] Stemmerick Supply Inc., 1018–20 Russell Blvd., St. Louis, Missouri.
[7] The Darco Corporation, 60 East 42nd Street, New York, New York.
[8] The Pittsburgh Coke and Chemical Company, Pittsburgh, Penna.
[9] Detailed drawings and descriptions of the turntable and of the glass parts of this and a somewhat similar fraction collector will be included in a forthcoming publication from this laboratory.

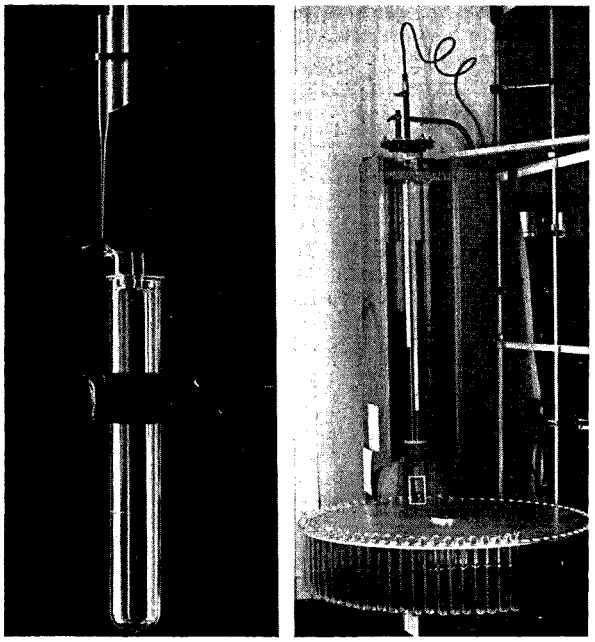

FIG. 1.—Chromatographic column and turntable with a detail of collection tube; the shoulder on the adapter reaches to the bottom of the tube.

ANALYTICAL

Analysis of Sample A.—A 25.0 g portion of sample A was pretreated and brought to a volume of 200 ml. The solution, adjusted to *p*H 7.0, was then placed directly on the carbon column by removing the flange. Dextrose was removed first by eluting the column with water under a pressure of 30 to 50 pounds; aqueous washing was continued until the effluent, concentrated 500-fold, showed no reducing sugar. Recovered dextrose was determined by reducing power calculated as the anhydrous sugar, after the total aqueous effluent (13.5 l) had been concentrated to a small volume. The total yield of dextrose, determined as the anhydrous sugar by the Munson-Walker and Lane-Eynon methods, was 10.20 g; calcd $[\alpha]_D^{25}$ in water: +51.8°.

The disaccharide portion of the hydrol was displaced with water containing 5% aqueous ethanol at a pressure of 5 to 8 pounds. In order to obtain some evidence of the complexity of the fraction without loss of any of the carbohydrate material, the effluent was collected on a turntable in 50 ml fractions and the course of the displacement was followed by measurement of the optical rotation of the fractions. Plotted, the rotations showed five sharp peaks. The total of 12.4 l of effluent was concentrated to sirup weighing 17.65 g. The index of refraction, n_D^{20}, was 1.3754; the total calculated yield of disaccharide sugars, 4.74 g; carbohydrate by anthrone (2), 4.86 g calculated as dextrose (equivalent to a disaccharide weight of 4.62 g).

Higher saccharides were displaced with 15% ethanol at a higher pressure of 30 to 50 pounds. The total volume of effluent, 3.75 l, was concentrated to a sirup

weighing 5.134 g; n_D^{20}, 1.3735. The total calculated yield of higher saccharides was 1.32 g and carbohydrate by anthrone, 1.34 g as dextrose. In the analysis of sample A, the total yield of sugars was 16.26 g.

Analysis of Sample B.—A portion of sample B weighing 69.0 g was pretreated, brought to a volume of one l, (*p*H 5.5), and fractionated on a series of three carbon-Celite columns (7) containing a total weight of carbon of 814 g. The dextrose was collected in the aqueous effluent and was followed polarimetrically, and by reducing power. The columns were washed with water as in the analysis of sample A. The total volume of effluent, 12.6 l, was concentrated *in vacuo* to a sirup weighing 49.95 g. The sirup, which had an index of refraction of 1.4416 (60% solids) was seeded with crystalline α-dextrose monohydrate and placed on a shaking machine at room temperature overnight. A heavy crop of sugar was obtained. This was separated by filtration, washed, and air-dried to constant weight; the crystalline sugar weighed 23.70 g. Successive crystallizations resulted in a series of eight smaller fractions of sugar; the total weight of all fractions was 31.95 g. The samples were collected and pulverized to a uniform mixture, identified as α-dextrose-monohydrate, m.p. 81–82°; $[\alpha]_D^{25}$ +47.6° (*c*, 2.456 g/100 ml; *l*, 2 dm; in water); reducing power as anhydrous dextrose, 90.7%. The total yield was 31.05 g of hydrate or 28.2 g of anhydrous dextrose.

The adsorbed sugars remaining on the carbon-Celite column were displaced with 4% aqueous phenol, the column was washed with 95% ethanol and the combined effluents were concentrated *in vacuo* to remove organic solvents. The resulting sirup was rechromatographed on a series of two smaller columns, 23 cm × 5 cm and 16 cm × 3.4 cm, the larger column placed on top. The disaccharides were displaced with 0.5% aqueous phenol after the columns were washed with 2% acetic acid. Table 1 records the fractions of phenolic effluent together with their respective saccharimeter readings and reducing powers.

RESULTS

The calculated rotations in the last column of Table 1 show a wide variation, suggesting the presence of several sugars. Four disaccharides

TABLE 1.—*Disaccharide displacement with 0.5 per cent phenol*

EFFLUENT SAMPLE	VOLUME	$R_{Cu}M\text{–}H_2O$[a]	°S(*l*,4)[b]	$[\alpha]_D^{25}$ [c]
	ml	*mg/ml*		
1	232	—	0.10	—
2	490	2.13	4.5	+183
3	270	3.02	4.55	131
4	330	1.87	2.80	129
5	322	1.98	2.80	122
6	300	2.00	3.20	138
7	360	3.25	4.45	118
8	238	3.11	2.60	72
9	232	2.91	2.00	60
10	236	2.71	1.80	58
11	290	2.82	1.60	49
12	320	1.83	1.63	77

[a] Reducing power as maltose hydrate.
[b] Observed degrees on saccharimeter having International Sugar Scale read in a tube 4 dm in length.
[c] Calculated for purposes of comparison on the assumption that the concentration of carbohydrate was equal to the reducing power expressed as maltose hydrate. Deviations from the maltose hydrate $[\alpha] = +130°$ provide an indication of the presence of other sugars differing in reducing power, optical rotation, etc.

were isolated and identified after second and third chromatograms were made. The fractions 1 to 6 (Table 1) were combined for a second resolution resulting in the identification of two sugars. The presence of α,α-trehalose in the first three new fractions was suggested by a positive rotation and negligible reducing power. Two later successive fractions of the effluent had reducing power and rotations which gave calculated specific rotations of +128° and +130°, suggesting the presence of maltose.

Acetylation of the combined nonreducing fractions from this second chromatogram led to the crystallization of an acetate; purified by recrystallization, 1.19 g of well-built crystals were obtained and were identified as octaacetyl α,α-trehalose, m.p. 100–101°; mixd. m.p. 99–100°; $[\alpha]_D^{25}$, +162.1° (*c*, 2.046; *l*, 2; U. S. P. chloroform).

Similarly, the sugar in the two reducing fractions mentioned was identified as the ester, octaacetyl β-maltose; the pure crystalline substance weighed 1.81 grams; m.p. 160°; m.m.p. 160°; $[\alpha]_D^{25}$, +62.7° (*c*, 2.047; *l*, 2; U. S. P. chloroform).

The remaining fractions from the second chromatogram were combined with fractions 7 to 12 from the first (Table 1) and refractionated as before by means of a third chromatogram. The calculated specific rotations of the fractions decreased progressively from +117° to +16° and then rose to +70° for two small fractions. Combination and acetylation of the major fractions (+117 to +16°) followed by fractional crystallization of the acetate obtained led to the isolation of the octaacetates of β-brachiose and β-gentiobiose as pure substances. The total yield of brachiose derivative, pure crystalline octaacetyl 6-[α-D-glucopyranosyl]-β-D-glucose, was 10.10 g; m.p. 142°; m.m.p. 142°; $[\alpha]_D^{25}$, +98.0° (*c*, 2.146; *l*, 2; U. S. P. chloroform). The total yield of gentiobiose derivative, pure crystalline octaacetyl 6-[β-D-glucopyranosyl]-β-D-glucose, was 3.37 grams; m.p., 192.5°; m.m.p., 192.5°; $[\alpha]_D^{25}$, −5.25° (*c*, 2.548; *l*, 2; U. S. P. chloroform).

The yields of crystalline octaacetates of these disaccharides were estimated by past experience in acetylating relatively pure sugars to be approximately 75 per cent. This figure was used, therefore, in calculating the original amounts of these identified disaccharides: *i.e.*, brachiose, 6.80 grams; gentiobiose, 2.26 grams; maltose, 1.24 grams; α,α-trehalose, 0.76 grams.

The unacetylated fractions (calculated $[\alpha]_D^{25}$, +70°)[10] together with final washings, were concentrated to a sirup weighing 1.424 gram; n_D^{20}, 1.3840; estimated yield of disaccharide, 0.45 gram.

The residual sirup from the dextrose crystallizations together with the concentrate of the washings preceding the disaccharide chromatogram section gave a sirup weighing 5.297 grams; n_D^{20}, 1.3815 or an estimated yield of additional disaccharides of 1.60 gram. A paper chromatogram prepared from this sirup showed that it contained disaccharides and traces of glu-

[10] Footnote *c*, Table 1.

cose. The combined weights of the disaccharides described above equal 13.11 gram.

Higher sugars were displaced from the column with the aid of 6 liters of 3.5 per cent aqueous phenol followed by 2 liters of 95 per cent ethanol. The concentrate of these solutions weighed 11.09 gram; n_D^{20}, 1.3816; estimated yield of saccharides 3.36 grams. Diluted to exactly 100 ml, the sirup had a rotation of +17.6° S (*l*, 1) and a reducing power equal to 0.69 gram as dextrose. An average $[\alpha]_D^{25}$ value of +180° may be calculated for the oligosaccharides present; from the weight and reducing power, the average degree of polymerization is calculated to be 5 if it is assumed that only reducing saccharides are present.

The total weight of carbohydrates isolated as dextrose, disaccharides, and higher saccharides was 44.7 gram.

A summary of all of the analytical data assembled on hydrol, both from the manufacturers and by chromatographic analyses of purified sirups, is shown in Table 2.

DISCUSSION

The relative amounts of mono-, di- and higher oligosaccharides (Table 2) are in excellent agreement in the chromatographic resolutions of samples A and B. The results expressed on the basis of the total sirups are more reliable than those calculated to a dry substance basis, since the accuracy of the individual moisture determinations is not too great. Thus, the moisture value of 26.0 used in the analysis of sample A is an average of values varying from 25.43 to 26.55, submitted by seven workers.

The total carbohydrate content of hydrol, an essential part of the analysis, appears to be well established at about 65 per cent. In the analysis of sample A, the sum of the mono-, di- and higher saccharide fractions was 65.1 per cent, which becomes 66.5 per cent when all weights are calculated to a dextrose unit basis. This last figure is in very good agreement with the value for total carbohydrate, calculated as dextrose, of 67.2 per cent obtained by the anthrone method on sample A.

The spectrophotometric anthrone method offers promise as a routine procedure for the determination of total carbohydrate in hydrol and for such use, it has three advantages: (*a*) it is a direct analysis involving complete breakdown of the carbohydrate; (*b*) it is quite insensitive to most non-carbohydrate impurities; and (*c*) it has been shown that with proper equipment and care the standard deviation on samples containing no interfering substances is 0.5 per cent[11]; the accuracy is estimated to be at least as great as ±1.5 per cent.

An alternative approach to the routine estimation of the total carbohydrate content of hydrol is suggested by the observation that the weight

[11] Private communication from work submitted for publication in *Anal. Chem.* by T. A. Scott, Jr., and E. H. Melvin of this laboratory.

TABLE 2.—*Analyses of the hydrols*

CONSTITUENTS	ANALYSES			
	HYDROL SIRUP BASIS		DRY BASIS	
	per cent		*per cent*	
Sample A. Non-carbohydrate[a]				
Moisture	26.0			
Protein	0.2		0.3	
Ash	8.8		11.9	
	35.0		12.2	
Carbohydrate[b]				
Dextrose ($C_6H_{12}O_6$)	40.8		55.2	
Disaccharides	19.0		25.6	
Higher Oligosaccharides	5.3		7.2	
	65.1		88.0	
Totals		100.1		100.2
Sample B. Non-carbohydrate[c]				
Moisture	25.5			
Protein	0.2		0.3	
Ash	9.6		13.0	
	35.3		13.3	
Carbohydrate[d]				
Dextrose ($C_6H_{12}O_6$)	40.9		54.9	
Disaccharide	19.0		25.5	
Higher Oligosaccharides	4.9		6.5	
	64.8		86.9	
Totals		100.1		100.2

[a] Averages of analyses by seven analysts in different laboratories including the Northern Regional Research Laboratory. Results supplied by W. R. Fetzer of Clinton Foods, Inc.

[b] By chromatographic analysis, reproducibility ±1%. Direct anthrone determination of total carbohydrate in sirup gave 67.2% calculated as dextrose. This figure agrees very well with the value of 66.5% obtained by chromatographic data if the di- and higher oligosaccharide weights are calculated as dextrose unit weights.

[c] Moisture and ash values determined by the authors. These are within the range found for similar samples by Corn Products Refining Company of Argo, Illinois. The protein value is an average of the company's results on similar samples.

[d] Dextrose determined as crystalline sugar, reproducibility ±0.5%. Disaccharide and higher oligosaccharide yields are calculated.

of sirup after subtraction of the water, ash, and protein content was essentially identical with the total carbohydrate weight recovered from the chromatographic analysis of sample A (Table 2). Thus, total carbohydrate might be estimated by difference in a manner analogous to the determination of nitrogen-free extract in foods (14), but with the advantage of a lesser complexity of non-carbohydrate constituents.

The total carbohydrates in hydrol cannot be determined by reducing power alone. We have shown that the sirup contains saccharides of several molecular sizes and that one disaccharide, α,α-trehalose, is non-reducing. Thus, the reducing power of hydrol sample A, expressed as dextrose, accounted for only about 85 per cent of the actual weight of saccharides shown to be present by chromatographic or anthrone analysis. It is apparent that, if reducing power is to be used, an acid hydrolysis step analogous to that used in the A.O.A.C. method for the determination of starch (9) would have to be incorporated in the procedure. Since the 1,6′-linked glucose polymers are less easily hydrolyzed than starch (mainly 1,4′-linked), suitable hydrolysis conditions would have to be established, along with the correction factor for the loss of dextrose by destruction and condensation during the hydrolysis.

Earlier reports on the composition of hydrol (10–12) have shown that it contains chiefly carbohydrates; these include a large amount of dextrose, a sizable fraction of disaccharides and smaller fraction of polysaccharides. The sirup thus resembles the type of mixtures obtained if dextrose is treated with aqueous mineral acid. Former workers, e.g., Wohl, Fischer, and Frahm (13) have shown that synthesis of polysaccharides from dextrose occurs in the presence of relatively high concentrations of acids (2.5 to 40 per cent) and leads to an equilibrium between dextrose and its polymers. Fetzer and co-workers (15) obtained similar equilibria by using lower acid concentrations of *p*H 1.0 to 2.5 as in the industrial hydrolysis of starch. Along with the re-synthesis some disintegration of sugars occurs, as shown by the presence of 5-hydroxymethylfurfural in industrial hydrols; recently the use of specially prepared carbons during the processing cuts the amount of it to about 0.3 per cent.

The authors were able to make better progress in the analysis of hydrol than earlier workers because of the chromatographic techniques now available; however, as a result of earlier attempts to resolve the disaccharide fraction into its components, one sugar, gentiobiose (6-[β-D-glucopyranosyl]-D-glucose) was isolated by Berlin (12) and the presence of an α-1,6′-linked disaccharide was at least suspected by Coleman (11).

Our use of carbon column chromatography has resulted in a more reliable resolution of a typical specimen of hydrol into mono-, di-, and higher saccharide fractions than has been yet published (10–12). In addition, four constituents of the disaccharide fraction were isolated and identified, *i.e.*, two new components, maltose and α,α-trehalose, one previously suspected, brachiose (11), and the one previously isolated, gentiobiose (12). The resulting semi-quantitative analysis of the disaccharide fraction is presented, therefore, in greater detail.

Experimental evidence (13) of the condensation of glucose in the presence of acid with the formation of glucosidic linkages points to a preference for condensation between the anomeric or hemiacetal hydroxyl and

the primary hydroxyls, yielding 1,6′ bonds; other linkages are formed but the quantity of these is less. Stereoisomerization takes place during the reactions resulting in the formation of both α- and β-linkages. Frahm (13) has presented evidence that the equilibrium is far to the α-side.

In agreement with his work, the present studies (Table 3) showed that the 1,6′-linked disaccharides, brachiose, and gentiobiose, accounted for about 71 per cent of the disaccharide fraction. The preferential formation of α-linkages is demonstrated both by the presence of three times as much of the α-1,6′-linked disaccharide, brachiose, as of the β-1,6′-linked disaccharide, gentiobiose, and by the fact that the other two disaccharides

TABLE 3.—*Composition of carbohydrate fraction of hydrol (Sample B) by chromatographic analysis and isolation*

COMPONENT	PER CENT OF TOTAL CARBOHYDRATE
Monosaccharide	
Dextrose (D-glucose)	63
Disaccharides	
Brachiose (6-[α-D-glucopyranosyl]-D-glucose	15.2[a]
Gentiobiose (6-[β-D-glucopyranosyl]-D-glucose	5.0[a]
Maltose (4-[α-D-glucopyranosyl]-D-glucose	2.8[a]
α,α-trehalose (α-D-glucopyranosyl-α-D-glucopyranoside)	1.7[a]
Others	4.7
Total Disaccharides	29.4
Higher Oligosaccharides	7.5

[a] Estimated on the basis that the isolated yield of pure crystalline octaacetyl derivative represented 75% of the total sugar present.

isolated, maltose and α,α-trehalose, are α-linked. Other disaccharides would be expected and were shown to be present in very small amounts on the basis of qualitative paper chromatograms. Their presence will not be discussed since these were not identified. One of these sugars possibly accounts for the fifth peak observed in the polarimetric measurements made on the disaccharide effluent in the analysis of sample A.

The small size of the higher oligosaccharide fraction, 8 per cent, found in the industrial hydrolyzate of starch as well as the calculated degree of polymerization, 5, also confirm the previous work of Frahm. His studies were carried out with methylated saccharides, however, whereas the present research represents actual saccharide fractionations, isolations, and identifications, and thus makes a start toward a true confirmatory investigation.

The excellent results obtained in the work described here justify the use of carbon column chromatographic techniques to resolve the mixture of

saccharides in hydrol. The analysis was complicated, however, by a variety of factors, *i.e.*, the presence of at least small amounts of the highly reducing 5-hydroxymethylfurfural, the large size of the mono- in comparison to the disaccharide fraction, the weak adsorption of some of the disaccharides, the presence of non-reducing sugars in the disaccharide fraction and the presence in the hydrol of extraneous materials introduced during the processing. For these reasons the use of fairly large samples (25 grams of sample A and 69 grams of sample B) adaptable to detailed examination and, if necessary, rechromatographing of the fractions, was desirable.

Several improvements and modifications of known methods were made during the course of the work.

(*a*) Pittsburgh carbon S.G.[8] was substituted for Celite[12] in the carbon column. Celite, frequently used with Darco[7] carbon G60 to facilitate the flow of solvent through the chromatographic column, is objectionable because of the soluble silicates found in the effluent. Pittsburgh S.G. carbon is a purified, granular, porous material;[13] used in place of the Celite as a diluent, it helped to produce a column with a satisfactory solvent flow rate and increased capacity without disrupting the fractionation according to molecular size. No evidence of 5-hydroxymethylfurfural was found in the paper partition chromatograms of the carbohydrate fractions. As a precautionary measure, about four times the amount of mixed carbon estimated to be necessary was used.

(*b*) Adjustment of the sample for analysis to *p*H 7.0 appeared to help in retaining the more weakly adsorbed disaccharides on the column. This adjustment was made in analysis of sample A previous to adsorption of the saccharides on the carbon and a sharp separation of mono- and disaccharides was effected. In analysis of sample B, adsorption was made at *p*H 5.5 and a portion of loosely held disaccharides desorbed into the dextrose aqueous effluent.

(*c*) The employment of nitrogen gas under pressure on a carbon (6, 7) column, as used by Claesson (8), and later by McDonald and Perry (6) in small-scale analyses of corn sirups, was applied with success on a larger scale suitable for preparative work. An automatic fraction collector with a simple device for reception of uniform samples without loss was used with the column (Fig. 1).

After the column had been in use for a few weeks and before the analysis of sample A, its efficiency was tested to determine loss due to adsorption, etc., with a mixture of 5.0 grams of Bureau of Standards α-dextrose and 5.0 grams of cellobiose (Difco standardized, which gave a paper chromatogram showing only one sugar). The recoveries, based on reducing power, were dextrose, 98.9 per cent; cellobiose, 99.0 per cent.

[12] Johns-Manville, 22 East 40th Street, New York, New York.

[13] The manufacturer specifies that particle size distribution, 8 by 30 mesh (U. S. Screen), is less than 5% plus 8 mesh, less than 5% through 30 mesh; ash about 12%; water-soluble ash, 0.2 to 0.3%.

CONCLUSIONS

(1) Chromatographic fractionations of the saccharides in two typical samples of hydrol sirup gave results in good agreement as regards the relative amounts of mono-(dextrose), di- and higher oligosaccharide fractions obtained from each (Table 2).

(2) In the disaccharide fraction four polymers of D-glucose (dextrose) were isolated and identified in good yields by means of their crystalline derivatives as brachiose (6-[α-D-glucopyranosyl]-D-glucose), gentiobiose (6-[β-D-glucopyranosyl]-D-glucose), 4-[α-D-glucopyranosyl]-D-glucose, and α-D-glucopyranosyl-α-D-glucopyranoside.

(3) The composition data obtained on hydrol has been discussed theoretically in order to bring out the dual catalytic action of the aqueous hydrochloric acid, *i.e.*, hydrolytic and synthetic, occurring in the industrial production of dextrose.

(4) The total saccharide content of hydrol (about 65 per cent), measured by chromatographic analysis on two samples, was confirmed by direct analysis by the anthrone method.

(5) For routine determination of total carbohydrate in hydrol, consideration should be given to the direct determination by the spectrophotometric anthrone procedure.

(6) The weight of hydrol minus the weights of protein, ash, and moisture furnishes an estimated figure of 65 per cent, also, for the total saccharides in hydrol. It is assumed, therefore, that only these noncarbohydrates are present in sizable amounts.

(7) The reducing power of hydrol, expressed as dextrose, accounted for only about 85 per cent of the carbohydrates present.

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REVIEW OF THE PROPERTIES OF GOSSYPOL AND METHODS OF ITS ESTIMATION*

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The potential use of cottonseed meals in the manufacture of mixed feeds for swine and poultry production necessitates the development of analytical methods for the determination of both free and bound gossypol. Such methods must be not only relatively rapid and precise but also accurate in order to be useful both for process control by the industry and for feed inspection purposes.

Satisfactory methods are available for the determination of gossypol in cottonseed meats, meals, and oil. Extension of their use to the analysis of mixed feeds containing cottonseed meal will require some modification to make them reliable for the determination of very small quantities of gossypol and to eliminate the influences of any interferences. This survey of the literature on the occurrence and properties of gossypol and of the methods proposed for its quantitative determination has been made as a preliminary step toward establishing satisfactory methods for the determination of both free and bound gossypol in mixed feeds containing cottonseed meals.

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Gossypol is the bright yellow pigment occurring in cottonseed in amounts reported to vary from 0.4 to 1.7 per cent of the moisture-free meats (35). It is contained in discrete morphological structures called pigment glands which are distributed throughout the kernel. The walls of these glands are quite resistant to rupture in the absence of water (13), and some of them withstand exposure to processing conditions and remain intact in cottonseed meals (5).

As early as 1915, Withers and Carruth (47) identified gossypol as the factor in cottonseed which is toxic to non-ruminants. They also demonstrated that certain processing conditions change the major portion of the gossypol to a non-deleterious form. This altered gossypol, designated as D-gossypol by Carruth (15), was characterized by its insolubility in ether, while the "free" or unchanged gossypol remained ether soluble. Later, Clark (17) postulated that D-gossypol was produced by condensation of gossypol with the free amino groups of the protein. He suggested the term "bound gossypol" for this inactive form. Thus for many years it has been recognized that cottonseed meals contain free gossypol, distinguishable by its solubility in certain organic solvents, and bound gossypol, insoluble in common organic solvents.

Cottonseed meal is produced by several methods of processing which include hydraulic pressing, screw pressing, and solvent extraction. Conditions of preparation vary widely among mills using the same general process. Variations in processing conditions as well as in the composition of cottonseed, due to varietal and environmental effects, cause variations in the free and total gossypol contents (38) and in the nutritional value (2) of cottonseed meals. The use of aromatic amines in the production of "degossypolized" meals introduces another compositional variation, since such meals contain gossypol combined with the amine used in addition to that combined with the amino groups of the protein.

Free gossypol in cottonseed meals has no apparent effect on growth when fed to ruminants, such as cattle and sheep, and is not a critical factor (18). In the diets of swine and poultry, however, evidence has been accumulating to indicate that the level of free gossypol which can be tolerated in the ration is limited. Recent investigations (2) indicate that cottonseed meals containing 0.03 per cent free gossypol can be fed safely to swine in quantities normally required to balance the diet with respect to protein.

Considerable attention is being given to the levels of free gossypol content of rations which can be fed to growing chicks, as well as to the relation between gossypol in the diet of laying hens and egg quality and hatchability (3). Although the investigations have not been completed, these results (23, 26, 29) indicate that the level in the rations at which free gossypol produces egg yolk discoloration is about 0.008 per cent, the level at which it lowers egg hatchability is between 0.012 and 0.016 per

cent, and a level of 0.014 per cent is not harmful to growth of chicks.

It is therefore obvious that suitable methods for the determination of gossypol in commercial mixed feeds must measure very small amounts of gossypol with good precision and accuracy. A method for gossypol in oils is also of importance since the fixation of color in crude cottonseed oils has been correlated with the gossypol content of the oils, particularly when the oils are stored at relatively high temperatures (8).

PROPERTIES OF GOSSYPOL

Since the original isolation in 1899 of gossypol, a polyphenolic plant pigment (28), much attention has been given to its properties and structure (8). It is a yellow crystalline material which has been reported to be soluble in cold dioxane, diethylene glycol, methanol, ethanol, isopropanol, *n*-butanol, diethyl ether, ethyl acetate, acetone, chloroform, carbon tetrachloride, and pyridine; slightly soluble in glycerol, cyclohexane, and high-boiling petroleum naphtha (b.p. 60–110°C.); and insoluble in low-boiling petroleum naphtha (b.p. 30–60°C.) and water (1).

Gossypol melts with decomposition when heated. Widely different decomposition temperatures, ranging from 180° to 214°C., have been re-

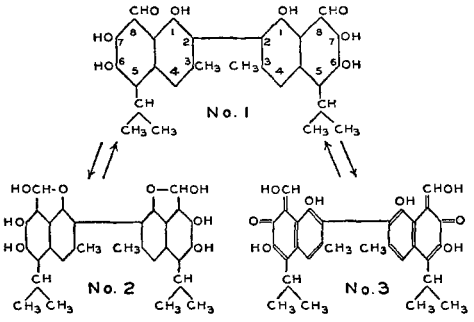

FIG. 1.—Tautomeric forms of gossypol.

ported by different investigators (1, 8, 17). This variation has been attributed to polymorphism (1). However, variations in the melting point of preparations of gossypol purified by recrystallization from the same solvents are no doubt due to the sensitivity of gossypol to such factors as light, heat, and oxidation (31).

The elementary composition, the results of alkaline titration, and the molecular weight of several derivatives all indicate that the molecular formula of gossypol is $C_{30}H_{30}O_8$ (16, 40). Adams and his coworkers (1) proposed, as a result of extensive studies of many reactions of gossypol and its derivatives and degradation products, three tautomeric forms for the molecular structure of gossypol. These structures are shown in Fig. 1.

Since the molecule contains acidic, phenolic, hydroxyl, and carbonyl groups as well as aliphatic side chains attached to a binaphthyl aromatic nucleus, gossypol is a highly reactive compound. In both the crystalline form and in a number of solvents it is highly photosensitive and is readily decomposed by heat (31). It reacts as a strong dibasic acid to form neutral salts when dissolved in dilute aqueous alkaline solutions. Lead and ferric gossypolate are insoluble, while the salts of sodium, potassium, and other common metals are soluble in water.

Alcoholic solutions of gossypol and its salts are extremely sensitive to oxidation; they are readily decomposed by Fehling's solution, ammoniacal silver nitrate, ferric chloride, hydrogen peroxide, and many other oxidizing agents including oxygen of the air (14, 28). Gossypol reacts with organic

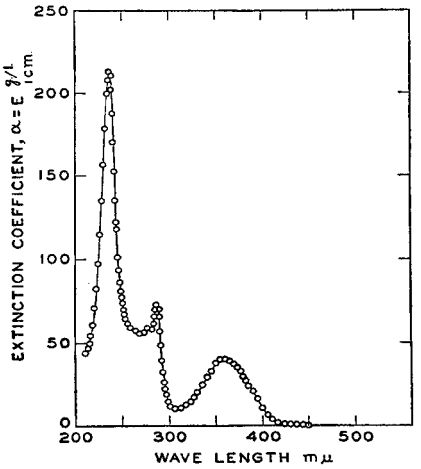

FIG. 2.—Ultraviolet absorption spectrum of gossypol in cyclohexane.

bases, such as aniline (15) or *p*-anisidine (34), and with liquid ammonia (1) to form derivatives. With organic acids it forms compounds which contain one molecule of acid for each molecule of gossypol and are readily decomposed by heat or moisture (1, 14). Stable derivatives are also formed with acetone and other simple ketones in the presence of small amounts of water (14, 34). The carbonyl groups of gossypol react with appropriate reagents to form a stable dioxine, dihydrazone, and di-2,4-dinitrophenylhydrazone (8, 16), while its phenolic groups react readily to form stable esters (16) and ethers (1). In common with many other polyphenols, gossypol exhibits antioxidant and antipolymerization activity (6).

Solutions of gossypol in cyclohexane (Fig. 2) show characteristic absorption in the ultraviolet region with well-defined maxima at 236, 286, and 358 mμ (39). Comparison of the absorption of gossypol in cyclo-

hexane, chloroform, and ethanol indicates a shift in the absorption bands toward longer wavelengths and a decrease in their intensity with increasing polarity of the solvent (8).

In addition to gossypol, cottonseed may contain very small amounts of a purple pigment, gossypurpurin (32), which has been assigned the empirical formula $C_{30}H_{32}O_7N$ on the basis of its elementary composition. It is readily converted to gossypol by mineral acids and gives derivatives with aniline and *p*-anisidine which are identical with those obtained from gossypol (34). Still smaller amounts of two other pigments, which are closely related to gossypol, have been found in cottonseed products. These include gossycaerulin, a blue pigment, which is reported to be formed in small quantities during the cooking of cottonseed prior to pressing (12), and gossyfulvin, an orange pigment, which has been isolated from the diethyl ether extracts of cottonseed (11). Spectrophotometric curves (12) of these pigments are compared with that of gossypol in Fig. 3. Since the reactions of these pigments are similar to those of gossypol, the small amounts of them which might be present in cottonseed meal would be determined as gossypol by most of the reactions found

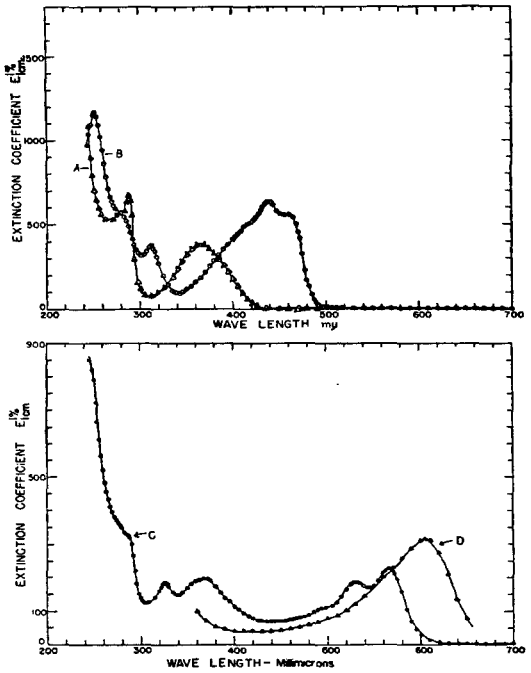

FIG. 3.—Ultraviolet absorption spectra of cottonseed pigments in chloroform. A. Gossypol. B. Gossyfulvin. C. Gossypurpurin. D. Gossycaerulin.

useful in analytical procedures. They are of little significance in the analysis of cottonseed meals and mixed feeds containing them since they are present in only trace quantities. In the discussion which follows, the term gossypol will be used to include both gossypol and these closely related pigments.

ANALYTICAL METHODS

Analytical methods for the determination of both free and total gossypol are important, since a major portion of the gossypol in cottonseed meal is bound during processing of the seed for oil and meal. Published procedures for each of these determinations are reviewed.

Free gossypol in cottonseed products. Several methods have been proposed for the determination of free gossypol in cottonseed meats and meal. All of them provide for the extraction of the gossypol from the sample, but they differ widely in the solvents used, the extraction procedure employed, and in the final evaluation of gossypol.

The first method, proposed by Carruth (15) in 1917, specified the extraction of the free gossypol with diethyl ether, followed by its determination gravimetrically as dianilinogossypol. This method as well as numerous modifications of it (19, 22, 41–43) involved several undesirable features. Long periods (forty-eight to seventy-two hours) of extraction with ether are necessary to remove all of the gossypol from cottonseed cake or meal. It was also found that the pigment is unstable in the ether extract during the prolonged heating unless special precautions are observed to stabilize it. For this purpose the use of peroxide-free ether containing small amounts of ethanol and water was recommended (22). The presence of water also assists in the extraction of the gossypol by rupturing the pigment glands. Other difficulties include the slowness of the precipitation of dianilinogossypol from the extracts and the frequent contamination of the precipitates.

Subsequent investigations (20–22, 43) showed that the separation of the free from the bound gossypol was not well defined. Variations in the amount of water present in both the sample and the ether, as well as the time of extraction, affected the amount of gossypol obtained. It has been suggested (20) that there was no sharp distinction between free and bound gossypol. However, in the light of recent findings (34, 36) it would seem more probable that the variations in results were caused by the hydrolysis of a portion of the bound gossypol.

Another proposed method (30) was based on the reducing action of gossypol, and provides for reaction of the extracted gossypol with Fehling' solution, followed by titration of the precipitated cuprous oxide. This method is subject to many of the limitations outlined above as well as to interference by the many other reducing substances which are present in extracts of cottonseed products.

Recognition of the need for more sensitive methods and of the difficulties inherent in determinations based on precipitation led to the development of colorimetric methods. In the first such method, proposed by Lyman, Holland, and Hale (27), the sample is extracted for seventy-two hours with a mixture of ether, water, and alcohol in a Butt-type extractor. An aliquot of the extract is heated with aniline and the absorption of the dianilinogossypol measured at 440 mμ. The absorption of a comparable aliquot, omitting the aniline treatment, is used to correct for the background absorption of the extract.

In order to reduce the extraction time, Smith (44) proposed a method in which a high speed blendor is used to extract the sample with a mixture of aqueous alcohol and ether. Free gossypol is determined in an aliquot of the filtered extract by a modification of the Lyman, Holland, and Hale colorimetric procedure (27). Limitations inherent in quantitative blendor extractions and in the manipulations involved have caused difficulties in the application of this method (7).

Boatner and coworkers (10) developed a method based on the reaction of gossypol with antimony trichloride. After equilibration of the sample with chloroform (twenty-four hours), an aliquot of the filtered extract was treated with antimony trichloride and the absorption of the red product was measured at 520 mμ. Interfering substances present in meal extracts required re-extraction of the gossypol from the chloroform solution with sodium hydroxide followed by transfer to an organic solvent prior to the development of the chromophore (12). Sensitivity of the reaction to moisture, and the critical reaction time limit the applicability of this method (7).

The use of aqueous acetone for the extraction of free gossypol was advocated by Pons and Guthrie (34) in a method which has been adopted by the American Oil Chemists' Society as official for the analysis of cottonseed cake, meal, and meats (4). The sample material is extracted with measured volume of 70 per cent aqueous acetone for one hour, using a mechanical shaker, and gossypol is determined colorimetrically in an aliquot of the extract after reaction with *p*-anisidine. Spectrophotometric curves of the product obtained from gossypol and several cottonseed materials are shown in Fig. 4, and illustrate the specificity of the method. Aqueous acetone offers several advantages over other solvents as an extracting agent. It is not only an excellent solvent for gossypol but the water in the mixed solvent facilitates the rupture of any unbroken pigment glands. (Since the gossypol is found in these pigment glands, they must be broken before it can be readily extracted.) Another property favoring the use of aqueous acetone is that its solutions of gossypol are stable because of the formation of an acetone-gossypol addition compound (14, 34). The extraction conditions employed are simple and reproducible, the small variations in extraction time do not affect the values obtained, and

the conditions of extraction minimize the hydrolysis of bound gossypol.

para–Anisidine offers several advantages over aniline as a reagent for color development. It is a white crystalline solid (m. p. 57°C.), easily purified and stable in the absence of moisture. In addition, the extinction coefficient of the reaction product of gossypol with *p*-anisidine is greater than that for dianilinogossypol (33); this makes it possible to determine smaller quantities of gossypol.

Total gossypol in cottonseed products.—Methods for the determination of total gossypol require the use of procedures which will recover both bound gossypol and free gossypol from the sample material without destruction of the pigment. Early investigators (42, 45) suggested methods

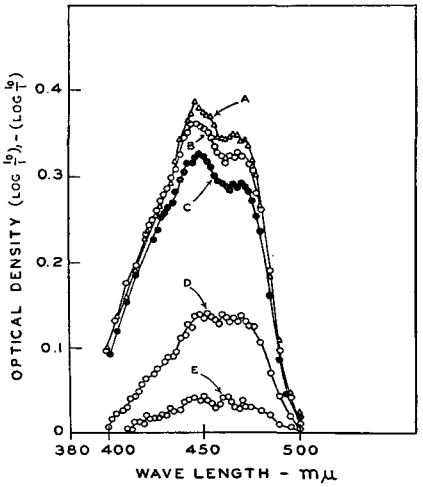

FIG. 4.—Absorption spectra of 70% aqueous acetone extracts of various cottonseed materials reacted with *p*-anisidine. A. Hexane-extracted meal; 0.2390 g (1.20% gossypol), 2/50 aliquot. B. Raw meats; 0.3276 g (0.837% gossypol), 2/50 aliquot. C. Pure gossypol; 0.0991 mg in 25 ml volume. D. Hydraulic-pressed meal; 2.000 g (0.060% gossypol), 2/50 aliquot. E. Screw-pressed meal; 2.000 g (0.022% gossypol), 2/50 aliquot.

requiring exhaustive extraction with hot aniline to remove the total gossypol pigments from cottonseed meals and cooked meats, followed by gravimetric determination of the dianilinogossypol. A more rapid method, which is applicable to cottonseed meats, cake, and meal and which requires less manipulation, was developed by Pons, Hoffpauir, and O'Connor (36). The bound gossypol is hydrolyzed to free gossypol by treatment with aqueous methyl ethyl ketone (11 per cent water) containing oxalic acid for six hours or overnight at 75°C. After removal of the oxalic acid by precipitation with barium acetate, the gossypol in the filtered extract is reacted with *p*-anisidine and determined colorimetrically.

For the determination of total gossypol in cottonseed oils, no acid treatment is necessary. The analytical sample is dissolved in a mixture containing 4 parts by volume of hexane to 1 part of isopropyl alcohol, and the gossypol present is reacted with *p*-anisidine and determined colorimetrically (37).

ANALYSIS OF MIXED FEEDS

No methods specifically designed for the analysis of mixed feeds for either free or total gossypol have been reported in the literature. In most nutritional investigations (9, 24, 26, 29), the gossypol content of the diet has been calculated from the analysis of the cottonseed meal used and its proportion in the diet. However, very little is known of the fate of the free gossypol in cottonseed meals incorporated in typical prepared feeds. A gradual disappearance of gossypol was noted in recent studies (23, 25) in which pure gossypol was added to typical chick feeds. This loss of added gossypol, as measured by the American Oil Chemists' Society method (4, 34), was correlated with the results of feeding experiments. No information is available as to whether a similar disappearance of gossypol would occur when cottonseed meal is used in the preparation of mixed feeds of commerce.

Although the method of the American Oil Chemists' Society has been shown by collaborative study (46) to give completely satisfactory results when applied to cottonseed meal, several factors should be considered in connection with its application to the analysis of mixed feeds. The possibility of interference by constituents of feeds, which are not present in cottonseed meal, has not been investigated. The effect of such interferences could be readily established by comparison of the analytical values obtained for cottonseed meals with those for feeds containing known proportions of these meals.

In any commercial use of amines in the processing of cottonseed for the production of meals of high nutritive value, the reaction product of the amines with gossypol would offer an additional complication. The diamino product formed would be extracted and would be present in the blank. Hence, it would be essential to use the same amine in the colorimetric determination of free gossypol, as the extinction coefficients of the several diamino derivatives differ. This consideration implies the need of knowing the amine used in processing the seed.

Another important factor to be considered is that the expected range of free gossypol content in mixed feeds is considerably below the optimum range of the method as developed for cottonseed meats and meals. Even in the absence of interfering substances, application of the procedure to samples containing smaller amounts of gossypol would require the use of increased sample weights or larger aliquots of the extracts. In a preliminary investigation, using cottonseed meal with a low gossypol con-

tent, it was found that when the sample weight was increased above 2.0 grams or when an aliquot larger than 2 ml was used, low results were obtained. However, if the temperature during the final color development with *p*-anisidine was increased from 60° to 80°C., sample weights as high as 5 grams and aliquots as large as 10 ml could be used. These preliminary results suggest that the method of the American Oil Chemists' Society may be suitably modified to be applicable to mixed feeds provided interfering substances are not present.

The total gossypol method of Pons, *et al.*, (36) should be applicable to mixed feeds with very little modification, since the amounts of total gossypol expected to be present are well within the optimum range of the method.

SUMMARY

The physical and chemical properties of gossypol and the analytical methods for its determination have been reviewed. Reports of nutritional investigations indicate that because of its critical effect in the diets of non-ruminants, the free gossypol content of mixed feeds prepared from cottonseed meal will be below the optimum range of published methods. However, preliminary experiments indicate that the method of the American Oil Chemists' Society may be modified so that it will be applicable. The method for total gossypol based on the hydrolysis of the bound gossypol with oxalic acid in aqueous methyl ethyl ketone should be applicable with very little modification. However, before applying these methods to mixed feeds containing cottonseed meal it would seem essential to re-examine and validate by collaborative study the extraction, the color development, and the effect of possible interferences.

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COLORIMETRIC DETERMINATION OF PROPENYLGUAETHOL IN IMITATION VANILLA

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The method described by Meyer (1) for determination of coumarin in Tonka beans (*Coumarouna odorata* Aublet) has recently been modified and adopted by the Association of Official Agricultural Chemists (2) as a First Action method for coumarin in imitation vanilla. In this application, vanillin or ethylvanillin is usually present and coparticipates in the chromogenic reaction, producing azo compounds of much lower intensity than the coumarin dye but of identical color ($A_{max}=495$ millimicrons).

In developing a technic for elimination of this source of interference, it was noted that propenylguaethol, a recently offered vanillin adjunct, also couples with a *p*-nitrobenzenediazonium chloride to form a chromophore, 1-ethoxy-3-(*p*)-nitrophenylazo-5-propenylphenol (Fig. 1, A) of intensity similar to the coumarin derivative. Utilization of this dye for analytical application was then undertaken.

It was found that propenylguaethol, coumarin, and ethylvanillin can be quantitatively steam-distilled from acidulated solutions containing milligram quantities of these phenols; vanillin, however, even in impractical excesses relative to the other synthetics, appears in the distillate in only small noninterfering amounts. Similar results were obtained when aliquots of vanilla extract steam-distillates were treated with diazotized *p*-nitroaniline. Detection of propenylguaethol in pure vanilla and its determination in the presence of an excess of vanillin therefore proved relatively rapid and simple.

Coumarin, an inner lactone, does not itself undergo azo-coupling unless hydrolyzed to *o*-coumaric acid, which in the Meyer method is accomplished by treatment with hot aqueous sodium carbonate. Since the presence of base is also required for neutralization of the hydrochloric acid generated during the azo reaction, it must not be omitted; however, when coumarin solutions were run without heating, only about 2 per cent lactone-ring opening was found to occur (Fig. 1, B and C). Incorporation of a blank containing the same amount of coumarin as in the unknown compensates for this low-order absorbance with only a minor error arising from any differences in the degree of hydrolysis between blank and sample. While such manipulation is not in itself laborious, coumarin must first be detected and determined (3, 4). Since the presence of propenylguaethol obviously precludes the use of the Meyer method, the gravimetric procedure of Hess and Prescott (5) should be used for this purpose.

Ethylvanillin, while forming only a weakly absorbing azo dye (in the visible region), will introduce a troublesome error if present in high concentration with respect to propenylguaethol (Table 1, footnote *b*). The

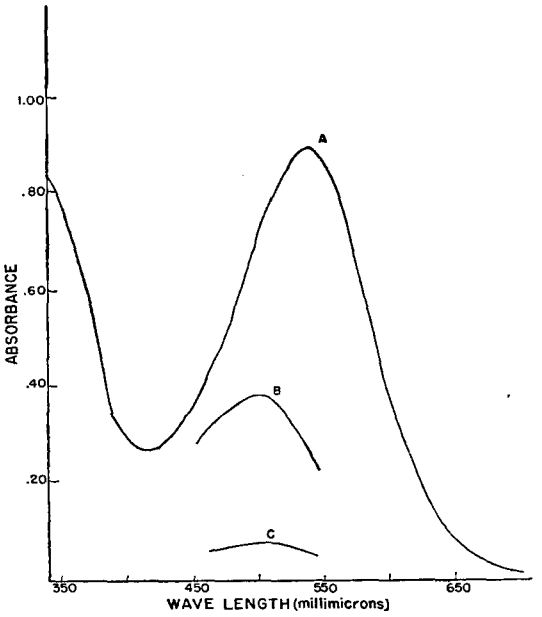

FIG. 1.—Effect of coumarin. A: Azo-coupled propenylguaethol (0.586 mg/100 ml); B: Azo-coupled coumarin (hot run) (0.398 mg/100 ml); C: Azo-coupled coumarin (cold run) (3.980 mg/100 ml (B×10)).

addition of the appropriate amount of ethylvanillin to the blank then becomes desirable. Detection and determination of this compound is now possible, using the Way and Gailey paper-chromatographic method (6). By substituting an ultraviolet lamp for the 2,4-dinitrophenylhydrazine spray recommended by these authors, propenylguaethol was found to migrate with the solvent front (R_F, 0.97) and thus does not interfere.

Like the coumarin dye, azo-coupled propenylguaethol does not follow Beer's law, so that graphical estimation is indicated. When the reagent system recommended by Meyer, also adopted by Roberts and Link (7), was used, the absorptivity at 530 millimicrons diminished from 1610 (102 micrograms/100 ml) to 1550 (502 micrograms/100 ml). When the A.O.A.C. proportions of reagents were employed, the highest value obtained was 1180. Varying the components randomly did not result in improved linearity over that obtained by Meyer's procedure.

Unlike coumarin, propenylguaethol does not form a light-fast dye. Detectable decomposition was observed on the spectrophotometer in samples exposed to normal room illumination after about ten minutes. Absorbance readings should therefore be taken without delay.

Because of the very low solubility of propenylguaethol in water, iso-

propanol was placed in the distillate receiver to prevent separation. The amount of alcohol employed in the blanks and in the calibration samples was adjusted to the same concentration, although only minor change in absorbance could be associated with an increase of isopropanol concentration from 0.5 per cent to 10 per cent (0.375 to 0.380 in an arbitrarily selected test sample).

The propenylguaethol used in this work was obtained from the Shulton Brothers Co., New York City. After three recrystallizations from 95 per cent ethanol, the material was oven dried (70°C.) for two hours and desiccated over sulfuric acid for three days (m. p., uncorr., 87.0–87.5°C.).

A Beckman Model B spectrophotometer, equipped with matched Pyrex cells (1 cm) was used for all optical measurements.

CALIBRATION CURVE

The calibration curve (Fig. 2) was obtained by using eleven different aliquots of an aqueous isopropanol (5 per cent v/v) propenylguaethol solution (50.2 mg per liter). Samples up to 10 ml were delivered to 100 ml

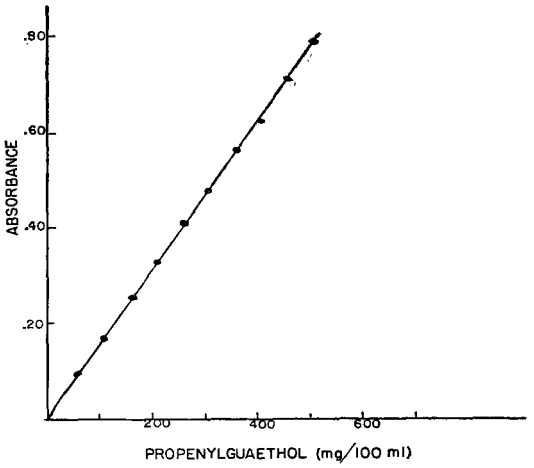

FIG. 2.—Calibration curve.

volumetric flasks from a buret. The isopropanol concentration was then adjusted to 0.5 per cent (v/v) and further treated with diazonium chloride solution, etc., as outlined in the procedure. After three weeks, recalibration from the same standard solution showed excellent agreement with the original curve. A known sample of varying concentration was routinely included in vanilla determinations to assure proper calibration. All such runs yielded data fitting the curve within 1.5 per cent.

METHOD

REAGENTS

(a) *p-Nitrobenzenediazonium chloride solution.*—Prepare as directed by Meyer (1), or Roberts and Link (7).

(b) *Sodium carbonate solution.*—Aqueous, 2% (w/v), A. C. S. grade.

(c) *Isopropanol.*—U. S. P. grade.

(d) *Coumarin and ethylvanillin solutions.*—U. S. P. grade, 200 mg/l, (5% isopropanol).

DETERMINATION

Pipet sample contg 2–10 mg of propenylguaethol into 100 ml round bottom flask and acidify with one drop of concd H_2SO_4. Connect flask to steam generator, short vertical Liebig condenser, and 250 ml conical flask marked to receive about 150 ml. Place 12.5 ml of isopropanol in the receiver and distill fairly rapidly to mark. Transfer distillate to 250 ml volumetric flask with rinse H_2O, make to mark, and mix. Pipet 10 ml aliquot into 100 ml volumetric flask, dil. with H_2O to 25 ml and add 5 ml 2% Na_2CO_3. After mixing, add 5 ml ice cold diazonium soln *via* chilled pipet while swirling contents. Make to mark with H_2O and mix. Fill sample-rinsed 1 cm cell and det. absorbance without delay at 530 mμ. In the absence of coumarin or ethylvanillin, H_2O may be used as the reference solvent. If either is present, prepare blank soln in a 250 ml volumetric flask, using the appropriate phenol soln. Adjust isopropanol concn to 5%. Run a 10 ml aliquot simultaneously with the unknown and use in the reference cell. In order to check calibration curve, a sample of known propenylguaethol concentration should be included in the determination.

RESULTS

The data obtained in testing five different imitation vanillas are shown in Table 1. The several determinations performed on each vanilla included individual steam distillations; only one distillate sample was run. The vanillas were prepared according to the following gallon recipes (each contained 6 oz. of added sucrose, and varying amounts of ethanol and water):

No. 1. Extractives from 1 lb. vanilla beans (60% bourbon, 20% Mexican, 20% Javanese), 3.5 oz. vanillin, 0.5 oz. propenylguaethol.

No. 2. Same extractives as shown above, 5 oz. vanillin, 4 grams propenylguaethol.

No. 3. Extractives from 8 oz. vanilla beans (composition as above), and

TABLE 1.—*Determination of propenylguaethol in imitation vanillas*

SAMPLE	NO. DETERMINATIONS	TAKEN[a]	FOUND	DEV.	RECOVERED
				per cent	*per cent*
No. 1	3	14.18	14.08	.85	99.3
No. 2	4	4.00	4.07	1.23	101.8
No. 3	3	10.00	10.02	1.69	100.2
No. 4[b]	4	10.00	10.00	1.10	100.0
No. 5	3	1.50	1.51	1.33	100.7

[a] Grams/gallon.
[b] Omission of ethylvanillin from the blank resulted in an apparent recovery of 10.71 g (107.1%) from three determinations (dev.=.92%).

from 8 oz. Tonka beans (Angostura), 8 oz. vanillin, 2 oz. coumarin, 10 grams propenylguaethol.

No. 4. Same extractives as in Nos. 1 and 2, 4 oz. vanillin, 2 oz. ethylvanillin, 3 oz. coumarin, 0.5 oz. piperonal (heliotropin), 10 grams propenylguaethol.

No. 5. Extractives from 1 lb. vanilla beans (ex-Tahiti), 3.5 oz. vanillin, 1.5 grams propenylguaethol.

SUMMARY

A method has been outlined for the determination of propenylguaethol in the presence of materials commonly associated with imitation vanilla.

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SEPARATION AND IDENTIFICATION OF COUMARIN AND FOUR OTHER VANILLA-LIKE FLAVORING SUBSTANCES BY PAPER CHROMATOGRAPHY

BY LLOYD C. MITCHELL (Division of Food, Food and Drug Administration, Department of Health, Education and Welfare, Washington 25, D. C.)

This paper describes a chromatographic separation and identification of coumarin and four other substances said to be currently used in food products as substitutes or adjuncts for vanilla. The five compounds are vanillin, ethyl vanillin, coumarin, piperonal (heliotropin), and 1-ethoxy-2-hydroxy-4-propenyl-benzene (Vanitrope®).

Gailey (1) has separated vanillin, ethyl vanillin, and piperonal on paper by using a solvent system containing about 15 ml of ammonium hydroxide (2 per cent by weight of NH_3) and 25 ml of butyl alcohol. He identified the compounds with 2,4-dinitrophenylhydrazine. Swain (2) has recorded the R_F values of coumarins and related compounds in twenty different solvent systems, together with the colors produced by a number of different chromogenic sprays. Svendsen (3) separated four coumarins, occurring naturally in two species of pimpinella root, with a solvent system of petroleum ether, benzene, and methanol, 25:20:10. The chromatograms were examined under ultraviolet light before and after spraying with 1

per cent alcoholic potassium hydroxide*. Bergner and Sperlich (4) separated vanillin, ethyl vanillin, piperonal, and coumarin on paper with petroleum ether. The moisture content of the paper was adjusted by leaving it in a water-saturated atmosphere in the chromatographic chamber for two to eight hours, or overnight, before adding the petroleum ether. Alcoholic potassium hydroxide, 0.1 *N*, was used as the chromogenic agent for coumarin, vanillin, and ethyl vanillin; and a saturated aqueous solution of hydrazine sulfate and 4 *N* hydrochloric acid, 90:10, for piperonal, vanillin, and ethyl vanillin. Sharp separations were obtained for vanillin and ethyl vanillin, but the coumarin and piperonal spots were more diffused. In paper chromatography, the moisture in the paper has been recognized as an important factor in many separations. One interpretation is that the water serves in the role of a stationary solvent, and that the system is one of partition chromatography.

In this work, pre-impregnation of the paper with formamide was found markedly to improve separations of vanilla-like compounds. Other details of the following method are helpful.

METHOD

(a) *Chromatographic tank and accessories* (5).

(b) *Ultraviolet light.*—RV "Black Light."[1]

REAGENTS

(a) *Mobile solvent.*—Commercial grade mixed octanes.[2]

(b) *Stationary solvent.*—Dissolve 2 ml formamide in 5 ml ethanol and dil. with ethyl ether to 100 ml.

(c) *Chromogenic agents.*—(1) To 11 g KOH add 5 ml H_2O, swirl, cool, and dil. with methanol to 100 ml (not applicable to piperonyl); (2) To 0.5 g hydrazine sulfate add 5 ml HCl, dil. with H_2O to 100 ml, and shake until dissolved (not applicable to coumarin and Vanitrope).

(d) *Standards*—Prepare 0.01 *M* solns of vanillin, ethyl vanillin, coumarin, piperonyl, and Vanitrope, and a mixt. of all five components, in ethyl acetate or ethanol. Keep in glass-stoppered vials.

PROCEDURE

Spot duplicate papers and develop chromatograms as previously described (6). When mobile solvent approaches, but does not reach, the top of the sheets (about 1½ hrs), remove papers from tank, mark solvent front, and hang papers from rod in hood until dry (about 5 min.). View papers under ultraviolet light. Wearing rubber gloves, spray one paper with chromogenic agent (c-1) and the other with agent (c-2), allow to dry (about 10 min.), and view under ultraviolet light. After examination, both papers may be sprayed with the chromogenic agent not previously used. The chromogenic agent last sprayed exerts a predominant effect; overspraying does not reveal all five compounds on the one paper but may be useful for obtaining check results.

DISCUSSION

On papers sprayed with potassium hydroxide and viewed under ultraviolet light, the coumarin spots fluoresced brightly; less than 0.01 micro-

* The authors' attention has been called to the work of K. Riedl and L. Neugebauer [*Monatshefte*, 83, 1083 (1952)] who studied the separation at 10° of a series of coumarins on paper impregnated with aqueous ethylene or propylene glycol, with petroleum ether (b. p. 60–70°) as the mobile solvent.

[1] 6-watt, model RV "Black Light," Vogel Luminescence Corp., San Francisco, California.

[2] Phillips Petroleum Co., Bartlesville, Oklahoma.

gram is detectable. Vanillin and ethyl vanillin, which may show as blue spots under ultraviolet light prior to spraying, are greatly intensified; Vanitrope gives a deep blue area which gradually fades; piperonal does not react with this chromogenic agent.

Hydrazine sulfate reagent in daylight gives yellowish spots with vanillin, ethyl vanillin, and piperonal. Under ultraviolet light, piperonal fluoresces brightly, but disappears if fumed with ammonia. On the other hand, ammonia fumes increase the intensity of the spots of vanillin and ethyl vanillin. The reaction is reversible, as the original spots return upon fuming with hydrochloric acid. There appears to be little, if any, difference in the intensity of the color produced by 0.5 per cent and saturated solutions of hydrazine sulfate.

R_F values obtained by the foregoing procedure are given in Table 1. The temperature was 26–27°C., and the concentration of each component of the mixed solution spotted was 0.01 molar.

TABLE 1.—*Separation of a mixture of vanilla-like flavoring compounds by proposed method*

COMPONENT	NO. OF OBSERVATIONS	R_F VALUES	
		AVERAGE	RANGE
Vanillin	36	0.06	0.05–0.08
Ethylvanillin	36	0.25	0.23–0.27
Coumarin	18	0.54	0.51–0.57
Piperonal	18	0.72	0.71–0.74
Vanitrope	18	0.96	0.94–0.98

Alternate chromogenic agents.—Other aldehyde reagents (7) such as *o*-dianisidine and 2,4-dinitrophenylhydrazine were tried. The former was prepared (fresh daily) by dissolving 0.5 g *o*-dianisidine in 10 ml glacial acetic acid and diluting with ethyl ether to 100 ml. The latter was prepared by dissolving 25 mg of 2,4-dinitrophenylhydrazine in 100 ml of 2 per cent perchloric acid (8).

Chromatograms developed with *o*-dianisidine are best viewed under ultraviolet light shortly after spraying. The sensitivity of 2,4-dinitrophenylhydrazine reagent is increased by overspraying the dried paper with potassium hydroxide (reagent c-1).

Alternate solvent systems.—Experience in paper chromatography has indicated that the resolving properties of similar or homologous solvents may differ materially. Hence, experiments were conducted with a number of aliphatic hydrocarbons.

Petroleum ether, with formamide as the immobile phase, caused coumarin to streak badly. When dimethylformamide was substituted for formamide, coumarin did not streak but its R_F value was only about 0.12. Addition of glacial acetic acid to the mobile solvent depressed the R_F

value of coumarin still further (to about 0.10 with 1 per cent acetic acid and to about 0.06 with 5 per cent).

As mobile solvents, acetonitrile, methanol, or methyl cellosolve (with varying proportions of water, and with or without added acetic acid) carried coumarin, vanillin, and ethyl vanillin to the upper fourth of the sheet without appreciable separation, with or without oil (9) as the fixed phase.

With formamide as the fixed phase, the mixed octanes (containing only a little 2,2,4-trimethylpentane) gave an excellent separation of vanillin, ethyl vanillin, coumarin, piperonal, and Vanitrope, as shown by R_F values in Table 1.

In Table 2 are presented R_F values for four of the five components obtained by the use of three mobile solvents which gave reasonably good separation with formamide as the fixed phase. Each value is the average of six determinations, three of them on the compounds spotted individually and three on spotted mixtures of the four compounds, each of 0.01 molar concentration. Temperature was 25–26°C.

TABLE 2.—*R_F values for vanillin, ethyl vanillin, coumarin, and Vanitrope for three mobile solvents*

MOBILE SOLVENT	R_F VALUES			
	VANILLIN	ETHYLVANILLIN	COUMARIN	VANITROPE
n-Hexane	0.08	0.29	0.66	0.97
n-Heptane	0.05	0.20	0.47	0.96
2,2,4-Trimethylpentane	0.05	0.18	0.41	0.95

n-Hexane gave somewhat more tailing and diffusion than the mixed octanes recommended.

A hundredth molar concentration is approximately optimum for solutions to be spotted. When 0.1 *M* solutions were used, overlapping tended to obscure differentiation, although the R_F values were apparently unchanged.

SUMMARY

Mixtures of coumarin and four other vanilla-like substances (piperonal, vanillin, ethyl vanillin, and Vanitrope) can be separated by paper chromatography. The sheets are impregnated with a solution of formamide in ethanol and ethyl ether, and are developed with mixed octanes. The components are detected on the paper by spraying one duplicate with potassium hydroxide (not applicable for piperonal) and the other with hydrazine sulfate (not applicable for coumarin and Vanitrope).

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THE SEPARATION AND IDENTIFICATION OF L-ASCORBIC, D-ISOASCORBIC, AND D-GLUCOASCORBIC ACIDS BY PAPER CHROMATOGRAPHY

By LLOYD C. MITCHELL and W. I. PATTERSON (Division of Food, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington 25, D. C.)

The effectiveness of isoascorbic acid in preventing the browning reaction in frozen fruits is well known, as is also its almost total lack of antiscorbutic activity. This preservative action, together with its relatively low cost, is an inducement to its commercial use in foods. Since the usual chemical methods for measuring ascorbic acid will not distinguish it from isoascorbic acid, a simple qualitative test for isoascorbic acid in the presence of ascorbic acid in food would be very useful. This paper describes the separation of mixtures of pure ascorbic, isoascorbic, and glucoascorbic acids by paper chromatography.

Earlier workers achieved the same goal with different solvent systems, using an atmosphere containing hydrogen cyanide to stabilize the ascorbic acids (1). In the present work the papers were impregnated with metaphosphoric acid solution which acted as a preservative for the ascorbic acids and did not interfere with development of the spots. Under the conditions described here, dehydroascorbic acid did not show a spot.

METHOD

APPARATUS

(a) *Glass jar.**—12 x 25 x 50 cm with flat ground top edges and flat glass cover, for 18¼ x 5½ inch paper. Bore ½ inch holes in the cover about 1¾ in. from each corner on the diagonal, and insert through stopper hook-shaped hanger rods to hold the horizontal rods to which the papers will be clamped.

(b) *Filter paper.*—Whatman No. 1 or 4, in 18¼ x 5½ inch strips cut from 18¼ x 22½ inch sheets.

(c) *Sprayer.*†—For application of stabilizing and indicator reagents.

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* Standard Scientific Supply Co., Cat. No. 7060, size #23.
† University Apparatus Co., Berkeley, California.

REAGENTS

(a) *Stabilizing reagent.*—1 g metaphosphoric acid (HPO_3) made to 100 ml with H_2O. Prepare daily.

(b) *Mobile solvent.*—Acetonitrile, acetone, H_2O, and glacial acetic acid, 80:5:15:1.

(c) *Indicator reagent.*—Ammonical silver nitrate. Dissolve 5 g $AgNO_3$ in H_2O, add 10 ml NH_4OH, and make to 100 ml with H_2O.

(d) *Standards.*—L-Ascorbic, D-isoascorbic, and D-glucoascorbic acids. Prepare 0.01 and 0.1 *M* solns of each acid and mixtures of the three acids in an aqueous soln contg 1 g HPO_3 and 1 ml acetic acid per 100 ml. Prepare fresh standards weekly or check for possible deterioration by the official method for ascorbic acid (2).

DETERMINATION

Rule the paper with a hard pencil 1½ inches from bottom of strip, clip top edge of paper to the rod which is to be used to suspend paper in jar, and clip bottom edge of paper to an auxiliary glass rod supported in a well-ventilated hood. Impregnate the filter paper with freshly prepared stabilizing soln, reagent (a), by spraying uniformly in horizontal strips beginning about ¾ inch below the marked base line and continuing to the opposite edge or "top" of paper, and allow to air dry (ca ½ hr). By means of capillary pipets, spot the various solns (preferably the 0.01 *M* solns on one sheet and the 0.1 *M* solns on a second sheet) at equal intervals along the line, transfer the papers to the jar contg ca half-inch layer of mobile solvent, reagent (b), adjust the hanger rods in their stopper mountings, and seal the cover with cellophane tape. Allow to stand until the mobile solvent front approaches (but does not reach) the top of paper (ca 5½ hrs). Remove papers from jar and hang from rod in hood until dry (ca ½ hr). Wearing rubber gloves, spray papers with indicator, reagent (c). With spots of size and intensity approximating those for 0.01 *N* concns or less, outline the spots with a soft pencil as soon as the papers are dry if they are to be preserved for future reference.

DISCUSSION

Detection of the ascorbic acids.—Ammonical silver nitrate oxidizes the ascorbic acids immediately on contact with the chromatogram. The 5 per cent solution produces a heavier spot than 0.05 *N* (0.85 per cent) $AgNO_3$ for quantities of 10 micrograms or less. The 5 per cent solution, however, causes the paper after a few hours' exposure to the light to become too dark to locate the spots of the 0.01 molar concentration of the ascorbic acids. If the papers are kept in the dark, this darkening of the papers does not occur rapidly nor to as great an extent. Under the conditions used here, the oxidation products of the ascorbic acids, as dehydroascorbic acid, do not produce spots.

Solvent systems.—Such water-miscible solvents as acetonitrile, acetone, ethanol, methanol, or methyl cellosolve, with some water but without acetic acid, cause streaking of the spots on the paper. The addition of 1 per cent acetic acid to the solvent systems containing acetonitrile, acetone, or methanol with some water, produces compact spots. Only those solvent systems tested which contain a high percentage of acetonitrile successfully separated the three ascorbic acids. The optimum solvent system contained acetonitrile, acetone, water, and glacial acetic acid in the pro-

portions by volume of 80:5:15:1. As the proportion of water was increased, the R_F values of the ascorbic acids moved closer together and higher up the paper. Omission of the water caused tailing of the spots even though acetic acid was present. The small amount of acetone serves to tighten the spots still more.

Stabilization of the Ascorbic Acids.—The ease with which solutions of ascorbic acids oxidize on exposure to air is well known. In this study, these acids had to be stabilized, both in their standard solutions and, particularly, on the paper chromatogram during their development. Experiments without any stabilizer on the paper produced very weak spots for the 0.01 molar concentrations in comparison with the corresponding spots in the presence of a stabilizer. Presumably the ascorbic acids in this concentration are mostly oxidized on the paper.

When the papers were sprayed with 1 per cent solutions, usually in ethyl ether or ethanol, of such antioxidants as α-naphthol, hydroquinone,

TABLE 1.—*R_F values of three ascorbic acids*

	0.01 *M* CONCENTRATION						0.1 *M* CONCENTRATION					
NO.	D-GLUCO-ASCORBIC ACID	L-ASCORBIC ACID	D-ISO-ASCORBIC ACID	ALL THREE ASCORBIC ACIDS			D-GLUCO-ASCORBIC ACID	L-ASCORBIC ACID	D-ISO-ASCORBIC ACID	ALL THREE ASCORBIC ACIDS		
1	.24	.40	.45	.23	.37	.45	.23	.37	.44	.23	.37	.44
2	.23	.37	.43	.23	.37	.44	.22	.37	.41	.22	.36	.42
3	.24	.39	.43	.22	.37	.45	.25	.39	.45	.22	.37	.45
4	.20	.34	.40	.20	.33	.40	.21	.36	.42	.21	.35	.43

pyrogallic acid, phloroglucinol, *p*-aminophenol, or 2,4-diaminodiphenylamine, and air-dried prior to spotting the solutions of the ascorbic acids, the developed spots were normal. The antioxidants moved faster than the ascorbic acids in most of the solvent systems and hence did not interfere with spot development when the chromatograms were sprayed with the ammonical silver nitrate. The official method for the quantitative determination of vitamin C uses metaphosphoric acid as the stabilizing agent (2). It was found that 1 per cent solution of HPO_3 in water sprayed on the paper and air dried prior to spotting of the ascorbic acids inhibited their decomposition. The HPO_3 moved more slowly than the ascorbic acids on the paper. It also gave a much lighter colored paper than the antioxidants listed above.

Table 1 shows the R_F values for four experiments.

SUMMARY

Mixtures of L-ascorbic, D-isoascorbic, and D-glucoascorbic acids are separated by paper chromatography. The paper is impregnated with a 1 per cent solution of metaphosphoric acid, air dried prior to spotting with

the ascorbic acids, and developed with a mixture of acetonitrile, water, acetone, and glacial acetic acid as the mobile solvent phase. These substances are detected on the paper by spraying with ammonical silver nitrate.

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THE AMMONIUM CHLORIDE-LIMING MATERIALS REACTION*

By CHARLES J. SCHOLLENBERGER and COLIN W. WHITTAKER
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Two properties of a soil liming material are of major importance: its total capacity to correct soil acidity (neutralizing value), and the speed with which the material will make that correction. The neutralizing value is readily determined by a simple and accurate chemical procedure. Satisfactory methods of predicting the speed of reaction (activity) of a liming material in the soil, however, have not been established. In practice, the activity is tacitly assumed to be proportional to the fineness of the material or, since fineness and geometric surface area are proportional to each other, to the surface area per unit weight as computed from particle diameters in the usual way. Trade specifications usually require that stated percentages of the liming material pass certain standard sieves.

At the same time it is recognized, at least in some quarters, that the sieve analysis does not reflect the intrinsic chemical reactivity of the material, its microcrystallinity, true surface, or any property that may affect the activity except the size composition. Many workers have recognized the need for a method that would measure the activity directly and thus automatically take into account all the various factors. A few such suggestions will be considered here.

The direct measurement of reaction rates in the soil is, of course, the most fundamental approach. Bear and Allen (4) pointed out that, in the absence of interfering factors, the diameters of all sizes of particles should be reduced by solution to the same extent in unit time. A composite of particle sizes, such as a ground limestone, could therefore be evaluated in a relative manner in terms of some standard particle size. If the stand-

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ard was merely assigned some arbitrary value, such a calculation offered little more than an improved interpretation of the sieve analysis. On the other hand, if the rate of reaction of the standard in the soil was known, then the rate of reaction of the complete ground limestone in the soil could be predicted in an absolute manner in terms of actual time units. In tests with soil-limestone mixtures under natural conditions, Bear and Allen found, from somewhat limited data, that the theoretical predictions were realized fairly well for the finer particle sizes but failed somewhat for the coarser particles. The finer fractions that obeyed the theory, however, would seem to include most of the material in the average agricultural limestone.

Schollenberger and Salter (12) in comprehensive experiments with several limestones and soils, established the average amounts of decomposition to be expected for dolomite and for high-calcium limestones in terms of diameter reductions in inches (a values), for various periods of time in the soil. Activity calculations based on these values are laborious and statistical in nature, and are not based on properties of the particular dolomite or high-calcium limestone under consideration. By making certain additional assumptions, these workers were able to devise a nomographic chart (12) for the rapid evaluation of agricultural limestones from limited sieve data. The chart has been widely used.

Activity determinations on individual samples are highly desirable but are practical only with some sort of an accelerated laboratory test. Rates of reaction in various media have been studied. These media include carbonated water (5, 7, and 10), and solutions of disodium citrate (15), sodium oxalate (2), ammonium chloride (13), and acetic acid-sodium acetate mixtures buffered in the soil pH range (1, 6, 8, and 9). Such tests rate the various samples with respect to each other, but in most cases the degree of correspondence of such a rating with relative activities in the soil was not determined. No quantitative relation between absolute reaction rates in the solvent and in the soil was established in any case.

Barnes (3) recognized that the reaction of limestone with oxalate ions was probably brought to a stop by the accumulation of insoluble calcium oxalate on the surface of the limestone, and used the oxalic acid-limestone reaction to obtain relative surface areas. This he did by simply determining the amount of oxalate formed on the surface under specified conditions. Thomas and Gross (14) revised the procedure so that the results on dolomitic limestones were more consistent with their observed activities in the soil relative to those of high-calcium limestones. Very recently, Webster, Evans, Volk, and Pratt (16) studied the relation of the Barnes relative surfaces to reactions of limestones with hydrogen-saturated clay. Although of a more fundamental nature than most of the chemical methods, the oxalate-relative surface procedures still afford only relative activities between individual samples from the same class of materials.

The present research was done to test the possibility of using an accelerated laboratory procedure to determine not only relative activities but also to predict absolute rates of reaction in the soil, thus establishing the needed quantitative relation between laboratory and practical field results. This paper presents the study of the reaction selected. Details of a practical routine procedure and tests of such a procedure are now being worked out.

Specifically, the problem consisted in the choice of a suitable reagent with which to test the activity of liming materials, in devising means for carrying out the reaction, and in determining the nature of its relation to the soil-liming materials reaction. The work of Shaw (13) on the evaluation of limestones with ammonium chloride, plus some preliminary tests by the senior author with that reagent, indicated the probable adaptability of ammonium chloride as the reagent, and that Shaw's apparatus and procedure, with necessary modifications, would be suitable for investigating the reaction. The point of main importance in comparing the ammonium chloride-liming materials and soil-liming materials reactions was whether the former, although of much higher velocity, followed the same general course as the latter. The ability of the ammonium chloride-liming materials reaction to reduce the diameters of all sizes of limestone particles at the same rate was taken as the main criterion of that point, and forms the major part of the research to be described. It is recognized that the theory of uniform rate of diameter reduction of particles of all sizes has not been fully demonstrated to be correct. It is, nevertheless, the most promising method of approach available at this time.

EXPERIMENTAL

Apparatus.—The apparatus shown in Fig. 1 is the result of trials with various designs, including the one used by Shaw. Steam was generated in the 3 l Erlenmeyer

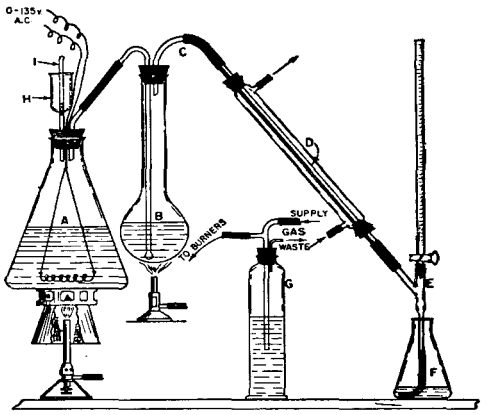

FIG. 1.—Apparatus for conducting ammonium chloride-limestone reaction.

flask, A, by passing current through the immersion heater composed of a coil of about 7 feet of No. 22 Nichrome wire welded to heavy copper leads. The delivery rate of steam, usually the equivalent of 20 ml of condensate per minute, was controlled by a variable voltage transformer. The water in the flask was maintained just at the boiling point by the burner so that the heating element supplied only the heat of vaporization, a feature that made for a uniform and readily adjusted rate of steam generation.

The steam passed to the bottom of the 800 ml Kjeldahl reaction flask B through a glass tube terminating in a small bulb with 2 mm perforations, one on the bottom and four spaced around the circumference, to give better dispersion of the entering steam and more thorough agitation of the reacting mixture. Flask B was connected to other units with flexible rubber tubing, and was swirled if necessary to reduce foaming or to wash down any material adhering to the walls above the zone of reaction.

To keep the volume of the reaction mixture constant, the contents of B were maintained just at boiling with the aid of the microburner shown. Gas pressure to both burners, each separately adjustable, was maintained constant by permitting a little gas to escape through a column of water maintained always at the same height in the container G. Steam, carrying ammonia from the reaction mixture, passed from B into the condenser made from an 18-inch length of 0.5 inch glass-jacketed copper tubing, D (for best thermal efficiency combined with small size). The final delivery tube E terminated in a rubber tube dipping into the mixture of condensate and standard acid in the 1000 ml receiver, F. This arrangement permitted swirling of F during a run. Additional standard acid (normal hydrochloric) could be delivered as required, without interrupting a distillation, from the buret directly into the stream of condensate. The tip of the buret was inserted into E through a tight-fitting rubber stopper to prevent loss of ammonia.

The apparatus differs from that used by Shaw in several respects, e. g., electrical heating instead of gas in the generation of steam, and provision for intermittent introduction of standard acid into the receiver without interrupting the distillation. Minor differences include the size of the flasks and the use of a more efficient condenser as required by the conditions of the present tests.

Procedure.—The ammonium chloride-liming material reaction was studied mainly from time-titration curves obtained by noting the time required for the ammonia liberated by the reaction, under constant distillation conditions, to neutralize successive measured increments of standard acid added to the receiver F. Details of the procedure follow:

The water in the flask A was brought to a boil and, with the immersion heater turned off, maintained just at boiling by means of the burner until ready to start the distillation. Any steam formed was allowed to escape through the filling funnel H. The required amount of standard acid was delivered to the receiver, F, from the 50 ml buret and bromocresol green indicator was added. A sample of 2 g was placed in the reaction flask, 200 ml of 5 *N* ammonium chloride solution at room temperature was added, and the flask was re-connected. The micro burner, previously adjusted to maintain the contents of flask B just at boiling, was put in position and the contents of the reaction flask were brought to boiling with a hand-held auxiliary burner. This was done quickly and at the same rate each time as far as possible. Four minutes was found to be a suitable time. As soon as condensate started to form in the condenser (when tube C became hot to the touch) rapid steam generation was begun by putting into operation the previously adjusted immersion heater and closing the neck of the filling funnel with the special stopper, I, composed of a tapered rubber plug fitted into the end of a short glass tube. Simultaneously, a timer was started and the hand-held burner removed from B. The receiver flask F was

shaken as required and the elapsed time for neutralizing the standard acid was noted to the nearest tenth minute. Without interrupting the distillation, another increment of acid was run into F, the total time elapsed from the beginning of the run was noted when this increment was neutralized, and so on until sufficient values had been taken. The total amounts of standard acid consumed at the end of each time interval were converted to fractions of the neutralizing power of the sample (activity) and plotted against the elapsed distillation times, forming a cumulative time-titration curve. For some purposes the acid was added to F in amounts computed in advance to be equivalent to certain definite degrees of decomposition of the sample. In making his tests Shaw used a smaller (0.25 g) sample, weaker ammonium chloride (2 *N*), and expressed amounts of ammonia in the first 100 ml of condensate from less active materials (dolomites) as percentages of that for high-calcium limestone and thus obtained a relative scale of activities.

Limestones.—For testing the effects of particle size and kind of material on the rate of diameter reduction in ammonium chloride, six particle-size classes were prepared from each of four materials. Prepared by the usual sieving technic, each class was defined by passage through one, and retention on the next finer member of the full series of U. S. Standard testing sieves. Since such adjacent members of the series differ in mesh-opening diameter by a factor of only 1.189 (fourth root of 2), each size class contained only a very narrow range of particle sizes. The mean diameter of the particles in each size class were taken as the square root of the products (geometric mean) of the limiting mesh diameters. The pairs of sieves were so selected that such mean diameters differed in the successive size classes by the constant factor of 2.

Selected large pieces of nearly pure, fine grained but porous Niagara dolomite, a fairly pure and porous Upper Columbus magnesic limestone, impure and dense high-calcium Vanport limestone, all widely used for liming the soil in Ohio, and a good quality Iceland spar were broken up and passed repeatedly through a laboratory "Chipmunk" crusher until nearly all passed a No. 7 sieve. About 200 grams of this material was placed on the No. 7 sieve which formed the top member of a stack of 8-inch sieves containing those required to produce the desired separates, and all were shaken together on the Ro-Tap machine for ten minutes. The finer mesh member of each pair of sieves was further shaken by hand to ensure good separation. The crushed Iceland spar was washed on a No. 270 sieve and dried prior to sieving to insure optimum sizing and removal of dust. The sieves used, the mean openings of each pair, and the chemical and mineralogical composition of each material are listed in Table 1. Note that the materials studied include representatives of the three types of limestone commonly used in agriculture together with Iceland spar, a massively crystalline form of calcium carbonate used as a reference material.

RELATION OF PARTICLE SIZE TO DIAMETER REDUCTION

To determine whether the ammonium chloride-limestone reaction was reducing the diameters of all sizes of particles at the same rate, and at the same time to estimate the degree of general correspondence with the soil-liming material reaction, use was made of the previously mentioned average a values of Schollenberger and Salter. Distillation times, designated "gauge points," corresponding to the various a values (diameter reductions occurring in the soil) were marked on time-titration curves obtained for different particle size classes. To do this it was necessary to convert diameter reduction values into fractions decomposed. This was done by assuming that the particles are, statistically, perfectly uniform

TABLE 1.—*Calcium carbonate equivalents and mineralogical composition of limestone separates*

SIEVE FRACTION	MEAN SIEVE OPENING DIAMETERS	CALCIUM CARBONATE EQUIVALENT	CALCITE[a]	DOLOMITE[a]
U. S. Sieve Nos.	*Inch* x 10^{-4}	*per cent*	*per cent*	*per cent*
Vanport High-Calcium Limestone				
170–200	32	86.8	86.8	—
80–100	64	87.8	87.8	—
45–50	128	89.2	89.2	—
25–30	256	90.2	90.2	—
14–16	512	90.5	90.5	—
7–8	1024	90.5	90.5	—
Upper Columbus Magnesic Limestone				
170–200	32	102.8	39.2	58.6
80–100	64	102.6	48.2	50.1
45–50	128	102.4	52.2	46.3
25–30	256	102.6	49.2	49.2
14–16	512	102.7	49.3	49.2
7–8	1024	102.7	46.7	51.7
Niagara (Guelph) Dolomite				
170–200	32	107.0	3.3	95.5
80–100	64	107.1	3.3	95.7
45–50	128	107.4	3.3	96.1
25–30	256	107.4	3.3	96.1
14–16	512	107.4	3.3	96.1
7–8	1024	107.3	2.5	96.6
Iceland Spar[b]				
170–200	32	99.9	100	—
80–100	64	99.9	100	—
45–50	128	99.9	100	—
25–30	256	99.9	100	—
14–16	512	99.9	100	—
7–8	1024	99.9	100	—

[a] All of the acid-soluble MgO is assumed to be combined as dolomite, and CaO in excess of that equivalent to the MgO is assumed to be present as calcite.
[b] Except for a determination of its $CaCO_3$ equivalent, this rather pure material was not analyzed.

spheres of diameter equal to the geometric mean of the nominal diameters of the limiting sieves used in preparing the size classes. The fraction decomposed $(1-R)$, corresponding to any a value, is then available from the relation $R=\left(\frac{d-a}{d}\right)^3$, where d is the particle diameter and R is the

fraction of total residual material. The *a* values for high-calcium limestone and dolomite, with corresponding gauge point designations, are listed in Table 2.

TABLE 2.—*Diameter reductions (a values) of limestones in the soil in various periods and gauge point designations*[a]

PERIOD IN SOIL	DIAMETER REDUCTION (1) AND GAUGE POINT DESIGNATION (2)			
	HIGH-CALCIUM LIMESTONE		DOLOMITIC LIMESTONE	
	(1)	(2)	(1)	(2)
Years	*Inch* x 10^{-4}		*Inch* x 10^{-4}	
0.25	22	C_1	11	D_1
1	46	C_2	30	D_2
4	69	C_3	55	D_3
16	82	C_4	74	D_4

[a] Suitable *a* values for magnesic limestones (those composed of a mixture of calcite and dolomite) are obtained by weighting the values in the table in accordance with the proportions of calcite and dolomite in the sample under consideration. Such values are designated DC_1, DC_2 etc., as in Table 3.

Figure 2 illustrates the type of plot required by the theory that particles of all sizes are reduced in diameter at the same rate. To draw these curves, a constant rate of diameter reduction of 173 x 10^{-6} inch per minute (a rate observed in tests with a dolomite) was assumed for all sizes of particles. Fractions decomposed corresponding to various diameter

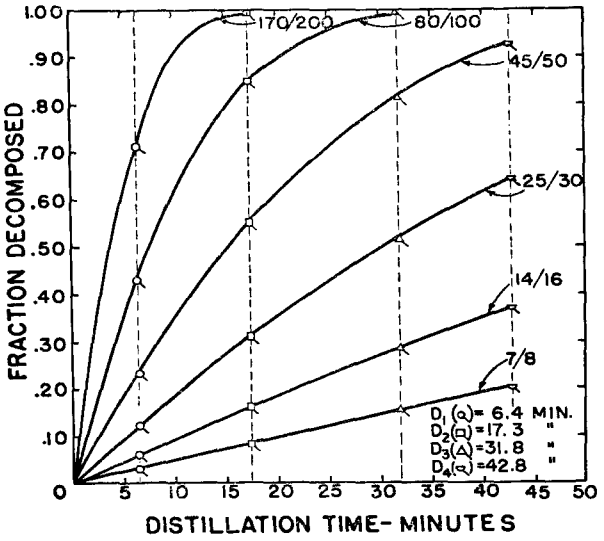

FIG. 2.—Theoretical decomposition of dolomite separated during distillation in ammonium chloride solution.

reductions were then plotted against the computed time of distillation, and a curve was made for each size class. The D_1 gauge points were plotted on the curve for each separate, and the series of points were connected by a lightly dashed line. This process was repeated for the D_2, D_3, and D_4 points. Note that, in this illustration of theory, the lines connecting the gauge points of the same subscript are straight, and parallel to the vertical axis.

Experimental results on the six size classes of each material are plotted in Fig. 3. As in Fig. 2, the various gauge points have been plotted on each curve and connected with dashed lines. (The closely spaced experimental points locating the curves are not shown.) Unlike the theoretical curves of Fig. 2, however, the dashed lines connecting the gauge points curve away from the vertical axis, indicating that the smaller particles are being reduced in diameter at a slower rate than are the bigger particles.

TABLE 3.—*Gauge times for various particle sizes (sieve fractions) of three limestones and Iceland spar in minutes*

GAUGE POINT DESIGNATION	SIEVE FRACTIONS (U. S. SIEVE NUMBERS)					
	170–200	80–100	45–50	25–30	14–16	7–8
Vanport High-Calcium Limestone						
C_1	6.4	5.6	3.7	2.7	2.3	—
C_2	7.4[a]	12.7	9.7	7.5	6.6	—
C_3	—	14.8[a]	15.9	12.7	11.4	—
C_4	—	—	19.5	15.8	14.3	—
Upper Columbus Magnesic Limestone						
DC_1	15.5	6.8	3.9	2.9	2.5	1.7
DC_2	27.1	23.7	11.4	8.5	6.8	5.4
DC_3	29.8[a]	35.5	21.4	15.7	12.5	9.7
DC_4	—	39.9[a]	29.7	20.6	15.9	12.7
Niagara (Guelph) Dolomite						
D_1	13.9	8.2	5.2	4.0	3.4	2.7
D_2	31.5[a]	26.6	18.4	14.1	12.3	10.2
D_3	—	42.7	38.8	29.5	25.7	21.5
D_4	—	44.3[a]	51.6	42.4	36.9	30.1
Iceland Spar						
C_1	7.1	5.6	4.2	3.2	2.4	1.7
C_2	8.7[a]	11.0	9.8	7.8	6.2	4.8
C_3	—	13.1[a]	14.8	12.5	10.4	8.0
C_4	—	—	17.4	15.2	12.8	9.9

[a] Completely decomposed in time indicated.

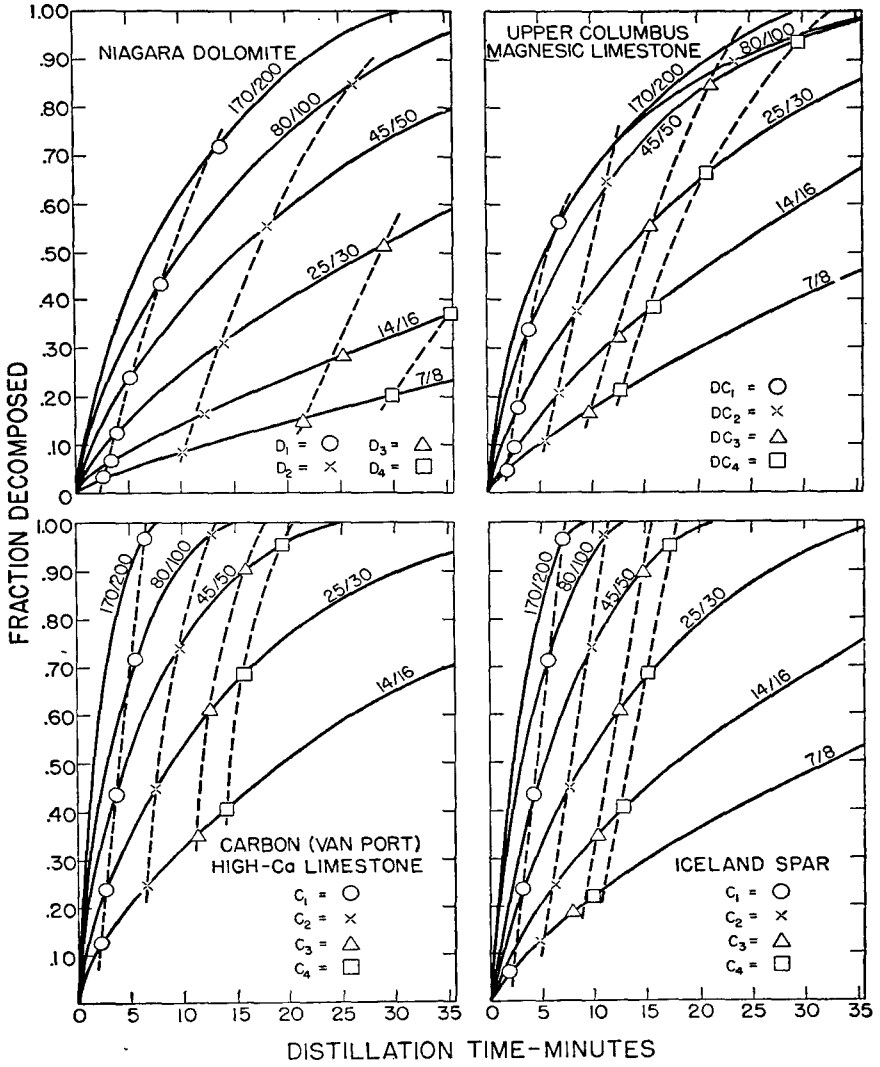

FIG. 3.—Decomposition of three limestones and Iceland spar during distillation in ammonium chloride solution.

This effect is further illustrated in Table 3 where the various gauge times have been listed. If the results conformed perfectly to the theory, the gauge times should be constant as one reads across the table.

Several possible causes of the apparent deviation from theory may be postulated. Variation in the relation between sieve-opening size and ef-

fective particle diameter, or in particle shape, as one passes from the larger to the smaller particle sizes, would change the effective surface area and hence the rate of reaction. Related to this is the surface roughness of the faces of individual particles which probably plays a large part in the initial rate of reaction. The magnitude of the surface irregularities constituting the roughness probably is largely independent of particle size until the particles become so small that the individual "hills and valleys" of the roughness become entire particle faces. The extent of the reacting surface would thus be at least partly governed by the roughness factor rather than entirely by particle size.

Foaming occurred in all distillations, but was troublesome only with the smaller particle sizes, and especially with materials of low magnesium content. The finer particles tended to become suspended in the foam and so are removed from the zone of most vigorous reaction. While such an effect would tend to cause a difference in the rate of diameter reduction of smaller and larger particles, its relative importance was not evaluated. The reaction flask was swirled frequently to break up the foam.

The gentle brushing required to get some of the materials through the finer sieves may have resulted in a slightly abnormal particle size distribution in such separates, due to forcing through particles that would normally have been too large to pass the sieve. The high-calcium limestone that was the most troublesome in this respect, however, shows little more departure from expected behavior than an Iceland spar that had been washed free of dust before sieving and required no brushing at all.

Breakup of larger particles during the distillation would increase the reacting surface and tend to make such particles react more rapidly than their initial size would lead one to expect. Such breakup could occur though penetration of the reagent into pores or through faster solution of more soluble substances contained in each particle—calcite intermingled with dolomite in magnesic stones, for example. Examination of residues from coarse separates of magnesic stone indicated that such breakup was perhaps an important factor in that case. The effect was also noticeable with dolomite.

The fact that the Iceland spar also shows slower diameter reduction of the smaller particles is highly significant. This material, with its perfect rhombohedral cleavage, tends to crush into particles having the same regular shape regardless of their size. Surface roughness in this material is certainly of a much smaller order of magnitude than in the limestone particles. Variation in the relation of sieve dimensions to particle size, if due to changes in particle shape with size, should be largely non-existent in this material. Effective surface should be more nearly determined by the particle size, due to much less surface roughness. It seems evident, that these various factors, while not eliminated as contributing to the effect noted, are certainly not the entire explanation.

In terms of grams of limestone decomposed per minute, the rate of reaction of the smaller particles is much greater than that of the larger ones. Reaction products from the smaller particles therefore accumulate in the reaction zone to a larger extent than from the larger particles and retardation of the reaction from this cause would therefore be relatively greater with smaller sized particles. It had been hoped that the removal of reaction products by the steam distillation procedure would be sufficiently rapid so that the reaction rate of all sizes of particles would be dependent solely on the intrinsic reactivity of the sample. Experiments to

TABLE 4.—*Influence of sample weight, reagent volume, and concentration on "gauge point" times (all distillations at 20 ml condensate per minute on 45–50 unwashed Iceland spar)*

CONDITIONS OF RUN			GAUGE POINT TIMES				TIME OF COMPLETE DECOMPOSITION
SAMPLE	NH_4Cl SOLUTION						
	VOL.	CONCN	C_1	C_2	C_3	C_4	
gram	*ml*	*Normality*	*min.*	*min.*	*min.*	*min.*	*min.*
0.250	200	5	1.5	4.0	6.1	7.2	8 1
0.500	200	5	2.2	5.2	7.8	9.1	10.8
1.000	200	5	2.9	6.9	10.7	12.2	14.9
2.000	200	5	4.4	10.2	15.6	18.2	23.1
2.000	50	5	8.0	21.4	34.2	41.0	51.0
2.000	100	5	5.2	13.0	21.3	24.4	30.5
2.000	200	5	4.3	9.9	15.1	17.9	23.5
2.000	400	5	3.6	8.4	12.5	14.7	19.5
2.000	200	1.25	7.3	17.5	26.8	31.3	40.5
2.000	200	2.5	5.2	12.3	18.7	21.9	27.5
2.000	200	4	4.4	10.2	15.5	18.1	23.4

test this factor (as well as those of sample size, and volume and concentration of the ammonium chloride reagent) were conducted on the Iceland spar, using the procedure already described except for the variations noted.

The effects of varying the distillation rate are shown in Table 5. The longer time required to reach gauge points on the smaller particles persisted even at the distillation rate which yielded 40 ml of condensate per minute, the highest rate possible with the equipment used. As the distillation rate increased, however, the spread in gauge times between larger and smaller particles tended to diminish, indicating that the more rapid removal of reaction products at the higher distillation rates was affecting the reaction rate.

Table 4 shows the results of tests on the effects of sample size and of reagent volume and concentration on the reaction. These tests, all conducted at a distillation rate yielding 20 ml of condensate per minute, are

not fully comparable to those of Table 5, because the sample used for the latter runs had been sieved from prewashed material.

Increasing the size of the sample increased the gauge times, a further indication that rate of removal of reaction products was affecting the results. When handling materials like agstones, however, the individual particles of which may weigh up to about 0.03 gram, it is hardly feasible to use a sample smaller than 2 grams. Increasing the reagent concentration and volume tended to decrease the gauge times but not enough to make them constant for all particle sizes. Adjustment of operating conditions so as to make the reactivity of the liming material the sole factor governing the reaction rate was apparently proved not to be feasible. The conditions as described under "Procedure" were therefore settled upon as approaching the desired conditions as closely as convenience and size of apparatus would permit.

The opposite approach to an explanation for the deviation from theory is that the theory itself is not correct. It seems logical enough that in the soil, the diameters of all sizes of particles would *tend* to be reduced at the same rate. But it is not difficult to imagine that this tendency could be modified by various factors, such as differences in the rate of diffusion of dissolved lime or carbon dioxide away from the particles in cases where the particles are very small and hence closely spaced and reacting rapidly in terms of rate per unit weight of material. This seems never to have been fully tested. Unpublished work of Schollenberger and Salter on incubated limestone-soil mixtures has indicated a somewhat similar effect. It may well be that the ammonium chloride-limestone reaction is actually closely parallel to the limestone-soil reaction, though not conforming perfectly to the theory of the latter reaction. In any event, the ammonium chloride-limestone reaction shows more promise than others of adaptability not only to the determination of relative reactivities but of exhibiting the needed quantitative relationship to the soil-limestone reaction.

RELATIVE ACTIVITIES INDICATED BY REACTION IN THE SOIL AND IN AMMONIUM CHLORIDE

Schollenberger and Salter, in determining their average a values, used six different limestones (see Table 6), No. 50–70, separates of which were available for use in the current study. These separates differed from those used in the original work in that they were more closely sized and had been washed free from adhering dust. Their activities, however, should be closely comparable to those of the original samples. The six separates were put through the ammonium chloride distillation procedure described. The results, expressed in terms of diameter reductions (a values), occurring in various periods of distillation, are plotted in the upper part of Fig. 4. The determined points (not shown) were closely spaced and located the curves accurately.

Individual a values for thirteen, fifty-two, and seventy-eight weeks in

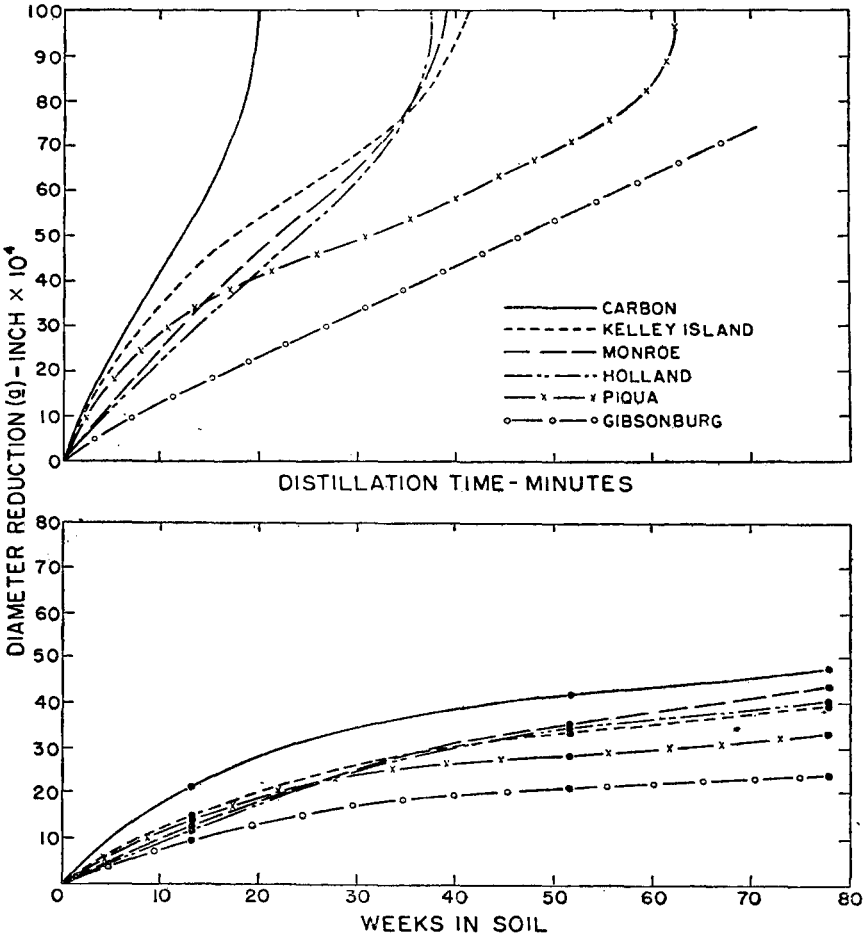

FIG. 4.—Particle diameter reductions of six limestones during distillation in ammonium chloride (upper) and in the soil (lower figure).

the soil for each of the limestones are plotted in the lower part of Fig. 4. These are average values based on numerous determinations of rate of carbonate disappearance taken at frequent intervals and have been corrected for particle size discrepancies. Note that the distillation curves show crossovers similar to those occurring in the soil curves, although the soil tests data were not sufficiently detailed to show accurately where the crossovers occur.

In Table 6 in columns headed "A," diameter reductions, expressed as a values, are listed for thirteen, fifty-two, and seventy-eight weeks in the

TABLE 5.—*Effect of distillation rates of 5, 10, 20, and 40 milliliters condensate per minute on gauge point distillation times for Iceland spar*[a]

SEPARATE	GAUGE POINT TIMES MINUTES															
	C_1				C_2				C_3				C_4			
	DISTN. RATES				DISTN. RATES				DISTN. RATES				DISTN. RATES			
	5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40
Sieve Nos.																
7–8	3.7	2.6	2.6	1.4	8.2	5.7	6.2	4.6	13.1	9.6	10.3	7.8	15.9	11.5	12.6	9.2
14–16	4.7	3.2	3.0	1.9	10.6	7.4	7.2	6.4	16.6	11.7	11.9	9.2	20.3	14.4	14.8	11.5
25–30	6.4	3.9	3.4	2.4	14.7	9.1	8.7	6.4	22.2	14.5	13.9	10.7	26.8	17.6	17.1	13.0
45–50	8.5	5.5	4.6	3.4	20.7	12.5	10.9	8.0	29.7	18.7	16.9	12.2	34.1	21.9	20.0	14.4
80–100	20.1	8.1	6.1	4.2	35.0	15.8	10.4	8.8	43.8[b]	20.0[b]	15.4[b]	11.0[b]				
170–200	20.5	10.6	7.9	5.4	30.5[b]	16.5[b]	10.4[b]	7.6[b]								

[a] Two-gram samples of washed material in 200 ml 5 *N* NH_4Cl.
[b] Completely decomposed in time noted.

TABLE 6.—*Diameter reductions (A) in inches* $\times 10^4$, *and activity rankings (B) of six limestones by soil tests and by ammonium chloride distillations*

NAME	LIMESTONES			TIME IN SOILS—WEEKS						DISTILLN. TIME—MINUTES					
	COMPOSITION			13		52		78		5		10		20	
	DOLOMITE	CALCITE	IMPURITIES	A	B	A	B	A	B	A	B	A	B	A	B
Carbon	0.4	92.6	7.0	21	1	41	1	47	1	23	1	43	1	100	1
Gibsonburg	94.7	4.7	0.6	9	6	21	6	24	6	8	6	13	6	23	6
Holland	73.5	7.6	18.9	11	5	34	3	40	3	12	5	24	5	42	4
Monroe	84.5	5.0	10.5	12	4	35	2	43	2	13	4	25	4	46	3
Kelley's Is.	26.9	69.8	3.3	15	2	33	4	39	4	21	2	35	2	54	2
Piqua	37.2	61.0	1.8	14	3	28	5	33	5	18	3	28	3	41	5

soil, and for five, ten, and twenty minutes distillation of ammonium chloride. Diameter reductions for five and ten minutes are, respectively, of about the same size as those for thirteen and fifty-two weeks in the soil. Those for seventy-eight weeks are, in some cases, about the same as for twenty minutes, but tend to be smaller. In the columns headed "B," the material showing the highest activity has been numbered 1, the next highest 2, and so on, for both of the tests.

The ranking for thirteen weeks in the soil is identical with that for five minutes' distillation; that for fifty-two weeks differs from the ten minute ranking only among the 4 stones showing similar activities. The seventy-eight week ranking approximates that for twenty minutes. In general, the distillation ranks the limestones in the same order as did the actual soil tests, regardless of the time elapsed and degree of decomposition of the liming material.

The foregoing observations indicate, at least so far as the 50–70 sieve separates are concerned, that the ammonium chloride-limestone reaction rate varies with the nature of the limestone in the same manner that the limestone-soil reaction varies with that nature. The former reaction should therefore be an accurate index of the activity of the limestone.

REACTION OF BLAST FURNACE SLAG WITH AMMONIUM CHLORIDE

The activities of eleven blast furnace slags were compared with those of a high-calcium limestone and a dolomite, using an earlier version of the ammonium chloride distillation procedure with a 0.5 gram sample and a much slower distillation rate. All samples were of the same size class; they passed a U.S. No. 140 sieve, but were retained on a No. 200. The slag samples were prepared from material remaining from an A.O.A.C.

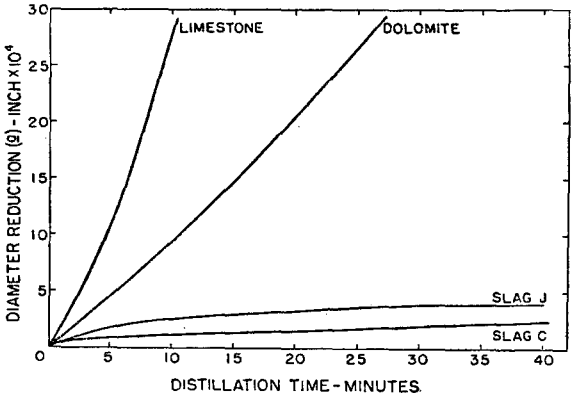

FIG. 5.—Decomposition of high-calcium limestone, dolomite, and blast furnace slags during distillation in ammonium chloride solution.

study of the neutralizing value of slags (11). Metallic iron in these samples was removed as completely as possible with a magnet to avoid the error that would result from reaction of the iron with ammonium chloride.

Indicated diameter reductions for two of the slags (the most active and the least active), with those for the limestone and the dolomite are plotted against the distillation times in Fig. 5. The activities of all the slags fall in a very narrow range and were very low compared to those of either the high-calcium limestone or the dolomite. General experience in the liming of soils with slags does not indicate any such extreme difference in the activities of slags and limestones. It is suggested, therefore, that the relation between the ammonium chloride-slag reaction and the soil-slag reaction is different from that between the ammonium chloride-limestone reaction and the soil-limestone reaction. This does not preclude the use of the ammonium chloride distillation for comparing the activities of different slag samples. Further data are needed, both on the slag-ammonium chloride reaction, and on the soil-slag reaction.

SUMMARY AND CONCLUSIONS

A study was made of the reaction between ammonium chloride and liming materials, and suitable apparatus and technic were developed for carrying out the reaction by a steam distillation procedure. The reaction rate was followed by determining the amounts of ammonia liberated in different time intervals. Tests on six limestones indicated that, in general, the ammonium chloride-limestone reaction rate varies with the nature of the limestone in the same manner that the limestone-soil reaction rate varies with that nature. The former reaction should, therefore, be an accurate index to the relative activities of different limestones.

The reaction rate per unit of particle surface area, computed from sieve dimensions defining particle-size classes, tended to decrease for a given limestone, or for Iceland spar, as the particle size decreased. Various factors may contribute to this effect. Inability to remove reaction products rapidly enough from the ammonium chloride reaction with finer particles is one; variation, with particle size, of the relation between sieve mesh dimensions and effective surface area, is probably another. This apparently slower reaction per unit of surface of the smaller particles might seem to result in a lower activity rating by the ammonium chloride reaction than would be consistent with the theory that particles of all sizes tend to be reduced in diameter in the soil at the same rate. Actually, it has not been fully proved that such a relation holds with the soil. This defect of the ammonium chloride-limestone reaction, if it is such, is of a minor nature and it is believed that the reaction has great possibilities for development into a satisfactory method for evaluating liming materials.

Blast furnace slags were rated with respect to each other by means of

this reaction but their rate of decomposition in ammonium chloride apparently does not bear the same relation to the soil-slag reaction that the ammonium chloride-limestone reaction does to the soil-limestone reaction.

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THE ELECTROMETRIC MEASUREMENT OF SOIL *p*H*

By LANNES E. DAVIS and JOHN M. RIBLE (Department of Soils, University of California, Davis, California)

For many years soil chemists have made electrometric measurements of the *p*H of soil pastes, and considerable study has been devoted to the detailed methodology in the attempt to obtain a *p*H value in any given soil sample which would be representative of the environment of plant roots in the field soil.

Some question may have arisen as to what is the basic significance of *p*H measurements in colloidal systems. Considerable progress in the early period was made on the theory of *p*H measurements in ordinary electrolytes. The very early concept, that $pH = -\log c_{H^+}$ where c_{H^+} is the concentration of H^+ ion, was replaced by the formulation $pH = -\log a_{H^+}$, where a_{H^+} is the activity of H^+ ion. As a result of ideas presented by Guggenheim (4) and others, it was realized that the concept a_{H^+} is not well defined as an independent variable of state under all conditions. For very

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dilute solutions of electrolytes, it can be defined in terms of the Debye-Hückel theory, a fact which has often been overlooked. For more concentrated solutions, it can be defined as a dependent variable, i.e., as a function of a methodologically defined concept, the pH itself. The pH is properly defined, according to MacInnes (6), as $pH=(E'-E_0)F/R'T$, where F is the Faraday equivalent, $R'=2.3026R$ (R is the gas constant), T is the absolute temperature, and E' is the measured EMF. E_0 may be formally defined as the EMF which would be obtained in a solution with $pH=0$.

In spite of the indefinite character of the concept of pH, it has generally been felt that a fair approximation is provided by the equation $pH=-\log c_H{}^+ f_\pm$, where $f_\pm$ is the mean ion activity coefficient for the acid present in a solution. This assumption has been based upon attempts to estimate the variability of the salt-bridge solution junction potential when certain standard buffers are compared with solutions of unknown pH. The pH of the standard buffers has been calculated according to methods employed by Bates and coworkers (1), as well as others.

RECENT THEORETICAL WORK WITH COLLOIDS

In general, colloid chemists have accepted the ideas presented by electrochemists with little critical investigation of their application to colloid systems. However, recently Jenny and his coworkers (2, 5) have shown conclusively that the junction potential is appreciable, variable, and unpredictable for certain types of colloidal systems. There has been considerable controversy about different aspects of their work, most of which is irrelevant to the present discussion of pH measurements in soil.

It is well known that the EMF in a pH electrometric setup is determined by the nature and concentration of the salt in the salt bridge. For many solutions, variation of the concentration of KCl in the bridge (the concentration of KCl surrounding the $Hg\text{-}Hg_2Cl_2$ electrode is standardized) produces no appreciable change in the EMF, provided that the KCl concentrations are high. Thus, saturated or near saturated KCl salt bridges are generally considered satisfactory. More precisely, lack of variation due to salt bridge concentration is a necessary but not certainly a sufficient condition for satisfactory results. It should be obvious that if EMF is plotted against KCl concentration and the curve has an appreciable slope as the concentration approaches saturation, considerable doubt is thrown upon the validity of the EMF measured with a saturated salt bridge as an estimate of the value of any variable of state in the system.

EXPERIMENTAL RESULTS

A preliminary report on this subject was presented by Davis (3) in 1951. No experimental work was done at that time, since it seemed desirable to await further clarification of the theoretical ideas presented by Jenny,

et al. The present authors decided recently that these ideas should be critically tested for soils, regardless of the status of ion exchange resins and certain clay minerals. Two possible lines of enquiry were considered: (*a*) A study of transference numbers for K^+ and Cl^- ions in soil pastes, similar to the work done by Jenny and his group with resins. However, the interpretation of the results in terms of *p*H theory would be dependent upon the adoption of one of several possible *ad hoc* hypotheses regarding the relation between transference in "perm-selective" materials and potentials at the junction: KCl- "perm-selective" material. (*b*) An investigation of the variability of *EMF* (or more practically, the related *p*H readings on a commercial *p*H meter) with concentration of KCl in the bridge. The latter line of work was carried out.

TABLE 1.—*Exchange resins (Ion-X)*

CATIONS	MEASURED pH, AVERAGE VALUES	
	SATURATED KCl	1/64 STANDARD KCl
H	1.31	2.70
Na	4.29	4.78
K	4.81	5.63
Ca	4.77	4.82
Mg	4.52	4.65

The setup included a Beckman Model H *p*H meter, an industrial model widely used for measurements in soil. It was employed for the work described here because it was believed that no gain in practical application of the results would accrue from an attempt at greater precision attained by use of a research model.

A series of mono-ionic resins H-Ion X, Na-Ion X, etc., was studied. For the soil work, 28 California soils were employed. There were 12 sandy loams, 4 loams, 8 clay loams, and 4 clays. The soils were made up as thick pastes. For the salt bridge, seven different concentrations of KCl were used: saturated, and $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, and $\frac{1}{64}$ saturated. For simplicity, only the contrast between saturated and $\frac{1}{64}$ saturated will be presented in the tabulation.

In Table 1 are shown the results obtained with granulated resins; it was difficult to get satisfactory duplications since there was considerable "suspension effect". However, it seems clear that variation of the concentration of KCl in the salt bridge produced appreciable variation of the estimated *p*H value in some of the materials.

Table 2 presents the results of the study of 28 soils. The average difference between the *p*H values with saturated KCl and $\frac{1}{64}$ saturated KCl was 0.11 ± 0.08 *p*H units. Experience indicates that this difference is not significant in terms of the chance observational errors which are commonly

TABLE 2.—*Natural California soils*

SOIL TYPE	MEASURED pH, AVERAGE VALUES	
	SATURATED KCl	1/64 SATURATED KCl
Ramona clay loam	7.21	7.18
Montezuma silty clay loam	7.69	7.58
Chino silt loam	7.72	7.63
Willows clay	8.05	8.20
Alamo clay	5.95	5.87
Hanford very fine sandy loam	7.21	7.07
Columbia very fine sandy loam	6.66	6.50
Redding gravelly loam	5.66	5.53
Ryde silty clay loam	7.70	7.59
Olcott fine sandy loam	6.02	5.88
Esparto silty clay loam	5.73	5.58
Hugo fine sandy loam	6.01	5.82
Clear Lake adobe clay	5.67	5.70
Capay silty clay loam	6.12	6.08
Capay loam	7.42	7.30
Capay clay	6.28	6.19
Nord fine sandy loam	7.85	7.62
Farwell loam	6.84	6.66
Conejo clay loam	6.75	6.69
Aiken clay loam[a]	6.01	5.81
Aiken clay loam[a]	5.96	5.77
Vina fine sandy loam	6.38	6.26
Goldridge fine sandy loam	5.57	5.46
Sites fine sandy loam	6.51	6.44
Tujunga fine sandy loam	6.91	6.76
Holland sandy loam	6.12	5.98
Rocklin fine sandy loam	6.04	5.87
Placentia sandy loam	6.21	6.03

[a] Aiken clay loams from two different locations were tested.

obtained. There was no consistent variation in the *p*H measurements when intermediate concentrations of KCl were used.

CONCLUSIONS

From these results the following conclusions may be tentatively advanced: there is no evidence of appreciable anomalous behavior for the 28 soil samples studied, which varied in *p*H from 5.57 to 8.05. It is possible that some systematic, undetermined error may invalidate the assumed significance of *p*H measurements in soil. However, this very general statement might also be made about the *p*H of any materials of commercial, agricultural, or general scientific importance. Accordingly, the soil chemist may proceed to study the methodology of *p*H measurements without apprehension that soils present a unique problem.

It may be worthwhile to comment that a fundamental aspect of soil

chemistry is the effect of soil properties upon the growth of crops. Further work on methodology should be determined by studies of correlations between soil *p*H measurements and the behavior of plants.

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FLAME SPECTRUM OF WHISKY*

DETERMINATION OF SODIUM AND POTASSIUM

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Methods of identifying brands of straight whiskies have always been of interest from a regulatory point of view. A means of identification of the metallic constituents normally present in these whiskies was considered desirable in connection with strengthening the present enforcement program. A Beckman DU flame photometer attachment employing an oxygen-hydrogen flame was selected because the spectra of straight whiskies could be determined with extreme rapidity, ease of manipulation, and accuracy. It would require a great deal more time to obtain the same data by chemical methods of analysis.

A rapid and accurate method for potassium is necessary in evaluating the age of whisky. According to Mathers (1), the potassium content of whisky can be definitely correlated with the previous usage of the barrel, due to the fact that the extractable potassium rapidly reaches an equilibrium value during the aging process. The previous usage of the barrel can in turn be used as a guide in predicting the amount of wood congeners which will be extracted by whisky during a definite period of aging. The ratio of the wood extractives to the potassium in whisky also provides an indication of quick aging and wood chip treatment designed to simulate natural aging processes.

In determining the flame spectra of straight whiskies, proper instrument setting (2) is essential. Adjustment of the potentiometer voltage by means of the sensitivity knob enabled detection of very low levels of light emission with maximum resolution. Selection of the best applicable

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slit width (3) was dependent on the element under consideration, since those metals with broad emission bands require wider slit widths than elements exhibiting lines. The potentiometer voltage was set with the sensitivity knob to provide a suitable galvanometer deflection.

The flame spectra of straight whiskies and the flame background at 0.5 mm slit width (midpoint sensitivity and optimum instrument adjustments) are represented in Fig. 1. (Relative intensities above 100 per cent T were obtained with the selector switch in the 1.0 position.) The hydroxy spectrum in the ultraviolet is noted from approximately 260 mμ to 360 mμ and has several clearly defined peaks in this region. No interference is offered by the hydroxy spectrum since few elements have emissions in this region. All straight whiskies examined exhibit a slight calcium resonance line at 422.7 mμ, a slight calcium oxide band at 554 mμ, and

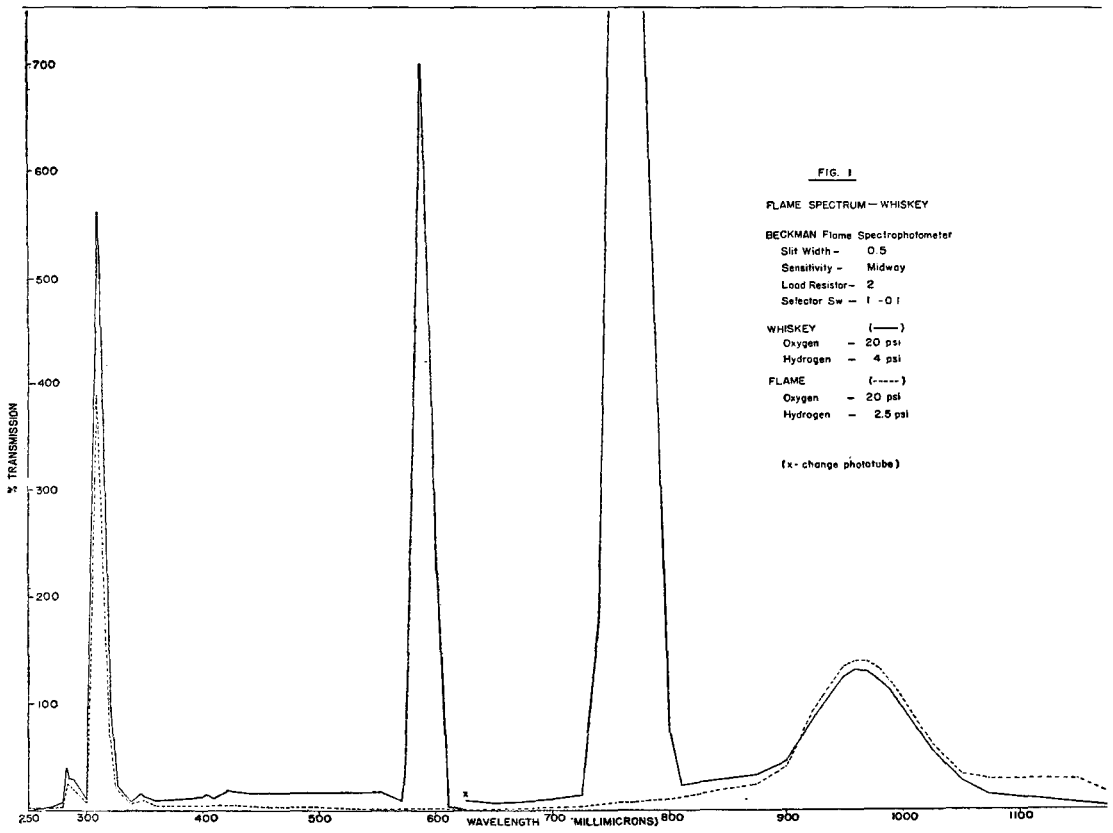

FIG. 1.—Flame spectrum of whisky.

the primary sodium line at 589 mμ. Potassium lines at 768 mμ and 404.6 mμ are noted for these whiskies. In the infrared region, molecular water bands are observed in the spectra and in the flame background from 850 mμ to 1100 mμ. These bands offer no interference in the detection of metallic elements.

DETERMINATION OF POTASSIUM AND SODIUM

INSTRUMENT AND APPARATUS

The Beckman DU spectrophotometer with flame photometry attachment and oxygen-hydrogen flame was employed. Polyethylene bottles were used for storing reagents and standards.

STANDARD REAGENTS

Sodium chloride.—Dry reagent grade sodium chloride crystals overnight in an oven, and cool in a desiccator. Make 2.5417 g to one l with distd water. The soln contains 1000 p.p.m. sodium.

Potassium chloride.—Dry reagent grade potassium chloride crystals overnight in an oven, and cool in a desiccator. Make 1.9066 g to one l with distd water. The soln contains 1000 p.p.m. potassium.

METHOD

Preparation of calibration curves.—(A study of the potassium and sodium spectra, illustrated in Fig. 2, indicates that these elements are determinable in the presence of each other without spectral line interference. To establish a method accounting for variations in background, readings are taken at a point before and after the wavelength of maximum brightness. These points represent the leveling-off portions of the curve in the vicinity of the emission line. The average back-

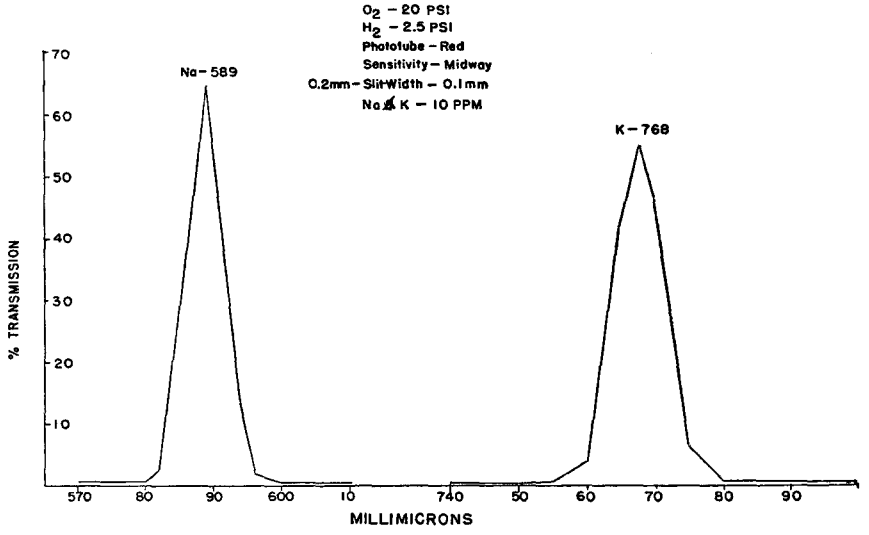

FIG. 2.—Flame spectra for sodium and potassium.

ground reading is subtracted from that obtained at the wavelength where maximum brightness is noted, and the difference is designated as "unit rise.")

Prepare standard solutions of both potassium and sodium to contain 10 p.p.m. in 50 per cent ethanol. Develop the "semi-permanent" standard curves (Fig. 3) for 1 to 10 p.p.m. potassium and sodium concentrations. For sodium, read at 570, 589, and 610 mμ and for potassium, read at 740, 768, and 790 mμ. Use proper phototube and instrument adjustment for each metal (Fig. 3), and plot as unit rise *vs.* p.p.m. (A straight line was obtained for potassium; the sodium curve was not quite linear.)

To determine sodium, fill a 5 ml sample cup with whisky and make the proper instrument settings. Place the sample in burning position and read at 570, 589, and 610 mμ. Repeat at least three times.

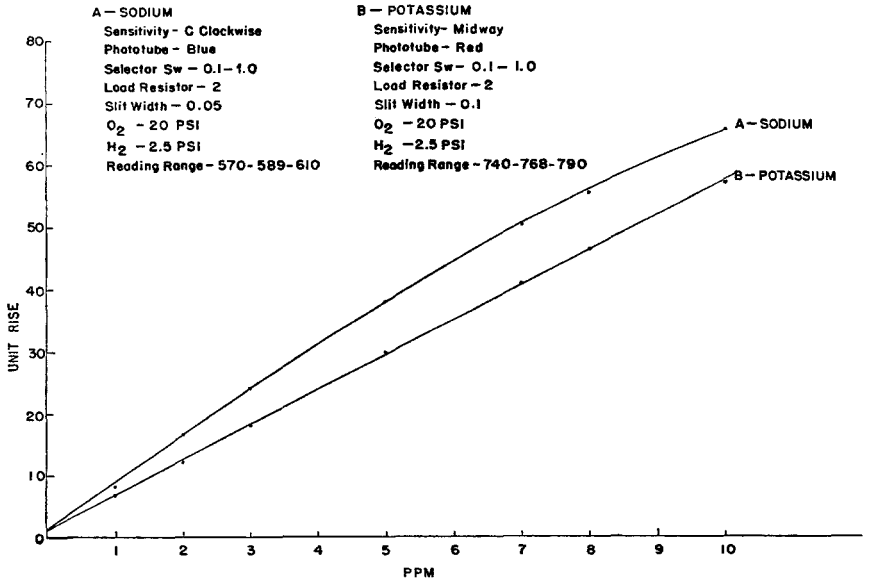

FIG. 3.—Calibration curves for sodium and potassium.

For potassium, pipet 10 ml of whisky into a 25 ml volumetric flask and make to mark with 50% ethanol. Fill the sample cup, make the proper instrument settings and place sample in burning position. Read at 740, 768, and 790 mμ, repeating at least three times.

(When determining either potassium or sodium, it is necessary to insert the standard solution immediately after the sample and to read under the same conditions.)

Jet operation and correction.—The degree of brightness exhibited by an element at its emission line or band depends upon the construction and condition of the jet. Since progressive clogging and incrustation reduces sensitivity, it is necessary frequently to check the "semi-permanent" calibration curve with standards. The percentage deviation, if any, is calculated and the correction is applied to the sample in question. Cleaning by the suggested procedures, sulfuric acid-dichromate solution (3) or F-3 carborundum-kerosene paste (4), is not always successful. Often ethanol siphoned by ordinary laboratory air pressure helps clear the bore from encrusted

material when other methods fail. A few drops of glycerin placed on the orifice of the jet, followed by siphoning ethanol with air pressure, is another method of restoring the original sensitivity of the jet.

CALCULATIONS

For Standard:

$$T_{max.} - \left[\frac{T_b + T_a}{2}\right] = \text{Unit Rise "A"}$$

For Sample:

$$T_{max.} - \left[\frac{T_b + T_a}{2}\right] = \text{Unit Rise "B"}$$

where: $T_{max.}$ = % transmission at maximum emission; T_b = % transmission before maximum; and T_a = % transmission after maximum.

Jet Correction:

$$\frac{\text{Theoretical Unit Rise from Calibration Curve}}{\text{Unit Rise A}} = \text{Correction Factor}$$

Then: Unit Rise "B" × Correction Factor = Corrected Unit Rise "B"
From Calibration Curve: Derive p.p.m. equivalent to Corrected Unit Rise "B"
And: P.P.M. × Dilution factor = P.P.M. in Sample.

ANALYTICAL DATA

Composites of straight whiskies and blends were prepared and analyzed for potassium and sodium. In addition, more than one hundred separate brands were checked quantitatively for these metals, and some of the typical results are presented in Table 1. Calcium was noted quali-

TABLE 1.—*Recovery of sodium and potassium*

SAMPLE	SODIUM			POTASSIUM		
	P.P.M. PRESENT	10 P.P.M. ADDED	20 P.P.M. ADDED	P.P.M. PRESENT	10 P.P.M. ADDED	20 P.P.M. ADDED
Straight Whisky						
Composite	10.0	20.2	29.8	28.5	38.6	48.5
Bottle #1	0.8	10.8	20.8	25.8	35.8	45.9
Bottle #2	2.2	12.2	22.3	21.3	31.4	41.4
Bottle #3	3.3	13.4	23.3	31.2	41.3	51.3
Bottle #4	1.7	11.8	21.9	28.3	38.4	48.4
Bottle #5	1.7	11.6	21.8	22.3	32.4	42.4
Blended Whisky						
Composite	3.9	13.8	23.9	26.3	36.4	46.4
Bottle #1	9.2	19.2	29.2	27.2	37.2	47.3
Bottle #2	2.2	12.4	22.2	28.0	38.2	48.3
Bottle #3	4.1	14.2	24.2	20.0	30.0	40.2
Bottle #4	10.0	20.1	30.0	26.0	36.0	46.1
Bottle #5	2.2	12.2	22.4	19.0	29.0	39.1

tatively because of its presence in trace quantities. Increment data presented in Table 1 indicate that the methods for sodium and potassium are accurate and reproducible.

INTERFERENCES

Certain organic molecules enhance and others inhibit (5) the amount of light emitted by potassium and sodium. The comparative results of the emissions from potassium and sodium in water, and in 50 per cent ethanol, with the oxygen-hydrogen flame, are presented in Table 2. Using 50 per cent ethanolic solutions as standards, it is noted that light intensity of aqueous solutions of potassium, in concentrations greater than 50 p.p.m., is reduced. Sodium intensity, from concentrations above 10 p.p.m. in water, is inhibited more than potassium.

TABLE 2.—*Effect of 50 per cent ethanol on potassium and sodium*

SOLVENT	POTASSIUM (P.P.M.)				SODIUM (P.P.M.)			
	10	30	50	100	10	30	50	100
Water	10.7	30.0	51.0	95.8	8.8	24.7	40.1	80.0
Ethanol, 50%	10.0	30.0	50.0	100.0	10.0	30.0	50.0	100.0

Organic congeners represent approximately 0.3 per cent of straight whisky. In a series of increment experiments, it was noted that light emitted by both potassium and sodium remained unaltered by normal congener concentrations.

West, *et al.* (6), with a natural gas-oxygen flame and a Beckman flame spectrophotometer, showed that emissions from 80 p.p.m. of potassium and sodium were not affected by the presence of 1000 p.p.m. of sulfate and chloride anions. Bicarbonate anions in the same concentration show an inhibiting effect on potassium and an enhancing effect on sodium. Parks, *et al.* (7), using a Model 18 Perkin-Elmer flame photometer, reported that 0.02 mole per liter of sulfate concentration slightly inhibited the emissions of potassium and sodium. Approximately 0.01 mole per liter of chloride and nitrate anions had a negligible effect on the emission of these metals.

Light emitted by potassium and sodium as excited by the oxygen-hydrogen Beckman flame was not affected by the presence of 1000 p.p.m of sulfate, chlorite, bicarbonate, and nitrate anions. The data presented in Table 3 were obtained under the same conditions used to construct the calibration curves.

Radiation interferences, or the positive or negative effect of a metal upon the net radiation of another metal being evaluated have been noted by several investigators. West, *et al.* (6), reported that potassium and sodium enhanced each other when excited by a natural gas-oxygen flame.

Mosher, *et al.* (8), noted that sodium in alcohol-plasma solution enhanced the potassium light 8 per cent. Knight, *et al.* (9), showed that sodium enhanced potassium, and attributed this effect to the instrument used.

TABLE 3.—*Effect of anions on potassium and sodium*

ANIONS 1000 P.P.M.	POTASSIUM P.P.M.		SODIUM P.P.M.	
	10	30[a]	10	30[a]
Sulfate	10.0	30.2	10.2	30.1
Bicarbonate	10.0	30.0	10.2	30.4
Nitrate	10.1	30.1	10.1	30.2
Chloride	10.1	30.0	10.1	30.4

[a] These concentrations required 1.0 selector switch.

Parks, *et al.* (7), with a Model 18 Perkin-Elmer flame photometer, showed that 80 p.p.m. of potassium can be added to 100 p.p.m. of sodium, and 80 p.p.m. of sodium to 100 p.p.m. potassium without effect. In the same article it is noted that 80 p.p.m. of potassium inhibits the light emitted by 1000 p.p.m. of sodium, and 80 p.p.m. of sodium slightly inhibits the degree of brightness exhibited by 1000 p.p.m. of potassium.

Experiments were conducted to discern the possible enhancement or inhibition of the light emitted by potassium and sodium in the presence of each other when excited by an oxygen-hydrogen flame of a Beckman flame spectrophotometer. Since potassium and sodium in straight whiskies are present in concentrations of less than 100 p.p.m., the effect of these

TABLE 4.—*Effect of potassium and sodium on each other*

POTASSIUM (OR) SODIUM P.P.M. (IN 50% ETHANOL)	SODIUM (P.P.M.)				POTASSIUM (P.P.M.)			
	10	30	50	100	10	30	50	100
10	10.0	30.2	50.0	100.0	10.1	30.0	50.7	100.0
30[a]	10.2	30.1	50.0	100.0	10.1	29.0	51.0	101.2
50[a]	10.3	30.2	50.0	100.0	10.4	30.0	50.7	102.0
100[a]	10.4	30.1	50.0	100.0	10.4	30.8	51.3	102.3

[a] These concentrations required 1.0 selector switch.

metals on each other was considered for quantities up to 100 p.p.m. The data presented in Table 4 were obtained under conditions stipulated in the preparation of the "semipermanent" calibration curves. No appreciable effect, within the accuracy of the instrument, was noted.

Radiation interferences have been mentioned by many authors using different flame spectrophotometers. Variations in data can be attributed to instrument modifications, individual techniques, and the types of samples. Dunker and Passow (10) indicate that emission is affected by a change in surface tension of a solution. This was observed in the course of

experimentation with whiskies. Viscosity changes were also noted and found to be extremely important when considering the effect of large concentrations of interfering ions.

SUMMARY

(1) The flame spectrum of approximately one hundred straight whiskies has been determined. These whiskies contained not more than 10 p.p.m. of sodium and not more than 35 p.p.m. of potassium. Calcium was present in trace quantities. Several blended whiskies were analyzed for potassium and sodium, and were found to contain similar metal concentrations. The potassium content of blends is partially due to the presence of blending agents high in potassium.

(2) An accurate quantitative method has been presented for the determination of potassium and sodium in the presence of each other.

(3) Application of these methods to other distilled spirits is feasible after appropriate modifications.

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THE CHEMICAL COMPOSITION OF CERTAIN GRADES OF PUERTO RICAN TOBACCO, TYPE 46*

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Type 46 cigar tobacco is produced principally in the central inland part of Puerto Rico. In 1950, approximately 25.5 million pounds of this type of tobacco were produced (11). This type of tobacco is used chiefly

* The investigation on which this report is based was conducted under the authority of the Agricultural Marketing Act of 1946 (RMA, Title II).

for cigar fillers; small quantities are used for cigar binders and for chewing tobacco.

There is very little information in the literature on the chemical composition of Puerto Rican tobacco, and the analytical data which have been recorded were obtained on tobacco not definitely identified as to type and grade (4–6).

The work described in this paper, which is of a preliminary nature, was undertaken for the purpose of determining the percentages of a number of constituents of several grades of Puerto Rican, Type 46, tobacco, and to correlate such findings with some of the physical characteristics which are important standardization factors of this type.

SELECTION AND PREPARATION OF SAMPLES

All the tobacco grades used for this investigation were from the 1949 crop. The tobacco, when received by the authors, had been air-cured in a manner customarily followed for this type; it had been farm sorted, but not subjected to "sweating" or bulk fermentation. In preparing the samples, the various farmers' grades, obtained from several farms, were carefully re-sorted leaf by leaf in order to attain the highest degree of uniformity, and any leaf not meeting the particular Federal grade specification was discarded. The several samples to be examined chemically were selected to represent the basic characteristics of their respective grades without making any allowances for mixtures commonly found in farm-sorted tobacco. Grades from two groups, namely, C and X, were selected. The C group includes tobacco which, because of its general quality, length, width, tensile strength, and relative freedom from defects, waste, and injury, can be stemmed by hand economically and is used as long fillers in the manufacture of cigars. The X group includes tobacco which cannot economically be stemmed by hand. It is commonly stemmed mechanically and is used as short fillers in the manufacture of cigars. Within each group, a number of grades of different qualities (indicated by Arabic numerals), and of light body (F) ("Fino") and heavy body (P) ("Pisado") were chosen.

X3S was selected because of its unusual characteristics in relation to the other tobacco grades. This tobacco is highly hygroscopic and of very heavy body; it is easily bruised; and, as the special factor "S" indicates, it is discolored or stained. The staining may be due to bruising or subjecting the tobacco to pressure in the course of handling, when it is in high order (high moisture content).

X3F normally appears in a variety of qualities, and, because of its special physical characteristics, it can be differentiated into several subgrades. The special factors (indicated by a capital letter in parentheses for the grade designation) have the following meanings:

The designation "(E)" is the abbreviation of the Spanish word "Espu-

milla," which refers to tobacco extremely tissuey in body and low in oil. It is also commonly referred to as "spent" or "pajizo."

The special factor "(H)" in the grade designation indicates that the tobacco had been subjected to microbial decomposition during air curing. Tobacco thus affected is commonly referred to as being "house-burnt" or "pole-burnt."

The factor "(A)" in the designation of the subgrade X3F(A) indicates that the tobacco is characterized by its yellow color ("A" is the abbreviation for the Spanish word "Amarillo," or yellow).

The special factor "(G)" designates tobacco having a definitely green or crude green color, and "(V)" in the grade designation refers to tobacco having a light greenish cast, as distinguished from a more definite green.

In all cases, the stems or midribs were completely removed; the residual leaf tissue of each grade was dried at room temperature, ground in a Wiley mill equipped with a 1 mm sieve, thoroughly mixed, and preserved in jars provided with airtight closures. The jars were stored in a metal cabinet.

METHODS OF ANALYSIS

All determinations were made in duplicate on dried (at room temperature) and ground stemmed tobacco, and the results were calculated on the basis of moisture-free and sand-free material except for sand which was calculated on a moisture-free basis.

Water-Soluble and Water-Insoluble Ash.—The total ash and 150 ml of distilled water were boiled, with stirring, over a small flame for three to five minutes. The mixture was filtered and the insoluble material was transferred to the filter paper and washed with hot water; the combined washings and filtrate (designated as "A") were retained for the determination of the "Alkalinity of Water-Soluble Ash." The filter paper and contents were transferred to a weighed platinum dish, dried, and heated for one hour at 600°C. in an electric muffle furnace. From the weight of ash obtained, the percentage of water-insoluble ash was calculated. The percentage of water-soluble ash was obtained by difference.

Alkalinity of Water-Soluble Ash.—The alkalinity of filtrate "A" was determined by the A.O.A.C. method (1, **29.13**), and the results were expressed in milliliters of normal acid required to neutralize the water-soluble ash from 100 grams of moisture-free tobacco.

Chlorine.—This was determined by the A.O.A.C. method (1, **6.41, 6.42**).

Petroleum Ether Extractives.—The ground sample (equivalent to 5 grams of moisture-free tobacco) was extracted for 24 hours in a Soxhlet extraction apparatus with petroleum ether (b.p. 30°–65°C.). The residue was dried to constant weight at 100°C. in an electric drying oven, weighed, and the percentage loss in weight calculated.

Nitrate Nitrogen.—Nitrate nitrogen was determined by the method of Vickery and Pucher (12).

Ammonia Nitrogen.—This was determined by the method of Pucher, Vickery, and Leavenworth (10). However, instead of colorimetrically estimating the ammonia that distilled over, it was determined by titration; a microburet was used with methyl red as the indicator. A blank determination was carried out, and a correction was made for the blank, as well as for the very small quantity of nicotine that distilled over.

Pectin or Water-Soluble Pectinic Acids.—The weighed sample (equivalent to 2 grams of moisture-free tobacco) was placed in the Weihe-Phillips extractor (13) and extracted successively, first for thirty minutes with 50 ml of hot (50°C.) 95% ethanol, and then for fifteen minutes with 25 ml of this solvent at the same temperature. The alcoholic extract was discarded. To the alcohol-extracted tobacco 100 ml of distilled water was added; the mixture was digested at room temperature for thirty minutes and stirred from time to time. The aqueous extract was removed by suction, and the extraction was repeated three more times or until a portion of the extract gave no precipitate of pectic material upon the addition of two volumes of 95 per cent ethanol. The combined aqueous extract was placed in a porcelain evaporating dish, concentrated on the steam bath to a volume of approximately 150 ml, and filtered into a 200 ml volumetric flask; the dish and filter paper were washed three times with distilled water and the solution was made to volume. Fifty ml of this solution was acidulated with 5 ml of 10 per cent HCl and diluted with water to a volume of approximately 100 ml; 200 ml of 95 per cent ethanol was added with stirring, and the mixture was allowed to stand overnight. The crude pectic material was filtered off, washed with a 1+2 (by volume) water-ethanol solution, and dissolved in hot water made alkaline by the addition of a few drops of NH_4OH solution. The pectic determination, as calcium pectate, was completed according to the method of Carré and Haynes (2) as modified by Kertesz (3).

Pectic Fraction Extractable with Hot Dilute Acid.—To the sample in the extractor from which the water-soluble pectin has been removed, as described above, 100 ml of 0.05 *N* HCl (previously heated to 85° C.) was added, and the mixture was digested for thirty minutes at 80°–85°C. with intermittent stirring. The extract was removed with the aid of suction and the digestion with hot 0.05 *N* HCl was repeated three more times. The combined extract was concentrated on the steam bath to a volume of approximately 150 ml, and the solution was filtered and made to volume in a 200 ml volumetric flask. The remainder of the procedure was the same as described for the determination of pectin and water-soluble pectinic acids, beginning with: "Fifty ml of this solution was acidulated. . . ." In this case also, the pectic material was calculated in terms of percentages of calcium pectate.

Pectic Acid and Pectates.—The procedure for the determination of pectic

acid and pectates was the same as described in the preceding paragraph, except that the residual tobacco sample from the extraction with hot 0.05 *N* HCl was extracted five successive times with a hot (80–85°C.) 0.5 per cent solution of ammonium citrate. In this case also, the pectic acid and pectates were determined as percentages of calcium pectate.

All the other analytical data recorded in Table 1 were obtained by methods described or referred to in a previous publication (9).

RESULTS AND DISCUSSION

The results obtained on the analysis of the several grades of Puerto Rican, Type 46, tobacco are shown in Table 1, where the several grades are arranged in four blocks. Blocks I and II consist of the thin body (F) and heavy body (P) tobaccos of the C group, respectively. Similarly, Blocks III and IV comprise grades of the X group, also subdivided on the basis of relative degrees of body.

Table 1 shows that the percentages of ash (sand-free), water-soluble ash, alkalinity of the water-soluble ash, and pectic acid and pectates were greater in the light-bodied CF grades than in the heavy-bodied CP grades. The reverse was true with respect to the percentages of the nitrogenous constituents, namely, total nitrogen, ammonia nitrogen, protein, and nicotine. In the X group of grades the percentages of ash (sand-free), water-soluble ash, and the alkalinity of the water-soluble ash were also greater in the four light-bodied X3F subgrades of Block III than in the heavier bodied grades of Block IV. However, the percentages of nicotine were greater in the grades of Block IV than in those of Block III. The very heavy bodied and stained tobacco, designated as X3S, had a much greater nicotine content than any of the other grades examined in this study.

The four subgrades of X3F differed in composition with respect to certain constituents. Thus, the percentages of ash (sand-free), alkalinity of the water-soluble ash, total nitrogen, and protein were significantly greater in X3F(G), than in the other three X3F subgrades. However, the nicotine content of X3F(G) was found to be extremely low, and was, in fact, the lowest of all the tobacco grades analyzed in this study. The extremely low nicotine content of X3F(G) can be attributed to the fact that this grade consisted of green, immature, second-growth leaf. However, it is rather difficult to account for the relatively high lignin content of X3F(G), since immature leaf would be expected to contain a smaller percentage of this constituent.

X3F(E) was found to contain a much greater percentage of oxalic acid than the three other X3F subgrades.

All the tobacco grades examined in this study were found to be entirely free of reducing sugars. The percentages of "Total Reducing Substances" recorded in Table 1 include various reducing substances, some of which may be of the nature of polyphenols.

TABLE 1.—*Composition of several grades of Puerto Rican tobacco, Type 46*[a,e]

U. S. GRADE	SAND	ASH (SAND-FREE)	WATER-SOLUBLE ASH	WATER-INSOLUBLE ASH	ALKALINITY OF WATER-SOLUBLE ASH[b]	CHLORINE	PETROLEUM ETHER EXTRACTIVES	TOTAL NITROGEN	NITRATE NITROGEN	AMMONIA NITROGEN
	per cent	*per cent*	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Block I:										
C1F	0.54	21.44	10.37	11.07	90	1.09	2.48	4.45	0.15	0.48
C2F	0.48	22.74	11.72	11.02	90	1.29	2.61	4.10	0.14	0.38
C2F(V)	0.37	21.78	11.33	10.45	90	—	0.61	4.58	—	—
C3F	0.90	22.36	10.89	11.47	97	0.78	2.12	3.72	0.10	0.31
Av.		22.08	11.08	11.00	92			4.21		0.39
Block II:										
C1P	0.40	18.34	8.22	10.12	50	1.33	2.26	5.41	0.15	0.72
C2P	0.45	17.39	7.22	10.17	43	1.07	3.20	4.63	0.08	0.53
C2P(V)	0.79	19.21	9.04	10.17	66	1.16	1.27	4.97	0.24	0.49
Av.		18.31	8.16	10.15	53			5.00		0.58
Block III:										
X3F(E)	0.68	21.90	9.10	12.80	49	1.65	2.41	2.77	0.03	0.17
X3F(H)	0.49	22.19	8.18	14.01	45	1.44	0.84	3.44	0.11	0.18
X3F(A)	0.63	20.62	8.35	12.27	38	1.02	4.54	2.24	0.14	0.22
X3F(G)	1.12	24.73	9.40	15.33	60	1.55	1.19	4.16	0.21	0.08
Block IV:										
X3P	0.36	17.84	6.01	11.83	13	1.63	1.81	4.64	0.07	0.37
X3S	0.80	14.98	6.95	8.03	34	1.37	3.17	5.09	0.09	0.61

TABLE 1—(*continued*)

U. S. GRADE	PROTEIN	NICOTINE	TOTAL REDUCING SUBSTANCES (AS GLUCOSE)[c]	PECTIN FRACTION EXTRACTED WITH ACID[d]	PECTIC ACID AND PECTATES (AS Ca PECTATE)	PENTOSANS	CELLULOSE	LIGNIN	OXALIC ACID	CITRIC ACID	*l*-MALIC ACID
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Block I:											
C1F	13.75	1.48	0.56	0.79	9.34	3.96	16.7	7.00	1.95	6.55	5.21
C2F	14.49	1.20	0.75	0.66	9.50	4.04	16.3	8.24	1.76	6.45	4.38
C2F(V)	16.27	1.15	0.38	—	—	—	—	7.06	2.05	5.95	4.28
C3F	13.88	0.95	0.76	0.76	9.29	4.19	16.5	7.97	2.42	5.77	5.77
Av.	14.60	1.19		0.74	9.38	4.06	16.5	7.57	2.04	6.18	4.91
Block II:											
C1P	15.78	2.90	0.56	0.88	8.22	3.62	13.0	5.78	1.55	6.85	4.00
C2P	14.47	2.00	0.93	0.86	8.94	3.93	16.2	7.37	2.58	5.39	4.72
C2P(V)	17.04	1.77	0.45	0.95	8.37	3.71	15.2	7.20	2.17	7.35	3.87
Av.	15.76	2.22		0.90	8.51	3.75	14.8	6.78	2.10	6.53	4.20
Block III:											
X3F(E)	11.56	0.94	0.99	0.89	9.30	4.89	19.2	7.99	3.67	6.20	3.16
X3F(H)	14.53	0.68	0.78	0.79	9.68	3.78	18.0	10.41	2.80	6.59	4.15
X3F(A)	7.93	1.19	1.63	1.56	6.20	4.95	17.6	5.97	2.72	4.59	4.37
X3F(G)	19.48	0.28	0.38	0.95	8.42	3.27	16.4	11.33	2.86	6.88	2.43
Block IV:											
X3P	18.02	1.79	0.48	1.09	7.47	4.20	16.5	8.01	3.97	5.92	2.32
X3S	12.10	4.13	0.92	1.46	6.96	3.15	12.2	5.39	3.18	3.89	5.61

[a] The tobacco was of the 1949 crop. Analyses were made only on stemmed tobacco.
[b] Alkalinity expressed in ml of normal acid required to neutralize water-soluble ash from 100 grams of moisture-free tobacco (reported to nearest ml).
[c] All grades were found to be entirely free of reducing sugars.
[d] All grades were free of pectin or water-soluble pectinic acids.
[e] Analytical data, except percentages of sand, calculated on moisture-free and a sand-free basis. The percentages of sand were calculated on a moisture-free basis.

By the analytical method used, all the principal pectic constituents, namely, pectin and water-soluble pectinic acids, the hot dilute acid-soluble protopectin, and pectic acid (and pectates) could be determined successively on the same weighed portion of sample. All the thirteen tobacco grades examined were found to be entirely free of pectin or water-soluble pectinic acids. In all cases, pectic acid was the principal pectic substance present. In this connection it may be pointed out that Neuberg and co-workers (7), who isolated methyl alcohol from tobacco smoke, postulated that the pectic substances are the precursors of this alcohol in the smoke. It is known that pectin, or water-soluble pectinic acids, and protopectin contain substantial quantities of ester methoxyl. Neuberg and co-workers considered that when tobacco is subjected to thermal decomposition, as in normal smoking, these ester methoxyl groups are split off and appear in the smoke as methyl alcohol. However, it is necessary to point out that pectic acid, unlike the other pectic substances, is substantially free of methoxyl. Accordingly, if the theory of Neuberg and co-workers as to the origin of the methyl alcohol in tobacco smoke is correct, then one would expect that the smoke from any of the thirteen grades of Puerto Rican tobacco examined in this study would be relatively free of methyl alcohol. Lignin is the only other substance found in tobacco which is known to contain methoxyl groups. However, all the methoxyl groups in the case of lignin are ether methoxyls, which are not so readily removed as ester methoxyl groups. Although lignin, when subjected to thermal decomposition or dry distillation, affords some methyl alcohol, a considerable portion of the methoxyl appears in the distillate as methyl ethers of various phenolic substances (8).

The relatively high pectic acid content in X3F(H), as compared with the three other X3F subgrades, may be attributed to the fact that this tobacco had been subjected to the microbial decomposition known as "house burn." It is known that certain microbial enzymes can degrade protopectin into pectic acid, and the latter constituent can thus increase at the expense of the protopectin. However, it may also be possible that pectic acid is relatively more resistant than some of the other tobacco constituents to the type of microbial decomposition causing "house burn" and this would result in an increase of this component percentagewise.

SUMMARY

The percentages of the following constituents (all calculated on a moisture-free and sand-free basis) of thirteen grades of Type 46 tobacco from which the midribs had been removed were determined: ash, water-soluble and water-insoluble ash, chlorine, petroleum ether extractives, total nitrogen, nitrate nitrogen, ammonia nitrogen, protein, nicotine, total reducing substances (as glucose), pectic substances, pentosans, cellulose, lignin, oxalic, citric, and *l*-malic acids. The percentages of ash, water-soluble

ash, alkalinity of the water-soluble ash, and pectic acid and pectates were greater in the light bodied (F) grades than in the heavy bodied (P) grades, while the reverse was true with respect to the percentages of total nitrogen, ammonia nitrogen, protein, and nicotine. In the X group of grades, the percentages of ash, water-soluble ash, and the alkalinity of the water-soluble ash were also greater in the light bodied (F) grades than in the heavy bodied (P) grades, while the percentages of nicotine were greater in the heavy bodied (P) grades. All thirteen grades were found to be free of pectin or water-soluble pectinic acids. In all cases, pectic acid was the principal pectic substance present.

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DICHLOROFLUORESCEIN AS AN INDICATOR IN THE DETERMINATION OF ANIONIC DETERGENTS

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The methods most frequently used for the determination of anionic detergents are the Jones colorimetric (1) and the Epton titration, both of which use methylene blue as the indicator (2). The latter method is rapid,

but difficulties are often encountered in determining the end point. Furthermore, carboxylates give no end point at all.

It was found that dichlorofluorescein might be used as an indicator for the determination of sulfated and sulfonated detergents and also for carboxylated detergents, including soap.

In the present experiments sodium lauryl sulfate and sodium lauroyl sarcosinate were used. The lauroyl sarcosine can also be determined provided it is converted by excess alkali into its sodium salt.

Dichlorofluorescein has been widely used as an adsorption indicator in the titration of halides (3, 4) and, more recently, high molecular quaternary ammonium halides (5), but it appears not to have been mentioned as an indicator for anionic surface active agents.

The procedure is that which is used in the Epton method and is based on a direct titration of the anionic detergent with a quaternary ammonium chloride in the presence of dichlorofluorescein. The latter produces a pink color with the cationic detergent. In aqueous solution, the change in color from the original greenish to pink is too gradual for estimation of the end point, but with chloroform a sharp color change is obtained. The appearance of the color should be observed with transmitted light.

EXPERIMENTAL

To determine optimum conditions, a series of tests were made with varying concentrations of cationic, as well as anionic, detergent. With a 0.01 *M* solution, the separation of the water-chloroform layer occurs very slowly. Therefore, the concentration of the cationic detergent was reduced to 0.008 *M*. Typical results are shown in Table 1. Similar results were obtained with the Epton titration.

TABLE 1.—*Titrations with 0.008 M cationic detergent*

SODIUM LAURYL SULFATE, 90% PURE G/250 ML[a]	CATIONIC DETERGENT ML		MG ANIONIC DETERGENT PER ML 0.008 *M* CATIONIC
0.2222	3.50	3.53	2.28
0.4447	6.95	7.00	2.29
0.6337	9.85	9.82	2.32
0.8276	12.57	12.57	2.37
1.0192	15.30	15.30	2.40

[a] 10-ml aliquots used.

Several routine analyses were made on various samples of commercial sodium lauryl sulfate, using both dichlorofluorescein and methylene blue (Epton) as indicators. A comparison of the results is given in Table 2.

To determine the standard deviation a series of determinations was made with 99.8 per cent pure sodium lauryl sulfate. Both dichlorofluorescein and methylene blue were used as indicators. Tables 3 and 4 show the results.

TABLE 2.—*Comparison between indicators*

SAMPLE NO.	WEIGHT OF SAMPLE IN G/250 ML	ACTIVE MATERIAL FOUND WITH		DIFFERENCE *per cent*
		DICHLOROFLUORESCEIN *per cent*	METHYLENE BLUE *per cent*	
1	0.3209	93.3	93.1	+0.2
2	0.5066	98.9	98.8	+0.1
3	0.3972	93.5	92.4	+1.1
4	1.4472	30.0	30.2	−0.2
5	0.4091	98.5	98.9	−0.4
6	0.4289	96.7	96.7	0.0
7	0.4163	100.3	100.0	+0.3
8	0.3247	98.4	97.7	+0.7
9	0.4113	90.3	91.0	−0.7

TABLE 3.—*Cationic detergent, 0.008 M; dichlorofluorescein indicator*

AMOUNT PRESENT GRAMS	AMOUNT FOUND GRAMS	RECOVERY PER CENT	d	d^2
0.00977	0.00972	99.28	0.75	0.5625
0.00969	0.00972	100.30	0.27	0.0729
0.01039	0.01044	100.47	0.44	0.1936
0.01127	0.01117	98.93	1.10	1.21
0.01013	0.01009	99.69	0.34	0.1156
0.01057	0.01059	100.18	0.15	0.0225
0.00890	0.00899	101.01	0.98	0.9604
0.00897	0.00899	100.22	0.19	0.0361
0.00897	0.00899	100.22	0.19	0.0361
		Av. = 100.03		$\Sigma d^2 = 3.2097$[a]

[a] Standard deviation: $\sigma = \sqrt{\Sigma d^2/N-1} = 0.63$.

TABLE 4.—*Cationic detergent, 0.008 M; methylene blue indicator*

AMOUNT PRESENT GRAM	AMOUNT FOUND GRAM	RECOVERY PER CENT	d	d^2
0.00938	0.00938	100.00	0.53	0.2809
0.00943	0.00940	99.67	0.86	0.7396
0.00786	0.00783	99.61	0.92	0.8464
0.00827	0.00852	103.02	2.49	6.2000
0.00908	0.00910	100.22	0.31	0.0961
0.01058	0.01040	98.29	2.24	5.0176
0.01035	0.01053	101.73	1.20	1.4400
0.01051	0.01067	101.52	0.99	0.9801
0.01150	0.01159	100.78	0.25	0.0625
		Av. = 100.53		$\Sigma d^2 = 15.6632$[a]

[a] Standard deviation: 1.40

The use of dichlorofluorescein as indicator appears to give better reproducibility than methylene blue.

Additional work (not quoted) indicated advantages for even lower concentrations than 0.008 *M*. Further determinations were therefore made with 0.004 *M* solutions of the cationic detergent.

Various chemists were asked to titrate amounts of the anionic detergent (sodium lauryl sulfate) unknown to them. The results appear in Table 5.

TABLE 5.—*Cationic detergent, 0.004 M; anionic detergent 100% pure*

CHEMIST	AMOUNT PRESENT	AMOUNT FOUND	RECOVERY	*d*	d^2
	GRAM	GRAM	PER CENT		
1	0.01094	0.01100	100.54	0.49	0.2401
	0.01065	0.01059	99.43	0.62	0.3844
2	0.01065	0.01067	100.19	0.14	0.0196
	0.01094	0.01088	99.45	0.60	0.36
3	0.01094	0.01088	99.45	0.60	0.36
	0.01054	0.01059	100.47	0.42	0.1764
	0.01077	0.01088	101.02	0.97	0.9109
4	0.01059	0.01065	100.57	0.52	0.2704
	0.01042	0.01044	100.19	0.14	0.0196
5	0.01088	0.01088	100.00	0	0
	0.00948	0.00944	99.46	0.59	0.3481
6	0.01071	0.01065	99.43	0.62	0.3844
	0.01082	0.01082	100.00	0	0
7	0.01065	0.01071	100.56	0.51	0.2601
	0.00967	0.00966	99.99	0.06	0.0036
			Av. = 100.05	$\Sigma d^2 = 3.3776$[a]	

[a] Standard deviation: 0.49.

On the basis of the data obtained, the following procedure is recommended:

METHOD

REAGENTS

(a) *Recrystallized quaternary ammonium chloride, M. W. 400.*—For a 0.004 *M* soln weigh accurately 0.3200 g and transfer to small beaker. Dissolve completely and transfer to 200 ml volumetric flask. Make to vol. (The soln is stable for 24 hrs.)

(b) *Dichlorofluorescein.*—Dissolve 0.05 g in 3 ml of 0.1 *N* NaOH and add 50 ml of distd H_2O. Filter if necessary. Keep in a stoppered bottle. (The soln is stable for 2 weeks.)

PROCEDURE

Pipet 10 ml of an approximately 0.004–0.006 *M* soln of the detergent into a 50 ml graduated, glass-stoppered cylinder. Add 15 ml of distd H_2O, followed by 3 drops of indicator and 10 ml of chloroform. Shake well. Add the standard cationic detergent from a 10 ml microburet almost to the end point. Avoid shaking during the early stages to prevent the formation of emulsion. Near the end point, the greenish-yellow color of the H_2O layer changes to pink. At this stage, apply mild shaking and titrate to the first appearance of pink color in the chloroform layer. Observe color in transmitted light.

COMMENTS

A concentration of 0.003 to 0.004 *M* is recommended in titrations of carboxylated types of detergents.

When the concentrations of anionic and cationic detergents are almost equal, danger of emulsion formation is decreased, but sometimes it may be necessary to clear the chloroform layer with the addition of 3–5 ml of chloroform.

The end point in the reaction between cationic and anionic detergent is stoichiometric. For convenience, the concentration of the anionic detergent should be in the same range as the cationic.

The reaction is favored in slightly alkaline media. The dichlorofluorescein indicator usually supplies the necessary alkalinity. Chlorides or sulfates do not affect the end point, but the presence of alcohol causes it to appear prematurely.

Soaps react like anionic detergents. This may be utilized practically: in mixtures containing soap and sulfated (and/or sulfonated) anionic detergents, the total detergent content can be determined by titration with dichlorofluorescein indicator. The sulfated (or sulfonated) anionic detergent can then be determined separately by the Epton titration and soap thus estimated by difference.

SUMMARY

(1) Dichlorofluorescein is recommended as indicator in the titration of anionic detergents.

(2) The preferred concentration for the standard cationic detergent solution is 0.004 *M*. The concentration of the anionic detergent should be in the same range. A proposed procedure and supporting data are given.

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A STUDY ON INTERFERENCES IN THE ANALYSIS OF ZINC ETHYLENEBISDITHIOCARBAMATE*

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During the time that the Rohm and Haas Company has been producing Dithane† Z-78, a careful check has been made on reports that formulations containing Dithane occasionally assayed below the expected values. In all cases the Dithane as supplied was found to be above specifications; thus it was felt that the low results found after formulation were due either to accelerated decomposition or to interference from some component of the diluent materials. After closer examination it was definitely shown that, at least in several cases, the low results obtained could be traced to the presence of copper contaminants. It was decided at this point to make a rather complete survey on interference materials that might be found with Dithane, either through planned addition or by chance contamination.

The question as to what materials should be included in this interference survey was decided on the basis of the probability of their occurrence as contaminants, diluents, dispersing agents, or added active ingredients. A micronized sample of Dithane Z-78, which contained 75.5 per cent zinc ethylenebisdithiocarbamate, was used in all of the tests and the analyses were run by the procedure of Clarke, *et al.*, (1) with the exception that the decomposition time was extended to one and one-half hours. The samples consisted of about 0.2000 grams of Dithane Z-78 to which was added approximately 0.0200 grams of the substance whose interference effects were to be examined. The results of this first survey are given in Table 1 under the heading *Sulfuric Acid.*

(For the purpose of this investigation, any analysis with a deviation greater than ± 1.5 per cent from the mean value of the standard (75.5 per cent) was considered to be evidence of interference. All runs were made in duplicate and those showing interference, i.e., deviation greater than ± 1.5 per cent, were again run in duplicate to check the findings. All values falling within ± 1.5 per cent of the value for the standard are noted by the letter A and those falling within ± 2.0 per cent but more than ± 1.5 per cent by the letter $\pm$B, the sign designating the direction. Materials that gave rise to a deviation greater than ± 2 per cent are designated by $\pm$C. It is with this "C" group of materials that the greater part of this paper is concerned.)

Examination of Table 1 shows that with the exception of sodium thiosulfate, the other interfering materials in the sulfuric method are sodium, lead, and calcium arsenates; copper sulfate; and cuprous oxide. While

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† Trademark of Rohm and Haas Company for fungicide based on zinc ethylenebisdithiocarbamate.

TABLE 1.—*Effect of various materials*[a]

10% ADDITIVE	SULFURIC ACID, 1.1 *N*	PHOSPHORIC ACID, 85%
Pesticides		
Calcium arsenate	−C	−B
Lead arsenate	−C	A
Aramite	A	A
Toxaphene	A	A
Chlordane	A	A
DDT (50% wettable powder)	A	A
Lindane	A	A
Dinitro-*o*-cresol	A	A
Kryocide	A	A
Wettable sulfur	A	A
$CuSO_4 \cdot 5H_2O$	−C	A
$NaHAsO_4 \cdot 7H_2O$	−C	A
Na_2SiF_6	A	A
Cu_2O	−C	A
Inorganic Contaminants		
$MgSO_4 \cdot 7H_2O$	A	A
$CaSO_4 \cdot 2H_2O$	A	A
$Al_2(SO_4)_3 \cdot 18H_2O$	A	A
$PbSO_4$	A	A
$Pb(C_2H_3O_2)_2 \cdot 3H_2O$	A	A
$MnSO_4 \cdot H_2O$	A	A
$Fe_2(SO_4)_3$	A	A
$CoCl_2 \cdot 6H_2O$	A	A
$NiCl_2 \cdot 6H_2O$	A	A
Na_2CO_3	A	A
$Na_2B_4O_7 \cdot 10H_2O$	A	A
$Na_2S_2O_3 \cdot 5H_2O$	−C	A
Diluents and Dispersing Agents		
Celite 545	A	A
Loomkill talc	A	A
Borden clay	A	A
Cherokee clay	A	A
Bentonite	A	A
Diluex	A	A
Walnut shell flour	A	A
Special tobacco carrier	A	A
Phospho dust	A	A
Tamol N	A	A
Starlac	A	A
Other		
Ethylene Thiourea	A	+C

[a] A—Assay within ±1.5% of average uncontaminated sample assay. B—Assay within ±1.6–2.0%. C—Deviation more than ±2.1%.

some tests were made on the C. P. grades of all of these compounds, most of the work was done on Orchard brand calcium arsenate, General Chemical Company; Ortho Brand lead arsenate, California Spray-Chemical Company; cuprous oxide, Rohm and Haas Company; and reagent grade sodium arsenate and copper sulfate pentahydrate. It is felt that the compounds used are representative of those likely to be found in practice and that the expected behavior of most other compounds in their class can be implied from the properties of the materials studied here.

In setting up criteria for suitable methods of eliminating the interferences, some thought had to be given to the expected concentrations of these materials. In all cases, the possibility of casual contamination existed, but there was also the question of intentional formulations of Dithane with large amounts of both copper and arsenate. Examination of the literature (2) showed that the maximum arsenate-Dithane ratio to be expected was about 300 per cent as calcium or lead arsenate, based on known formulations of these arsenates with zinc dimethyldithiocarbamate and zinc ethylenebisdithiocarbamate. Similarly, the maximum copper-dithiocarbamate ratio brought to the attention of the authors was about 200 per cent as basic cupric carbonate or about 100 per cent as copper. These, then, were the upper concentration limits aimed at in developing methods for eliminating these interferences.

The methods developed for eliminating, or in some cases minimizing, these interferences are based essentially on the conventional procedures (1, 3) which call for decomposing the dithiocarbamate in acid medium to yield carbon disulfide which is then washed with lead acetate, converted to the xanthate, and titrated with iodine. The fundamental modification, called the "basic method," is the substitution of 85 per cent *ortho*-phosphoric acid for 1.1 *N* sulfuric acid. This change eliminates interferences of the "C" group of materials when the latter are present in approximately 10 per cent concentration. In order to eliminate interferences due to higher concentrations of these materials, it was necessary to employ pretreatment of the sample and, in one case, slight changes in the basic procedure. These are the "modified methods" which are given immediately after the "basic method." The exact magnitude of the interferences due to the presence of various amounts of the "C" group of materials as well as the corrections obtained by using the various procedures employed is given in Figs. 1 and 2.

After "the basic method" was found to be satisfactory, it was used in another interference study made on the same group of materials tested with the sulfuric acid method. The results are given in Table 1, under the heading *Phosphoric Acid.* It was at this point that the interference of ethylene thiourea with the phosphoric acid method was noted; this problem was solved successfully by the modified method used for calcium and sodium arsenate.

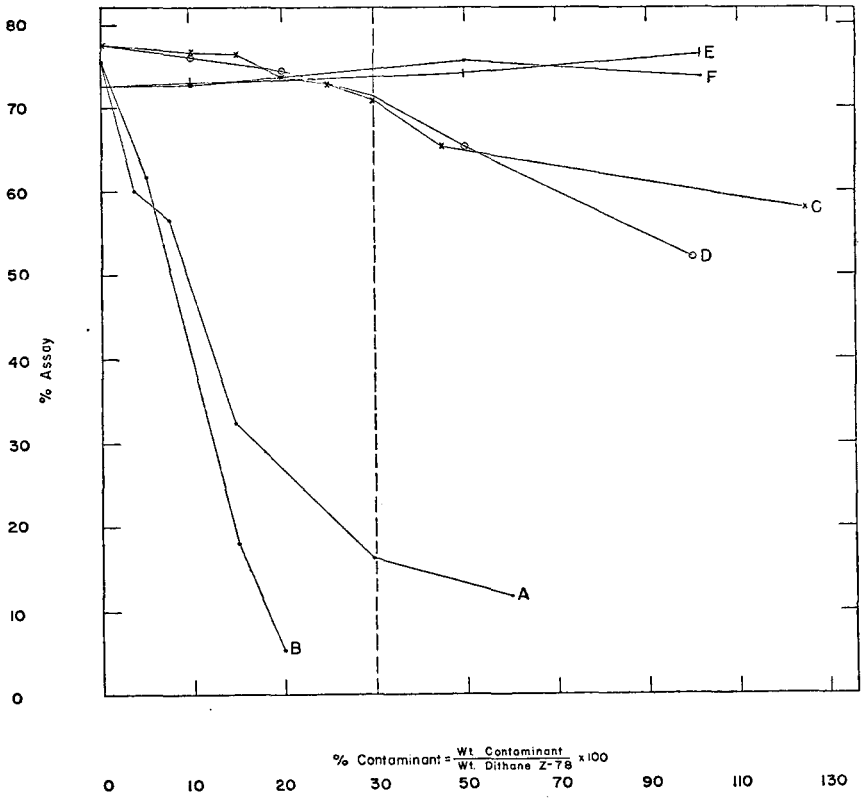

FIG. 1.—A: Dithane Z-78 + $CuSO_4 \cdot 5\ H_2O$—1.1 *N* H_2SO_4.
B: Dithane Z-78 + Cu_2O—1.1 *N* H_2SO_4.
C: Dithane Z-78 + $CuSO_4 \cdot 5H_2O$—H_3PO_4
D: Dithane Z-78 + Cu_2O—H_3PO_4
E: Dithane Z-78 + $CuSO_4 \cdot 5\ H_2O$ + 3 ml Na_2S—H_3PO_4
F: Dithane Z-78 + Cu_2O + 3 ml Na_2S—H_3PO_4
The dotted vertical line marks a change in the scale used in plotting per cent contamination.

Two problems related to this work were investigated at some length:
(1) The decomposition of ethylene thiourea in acid medium.
(2) The role of the "solvent medium" in the decomposition of zinc ethylenebisdithiocarbamate. Both topics are briefly discussed after the presentation and discussion of the methods.

Throughout this paper, the material used as the reference standard is the Dithane Z-78 sample employed in the interference tests. In the few cases where other samples were used, the results are presented on the

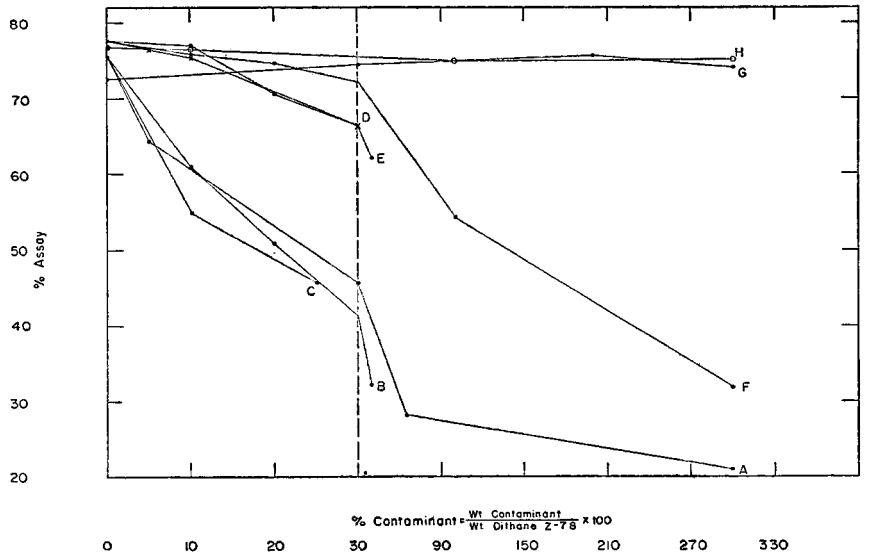

FIG. 2.—A: Dithane Z-78+calcium arsenate—1.1 *N* H_2SO_4
B: Dithane Z-78+sodium arsenate—1.1 *N* H_2SO_4
C: Dithane Z-78+lead arsenate—1.1 *N* H_2SO_4
D: Dithane Z-78+calcium arsenate—H_3PO_4
E: Dithane Z-78+sodium arsenate—H_3PO_4
F: Dithane Z-78+lead arsenate—H_3PO_4
G: Dithane Z-78+lead arsenate (30% $HClO_4$ wash)—H_3PO_4
H: Dithane Z-78+calcium arsenate (25% acetic acid wash)—H_3PO_4

$$\%\ \text{Assay } \textit{vs.}\ \%\ \text{contaminant} \left(\frac{\text{wt contaminant}}{\text{wt Dithane Z-78}} \times 100 \right)$$

The dotted vertical line marks a change in the scale used in plotting per cent contamination.

basis of this standard. When references are made to deviations or recoveries they are based on this material which assayed 75.5 per cent by the normal sulfuric acid procedure and 77.5 per cent by the basic phosphoric acid method. The difference between these two standard values is thought to be due to the presence of small amounts of ethylene thiourea and sodium thiosulfate, which will be discussed. The phosphoric acid value is an average based on about twenty determinations performed over a period of two weeks. The standard deviation is ± 0.63 and while on any one day replicate analyses show considerably less scatter, it is felt that the results of analyses over a period of time give a much closer approximation to the true precision of the method as ordinarily applied. All of the data plotted in Figs. 1 and 2 are the averages of at least duplicate runs.

METHODS

BASIC METHOD

(The "basic method" will eliminate interference of cuprous oxide up to 15% by weight; copper sulfate pentahydrate, up to 15%; lead arsenate, up to 10%; calcium arsenate, up to 9%; and sodium arsenate, up to 10%. Ethylene thiourea will give a positive interference which can be eliminated in the modified acetic method. For larger amounts of these contaminants, this method must be modified as discussed in the notes given at the end of the method.)

REAGENTS

(a) *Acetic acid*, 30%.

(b) *Lead acetate (aqueous)*, 10%.

(c) *Ortho-phosphoric acid*, 85%.

(d) *Methanolic potassium hydroxide*, 2 *N*. Dissolve 112 g of KOH pellets in 500 ml of anhyd. methanol, filter through cotton, and then add an addnl 500 ml of methanol. Run a blank on this soln by pipetting 25 ml into a 500 ml Erlenmeyer flask contg 150 ml of H_2O, add 3 drops of phenolphthalein indicator, neutralize with 30% acetic acid, add 5 ml of starch indicator and 250 ml of H_2O, and titrate with 0.1 *N* iodine.

(e) *Iodine*, *N*/10.

(f) *Phenolphthalein indicator solution.*

(g) *Starch indicator solution.*

APPARATUS

(a) *Automatic buret.*—For 0.1 *N* iodine.

(b) *Decomposition apparatus.*—The apparatus used has been previously described (1). The only modification is in the lead acetate trap which contains a small amount of glass wool at the bottom surrounding the inlet tube. In addn, where 100 ml of acid is called for (see note 1) a 300 ml round bottom boiling flask must be used and the inlet tube into this flask must be lengthened so that it will extend to within 1 cm of the bottom of the flask.

PROCEDURE

(The method is based on the fact that the sample decomposes on heating in an acid medium with the evolution of CS_2. This is aspirated through a trap contg lead acetate soln to remove the H_2S and SO_2 formed from impurities in the sample. The CS_2 is then trapped in methanolic KOH and forms K xanthate. This soln is then neutralized to phenolphthalein with dil. acetic acid and titrated with 0.1 *N* iodine soln to a starch end point.)

Accurately weigh 0.2 to 0.3 g (Note 1) of sample into a small glass vial (e.g., 12×25 mm) and immediately place in the boiling flask. Transfer 15 ml of the 10 % lead acetate to the first absorbing trap, which has a quantity of glass wool in the bottom to break up the larger bubbles formed on aspiration. Pipet 25 ml of the 2 *N* methanolic KOH into the second absorbing trap and start aspiration at a moderate rate. Add 50 ml (Note 2) of hot 85% *o*-phosphoric acid through the inlet tube and heat the flask with a Bunsen flame as rapidly as possible. Loosen the clamp holding the condenser and swirl the flask in such a manner that the sample becomes completely wet. Care must be taken to avoid bumping until the soln begins to boil. The H_2O originally present in the phosphoric acid condenses, and upon dropping back into the hot phosphoric acid, may cause sudden changes in pressure which tend to make the soln back up into the inlet tube. When this occurs to a large

degree, temporarily remove the flame or increase the aspiration rate until boiling begins. Continue boiling the flask for 1.5 hrs (Note 3).

Disconnect the methanolic KOH absorption trap and rinse the contents into a 500 ml wide-mouth Erlenmeyer flask, using ca 250 ml of distd H_2O. Add 3 drops of phenolphthalein indicator soln and titrate with 30% acetic acid until the soln is just neutral. Immediately titrate the neutralized sample with 0.1 *N* iodine. When near the end point, add 5 ml of starch soln and titrate to a faint, but definite, color change. Subtract the blank, as obtained from titrating 25 ml of the methanolic KOH with 0.1 *N* iodine, from the vol. of iodine used in the titration of the sample. Calculate the percentage of zinc ethylenebisdithiocarbamate from the relation:

$$\frac{(\text{Sample titration} - \text{blank})(I_2 \text{ normality})(\text{Eq. wt of dithiocarbamate})}{\text{Wt of sample} \times 10}$$

NOTES

Note 1: Correspondingly larger samples must be taken if analysis is run on a formulation; e.g., for formulations containing 3.9% zinc ethylenebisdithiocarbamate, a 2.0–3.0 g sample should be used.

Note 2: As discussed later, the boiling temperature of the decomposition medium is fairly critical. Therefore when large amounts of inert impurities or water are present in the sample, it is advisable to use a 300 ml boiling flask and add 100 ml of hot phosphoric acid in place of 50 ml.

Note 3: For uncontaminated samples, decomposition is complete after 0.5 hr of boiling and the solution is free of solid impurities.

(A) ELIMINATION OF INTERFERENCE DUE TO MORE THAN 10 PER CENT COPPER

Procedure.—Use two lead acetate traps to which 3–5 g of solid lead acetate are added in addn to the normal amount of 10% lead acetate soln. Then treat the sample in exactly the same manner as in the "basic method," except that after placing the sample in the decomposition flask, add 3 ml of a satd sodium sulfide soln, swirl the flask until the sample is thoroly wet, and then wait 5 min. before adding the hot phosphoric acid. Caution must be used in adding the initial portion of phosphoric acid, since a large vol. of H_2S will be given off and dropwise addns of the first 3 ml of acid is recommended. (The results obtained with the use of sodium sulfide must be corrected in accordance with the amount of copper present (Fig. 1). This correction ranges from +2–4% over the range of copper contamination to which this procedure is suited, and can be estimated more closely, of course, if the amount of copper present is approximately known.)

(B) ELIMINATION OF INTERFERENCE DUE TO ETHYLENE THIOUREA AND MORE THAN 9 PER CENT CALCIUM ARSENATE, OR 10 PER CENT SODIUM ARSENATE

(Since the use of phosphoric acid alone will eliminate only the interference of 9% calcium arsenate and 10% sodium arsenate, some other means had to be devised to handle larger amounts of these compounds. Washing the sample with 25% acetic acid and then decomposing the sample plus the filter paper with phosphoric acid proved an excellent solution. This wash was also successful in eliminating any interference due to ethylene thiourea.)

Procedure.—Weigh the sample directly into a 50 ml beaker, and add 10 ml of 25% acetic acid. Disperse the sample, making sure it is wet very thoroly, allow 10 min. total contact time, and then transfer the contents of the beaker to a filtration apparatus. The filter paper should be of a very low porosity (Whatman #42) and

mild suction is recommended. Complete the transfer, using 5 ml of 25% acetic acid, followed by minimum quantities of H_2O. If the sample size is such that the quantities of wash liquids are not sufficient to wet and transfer the sample efficiently, increase the amounts used. Place the filter paper containing the sample into the decomposition flask and proceed according to the usual phosphoric acid method. Although no decomposition of zinc ethylenebisdithiocarbamate in 25% acetic acid was observed over a period of an hour, it is best to complete the washing operation as rapidly as possible.

In comparing these results to the usual phosphoric acid determination of an uncontaminated[1] sample, it is found that these values are slightly lower. Presumably this is due to the fact that the washing step results in slight decomposition of the sample and also the elimination of the small amounts of ethylene thiourea normally present. It also follows, therefore, that the results obtained will be lower than the straight phosphoric acid decomposition of the same sample. Corrections can be obtained from Fig. 2.

Frothing of the solution may occur during the decomposition after 15–30 min. of digestion. Care should be taken at this point that this froth is not carried over into the lead acetate trap. When this cannot be prevented by reasonable variation of aspiration and heating rate, the decomposition should be stopped. Good results are obtained even after only 0.5 hr of heating time.

(c) ELIMINATION OF INTERFERENCE DUE TO MORE THAN 10 PER CENT LEAD ARSENATE

Lead arsenate also interferes with the assay of zinc ethylenebisdithiocarbamate, but it is insoluble in 25 per cent acetic acid under the conditions used. An effective means of removing large amounts of lead arsenate is to wash the sample with 30 per cent perchloric acid.

Procedure.—Treat the sample in the same manner as with the acetic acid modification, using two portions of 10 and 5 ml of 30% perchloric acid,[2] followed by an addnl H_2O wash; then decompose the sample and filter paper with phosphoric acid. Any effects of trace amounts of arsenate remaining after the washings are eliminated by the use of phosphoric acid. The washing steps should be completed within 15 min., as significant amounts of the sample decompose during treatment. Apply corrections from Fig. 2 as explained in the following discussion of the method.

DISCUSSION OF THE METHODS

Figures 1 and 2, which are largely self explanatory, are a summary of data obtained by the above methods. The data for Dithane Z-78 plus 230 per cent yellow cuprocide, Dithane Z-78 plus 275 per cent cupric carbonate and Dithane Z-78 plus 200 per cent copper in basic copper sulfate are not included. These three samples all assayed 72.5 per cent. From these data the corrections needed for specific samples and methods may be obtained. Caution should be applied in transferring these figures directly to other compounds but by an examination of these data it should be possible to pick the modified method best suited to a particular sample. However, as discussed subsequently, in certain special cases the use of a decomposition

[1] Taken to mean that no thiosulfate is present since the latter, which is a suspected decomposition product in certain cases, interferes in the sulfuric acid method.

[2] While 30% perchloric acid has been used in this laboratory for numerous determinations, it should be remembered that this acid, especially in more concentrated solutions, is potentially dangerous.

medium other than 85 per cent phosphoric acid may be desirable and this should be checked (see discussion on the role of the medium on the decomposition of zinc ethylenebisdithiocarbamate).

Glass wool is needed in the lead acetate traps because the rate of aspiration must be increased over the conventional rate when using phosphoric acid in order to minimize bumping and backing up of the sample into the inlet tube. The glass wool is also needed to increase the efficiency of absorption of H_2S which is given off at a faster rate with this method. A perforated disc, or traps, as recommended by Lowen (4) can accomplish the same purpose. Glass wool and the traps shown were used merely because of availability and ease of cleaning between runs.

The effect of varying the rate of air passage through the potassium xanthate was examined to see if it was a significant variable. A standard solution of potassium xanthate was prepared by adding carbon disulfide to methanolic potassium hydroxide. Aliquots were added to the traps used in the conventional setup. Air was drawn through the apparatus at two widely different rates for equal periods of one and one-half hours and a blank was run, using only the methanolic potassium hydroxide. The results are summarized in Table 2, and it is apparent that the error due to the passage of air through the xanthate trap is negligible.

TABLE 2.—*Effect of varying rate of air passage through potassium xanthate*

RATE OF AIR PASSAGE	STD. POTASSIUM XANTHATE	METHANOLIC KOH	STD. ALIQUOT (NO AIR)
2–4 bubbles/sec.	24.97 ml $N/10$ I_2	Blank	25.05 ml $N/10$ I_2
10–15 bubbles/sec.	25.10 ml $N/10$ I_2	Blank	

To clarify the effect of the rate at which air is drawn through the system on the recovery of carbon disulfide, a weighed amount of carbon disulfide was placed in the empty reaction vessel of a conventional setup and air was drawn through the system at two different rates, as before. Complete recovery was noted in both cases. This shows further that no losses are incurred when the air stream is varied from a slow to a moderately fast rate.

When washing a sample suspected of containing sodium or calcium arsenate with 25 per cent acetic acid, care must be used to insure that all the sample is dissolved or suspended in the wash media. In running standards, it was found that the C. P. calcium arsenate is less soluble in 25 per cent acetic acid than is technical calcium arsenate manufactured for spray purposes and complete elimination of the interferences due to C. P. calcium arsenate is possible only when the sample is adequately mixed with the acetic acid prior to filtration.

During analysis of several uncontaminated standard samples by the perchloric acid modification, some deviations were encountered which suggested that the sample was decomposing during the wash period. The effect of 30 per cent perchloric acid and 25 per cent acetic acid on Dithane in relation to time was studied and is summarized in Table 3.

TABLE 3.—*Effect of perchloric and acetic acids on Dithane*

WASH	SAMPLE SLURRIED[a] AND FILTERED AS SOON AS POSSIBLE	SLURRIED FOR ½ HOUR,[a] THEN FILTERED	SLURRIED FOR 1 HOUR,[a] THEN FILTERED
25% Acetic acid	75.3 Assay	75.3 Assay	74.6 Assay
30% Perchloric acid	72.4 Assay	68.9 Assay	61.8 Assay

[a] Slurrying and filtering the sample as rapidly as possible took 10 to 15 minutes. All times given are in addition to this 10–15 minute period.

It is evident from these results that use of 25 per cent acetic acid causes no decomposition in the time normally needed to treat a sample. However, when using perchloric acid, the washing must be done as quickly as possible. By extrapolating back to zero time with the perchloric acid washes, a value very close to that obtained with acetic acid is found.

While the main portion of this work has been done on Dithane Z-78, the methods have also been found applicable to commercial mixes of Dithane dusts. Some of these were mixes accidentally contaminated by a formulator with small amounts of copper salts; as a result, they assayed very low by the conventional procedure (1). However, when the basic phosphoric acid method was used, the assay was satisfactory.

Effects of Impurities Associated with the Decomposition or Manufacture of Zinc Ethylenebisdithiocarbamate.—It had previously been noted (1) that sodium thiosulfate caused low results in the assay of disodium ethylenebisdithiocarbamate. In the case of zinc ethylenebisdithiocarbamate, the effect of sodium thiosulfate on the assay is similar and of the same order of magnitude if the sample is decomposed in 1.1 *N* sulfuric acid. When the phosphoric acid method is used, however, there is no measurable interference due to the amount of sodium thiosulfate normally present. Even with large amounts, e.g., 50 per cent sodium thiosulfate, the recovery was 97 per cent.

Ethylene thiourea has been postulated by several authors (5, 6) as a decomposition product of Nabam (disodium ethylenebisdithiocarbamate). In the present tests, 10 per cent of ethylene thiourea did not interfere with the analysis of Dithane by the sulfuric acid method. However, this was not the case when phosphoric acid was used. Under these conditions, ethylene thiourea decomposes, producing a gas that passes through the lead acetate trap and is absorbed by the methanolic potassium hydroxide

to yield a titratable product. On a weight basis, one part of ethylene thiourea assays the same as 1.2 parts of zinc ethylenebisdithiocarbamate by the basic phosphoric acid method. However, this difficulty can of course be easily remedied by a prewash with acetic acid, as is done in the modified procedure.

One important point that remains to be discussed is the difference between the values obtained for the standard by the various methods. It is believed that differences are due primarily to the presence initially, or the production during decomposition, of small amounts of ethylene thiourea. If the ethylene thiourea is present initially, the explanation of the discrepancy is obvious and leaves only the second case to discuss. Clarke (1) postulates two alternative methods by which ethylenebisdithiocarbamate can decompose and states that (*a*) is the one that greatly predominates.

(*a*) $2H^+ + Zn(CS_2 \cdot NHCH_2CH_2NHCS_2) \rightarrow 2CS_2 + NH_2CH_2CH_2NH_2 + Zn^{++}$

(*b*) $2H^+ + Zn(CS_2 \cdot NHCH_2CH_2NHCS_2) \rightarrow CS_2 + H_2S + \overline{NHCH_2CH_2NH\overset{\overset{S}{\|}}{C}} + Zn^{++}$

However, if one assumes that (*b*) proceeds to some extent, then the sulfuric acid method will give values below the theoretical while the phosphoric method will be unaffected, since the ethylene thiourea produced in (*b*) decomposes in the latter medium. Small amounts of thiosulfate present initially or formed by decomposition could also account for the discrepancies.

Decomposition of Ethylene Thiourea.—As described, 85 per cent phosphoric acid will decompose ethylene thiourea to give a titratable product. It was noted that when a pure sample of ethylene thiourea was decomposed by the normal phosphoric acid method, no white precipitate was formed during the course of the iodine titration as was observed invariably when a dithiocarbamate was analyzed. It was believed that the ethylene thiourea produced carbonyl sulfide, and it was decided to investigate this point. Carbonyl sulfide was prepared by the method of Klason (7), with modifications as suggested by Mellor (8), for purifying the gas stream. The gas obtained by this procedure was distilled at low temperatures and the volatile fractions were collected. Small amounts of the carbonyl sulfide were reacted with methanolic potassium hydroxide and the resulting solutions were then titrated in the usual manner with iodine solution. Again no white precipitate was obtained. This, together with consideration of the chemistry involved, indicates that the gas produced by the decomposition of ethylene thiourea is probably carbonyl sulfide.

Study of Various Decomposition Media and Effect of Temperature on Decomposition.—When it was found that 85 per cent phosphoric acid caused a more rapid decomposition of zinc ethylenebisdithiocarbamate than did

1.1 *N* sulfuric acid and also eliminated certain interferences, it was decided to attempt to characterize the important and necessary properties of a good decomposition medium. A number of experiments were designed to observe the effect of the boiling temperature, nature of the acid, concentration of the acid, presence of water, and strength of the acid.

Eighty-five per cent *o*-phosphoric acid boils around 165–170°C. while 1.1 *N* sulfuric acid boils at approximately 101–102°C. The composition of these acids was varied to give solutions having a range of boiling points. In Table 4 are summarized the approximate boiling temperatures of various concentrations of sulfuric and phosphoric acids after the addition of about 0.2 gram of Dithane.

TABLE 4.—*Boiling temperature of sulfuric and phosphoric acids after addition of Dithane*

Dithane sample +1.1 *N* sulfuric acid	101–102°C.
Dithane sample +50% sulfuric acid	135–140°C.
Dithane sample +60% sulfuric acid	155–163°C.
Dithane sample +80% sulfuric acid	175–185°C.
Dithane sample +85% *o*-phosphoric acid	150–155°C.
Dithane sample +45% *o*-phosphoric acid	114–118°C.
Dithane sample "180°" *o*-phosphoric acid[a]	168–172°C.

[a] The "180°" phosphoric acid was obtained by distilling water out of 85% *o*-phosphoric acid until the temperature reached 180°C.

The 80 per cent sulfuric acid was eliminated immediately as there was poor recovery of zinc ethylenebisdithiocarbamate due to oxidation. All the other acid mixtures gave good assays for uncontaminated zinc ethylenebisdithiocarbamate.

As the temperature of the medium went up, the samples digested at a faster rate in both acids and it was also noted that with a given copper contamination, the dithiocarbamate assay value increased with the temperature of the decomposition medium. Relative assay values of Dithane plus 50 per cent copper sulfate contamination are: 1.1 *N* sulfuric, 13 per cent; 45 per cent phosphoric, 40 per cent; 50 per cent sulfuric, 60 per cent; 80 per cent phosphoric, 66 per cent; 60 per cent sulfuric, 69 per cent; and 180° phosphoric, 71 per cent. Thus, it can be seen that the important factor in these mixtures is not the type or the concentration of acid, but rather the decomposition temperature.

The reason for the choice of 85 per cent phosphoric (purchased as such) rather than 60 per cent sulfuric or "180°" phosphoric, was one of convenience, though the latter acids were slightly better in eliminating the copper interference in certain cases. In the range of casual contamination all three are equivalent, while at the 50–100 per cent level, none is as successful in removing the interference as the sodium sulfide-phosphoric combination. However, 60 per cent sulfuric and "180°" phosphoric were

also found to be quite successful, and it is conceivable that in certain situations, i.e., 50 per cent copper sulfate contamination, the use of these acids would be more convenient than the Na_2S-phosphoric method. It is also to be noted that ethylene thiourea in 60 per cent sulfuric only gives half the interference that is found in 85 per cent phosphoric, although sodium thiosulfate interferes significantly in 60 per cent sulfuric.

All three acids were used in analyzing samples containing either 40 per cent calcium arsenate or 40 per cent lead arsenate without pretreatment of the sample. The results obtained with 60 per cent sulfuric are about 5 per cent lower than those found with 85 per cent phosphoric while those obtained with "180°" phosphoric acid are about 4 per cent higher. It is believed that all the methods described could be used with either the 60 per cent sulfuric or "180°" phosphoric acids in place of 85 per cent phosphoric acid.

To obtain some further evidence as to the importance of temperature *vs.* amount and type of acid used in the decomposition and also to get some idea of the role of water, two other media were used for decomposition. These materials were *p*-cymene, b.p. 172–173°, and caprylic acid, b.p.174° C.; the recovery values were 40 and 60 per cent respectively.

CONCLUSIONS

Some conclusions that may be drawn from this work concerning the importance of various factors on the decomposition of zinc ethylenebisdithiocarbamate to yield carbon disulfide are:

(1) Free water is not a necessary condition for the decomposition of zinc ethylenebisdithiocarbamate, cf. 180° phosphoric acid and *p*-cymene.

(2) Acid is not essential for the decomposition, although it probably acts as a catalyst promoting the desired mode of decomposition, cf. *p*-cymene.

(3) The temperature of decomposition is a very important factor, cf. all runs.

(4) The type of acid is not critical, though strong acids appear to promote the decomposition.

ACKNOWLEDGMENT

The authors are indebted to Dr. C. P. Lo for his help in planning the interference tests and to G. Brandes and T. Callan for their aid in collecting and examining certain commercial samples.

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SEPARATION AND IDENTIFICATION OF CHLORINATED ORGANIC PESTICIDES BY PAPER CHROMOTOGRAPHY

III. ALDRIN, ISODRIN, DIELDRIN, AND ENDRIN

By LLOYD C. MITCHELL (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

The solvent systems used to separate aldrin and dieldrin (1) also separate isodrin and endrin; however, they do not separate aldrin from isodrin or dieldrin from endrin. In this paper, the separation and identification of all four components by paper chromatography are described.

METHOD

Apparatus, reagents, and procedure used in the present method are the same as previously described (2), and the method for locating the developed spots is essentially the same. Other changes in the original procedure are as follows:

APPARATUS

The glass jar was replaced by a stainless steel tank (3), and the filter paper was not washed.

REAGENTS

The mobile solvent used here is pyridine and water, 3+2, v/v; the potassium hydroxide is increased in strength to 2 *N*, and is sprayed on both sides of the paper instead of only on the front side; the standards include isodrin and endrin in addition to aldrin and dieldrin.* All of the standards are of greater purity than those previously used (1).

DISCUSSION

It was found unnecessary to wash the filter paper, since the silver reactants normally present moved appreciably ahead of the four pesticides and formed a band across the upper portion of the chromatogram. Stronger potassium hydroxide was used because it apparently produced somewhat heavier spots on the paper. The paper was sprayed on both sides because the reagent did not always soak through, as evidenced by a "peppered" appearance on the side not receiving the spray.

* Highly purified samples of these substances were supplied by Shell Chemical Corporation, Julius Hyman Division, Denver, Colo.

TABLE 1.—*R_F values of aldrin, isodrin, dieldrin, and endrin spotted in all possible combinations, each component of 0.01 M concentration*

NUMBER	ALDRIN	ALDRIN DIELDRIN	ALDRIN ISODRIN DIELDRIN	ALDRIN ISODRIN	DIELDRIN	ALDRIN ENDRIN	ALDRIN DIELDRIN ENDRIN	ALDRIN ISODRIN DIELDRIN ENDRIN	ALDRIN ISODRIN ENDRIN	ISODRIN DIELDRIN	ISODRIN	DIELDRIN ENDRIN	ISODRIN DIELDRIN ENDRIN	ISODRIN ENDRIN	ENDRIN
A 1	.40	.37	.36	.35		.37	.37	.36	.36						
			.45	.44				.44	.44	.45	.44		.45	.45	
		.61	.60		.59		.60	.61		.61		.58	.61		
						.66	.66	.67	.67			.65	.66	.66	.70
A 2	.40	.39	.36	.35		.35	.35	.35	.35						
			.45	.43				.43	.43	.45	.43		.44	.44	
		.61	.61		.59		.59	.58		.61		.59	.59		
						.65	.64	.64	.65			.64	.64	.67	.70
B 1	.34	.36	.35	.35		.37	.36	.36	.35						
			.43	.43				.45	.44	.46	.45		.44	.47	
		.61	.60		.59		.61	.63		.63		.61	.61		
						.67	.67	.68	.69			.68	.68	.70	.72
B 2	.41	.40	.39	.39		.39	.38	.37	.37						
			.47	.48				.46	.46	.46	.46		.44	.45	
		.67	.65		.65		.64	.62		.63		.61	.61		
						.70	.70	.70	.69			.67	.67	.69	.71
C 1	.39	.39	.37	.37		.37	.36	.36	.36						
			.45	.44				.45	.44	.44	.44		.43	.45	
		.63	.61		.61		.61	.61		.61		.59	.59		
						.67	.67	.67	.67			.65	.65	.67	.71
C 2	.41	.40	.39	.39		.39	.39	.38	.39						
			.48	.50				.47	.47	.47	.48		.46	.47	
		.69	.68		.67		.66	.65		.67		.65	.63		
						.73	.72	.72	.71			.70	.69	.70	.74
D 1	.37	.38	.38	.38		.37	.37	.38	.37						
			.46	.47				.47	.46	.48	.47		.47	.47	
		.63	.62		.63		.63	.64		.64		.62	.63		
						.69	.69	.69	.69			.69	.69	.70	.72
D 2	.44	.44	.42	.41		.42	.42	.41	.41						
			.52	.52				.51	.50	.50	.50		.50	.51	
		.71	.69		.67		.68	.67		.66		.64	.66		
						.72	.73	.72	.72			.70	.71	.73	.73

TABLE 2.—*R_F values of aldrin, isodrin, dieldrin, and endrin spotted in all combinations of two, three and four components, each component of 0.01 molar concentration*

NUMBER	ALDRIN DIELDRIN	ALDRIN ISODRIN	ALDRIN ENDRIN	ALDRIN ISODRIN DIELDRIN ENDRIN	ISODRIN DIELDRIN	DIELDRIN ENDRIN	ISODRIN ENDRIN	NUMBER	ALDRIN ISODRIN DIELDRIN	ALDRIN DIELDRIN ENDRIN	ALDRIN ISODRIN ENDRIN	ISODRIN DIELDRIN ENDRIN	ALDRIN ISODRIN DIELDRIN	ALDRIN DIELDRIN ENDRIN	ALDRIN ISODRIN ENDRIN	ISODRIN DIELDRIN ENDRIN
A 1	.25	.25	.26	.25				A 2	.22	.26	.25		.27	.25	.26	
		.32		.32	.32		.32		.29		.32	.33	.33		.33	.33
	.42			.45	.47	.45			.43	.46		.45	.47	.45		.46
			.53	.51		.52	.49			.51	.52	.51		.51	.52	.52
B 1	.25	.26	.25	.26				B 2	.24	.26	.26		.27	.26	.27	
		.34		.32	.33		.31		.31		.35	.34	.35		.35	.32
	.42			.48	.48	.46			.49	.49		.49	.51	.46		.47
			.53	.54		.51	.50			.55	.56	.55		.53	.54	.53
C 1	.27	.28	.27	.24				C 2	.26	.26	.26		.27	.27	.27	
		.34		.32	.32		.30		.32		.32	.35	.35		.33	.33
	.47			.43	.46	.45			.49	.49		.49	.50	.49		.47
			.53	.51		.52	.50			.54	.54	.55		.55	.53	.53
D 1	.42	.41	.40	.39				D 2	.42	.41	.44		.43	.42	.42	
		.50		.52	.47		.49		.54		.54	.51	.52		.53	.55
	.71			.67	.68	.69			.75	.73		.71	.72	.71		.74
			.72	.73		.76	.76			.80	.81	.78		.78	.78	.81
								Repeat Runs								
A 3	.25	.26	.25	.23	.30		.30	A 4	.27	.27	.27		.26	.28	.27	
		.31		.31					.35		.34	.33	.32		.33	.34
	.46			.44	.44	.44			.49	.47		.47	.49	.47		.49
			.51	.51		.51	.49			.54	.52	.53		.53	.54	.55
B 3	.29	.28	.26	.23				B 4	.28	.30	.30		.30	.28	.29	
		.35		.33	.35		.33		.36		.37	.37	.37		.36	.36
	.49			.48	.50	.47			.51	.50		.50	.51	.50		.51
			.56	.54		.53	.54			.56	.57	.57		.56	.55	.57
C 3	.29	.26	.27	.25				C 4	.28	.28	.26		.26	.28	.26	
		.33		.32	.30		.31		.34		.34	.35	.34		.33	.33
	.46			.44	.44	.44			.48	.49		.48	.49	.49		.47
			.51	.50		.52	.51			.54	.54	.54		.55	.54	.52
D 3	.42	.40	.41	.39				D 4	.41	.41	.40		.41	.41	.41	
		.49		.49	.50		.51		.52		.49	.49	.49		.49	.54
	.62			.67	.68	.68			.71	.67		.67	.68	.68		.73
			.74	.73		.73	.75			.74	.74	.74		.75	.75	.78

In Table 1 are presented R_F values obtained for the four components when spotted in all fifteen possible combinations, on duplicate sheets (1 and 2) in the same tank, and in quadruplicate tanks (A,B,C,D.) All tanks were lined with blotting paper (3).

Further R_F values obtained for all possible combinations of two, three, and four components are given in Table 2. Three-component combinations were duplicated on the same sheet, in quadruplicate tanks, and in duplicate runs. Two- and four-component combinations were repeated in quadruplicate tanks and in duplicate runs. Tank D was lined with blotting paper; the other tanks were unlined. Results for tank D are consistent with those reported in Table 1, and are substantially higher than results obtained with unlined tanks. Time required for the solvent front to approach the upper edge of the 8-inch sheet in unlined tanks was approximately four hours—in lined tanks, approximately six hours. The band of silver reactants normal to the paper was substantially narrower when tank lining was used.

The results were obtained over a period of about seven weeks, during which time the temperature of the laboratory ranged from 23° C. for results in Table 1, to 28°C. for the initial runs of Table 2, and 30°C. for the repeat runs. The R_F values were apparently uninfluenced by this temperature variation.

A hundredth molar concentration of each component in the solution spotted is about optimum. When 0.1 molar concentrations were spotted on the 8-inch paper, aldrin and/or isodrin were separated from dieldrin and/or endrin, but there was overlapping of the spots of aldrin and isodrin, and of dieldrin and endrin, respectively. Use of paper longer than 8 inches produced unsatisfactory separations; excessive diffusion obscured any further separation which may have been achieved.

SUMMARY

R_F values are presented which show the separation, by a previously described technique, of the four components, aldrin, isodrin, dieldrin, and endrin, in all possible combinations. Somewhat better separations were obtained when the tanks were lined with blotting paper than when they were unlined, but a longer period of time was required to complete the development of the chromatograms in the lined tank.

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NOTES

A TANK FOR 8×8 INCH PAPER CHROMATOGRAMS

By LLOYD C. MITCHELL (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

In applications of paper chromatography, various containers have been used. The one illustrated in Figure 1 is now preferred for our purposes.

Details of construction are explained by the diagram. In use, the glass cover is sealed on with 1-inch cellophane tape. Glass troughs to hold the mobile solvent may be obtained from companies handling equipment for paper chromatography.

The 8×8 inch sheet was selected because time for development with many mobile solvent systems is within a one to four hour range and most tests can thus be completed within a working day. On each 8×8 inch sheet 7 or 8 samples can be spotted, without crowding, for one-dimensional development. This allows an adequate number of controls to be included along with unknown samples—a provision especially important where temperatures vary. Another important factor in paper chromatography is diffusion of the spot; in general, diffusion is proportional to the distance traveled on the sheet. In 6 to 6½ inches of travel (the usual maximum distance for 8×8 inch paper) diffusion is ordinarily not excessive. Although the size of the developed spot may limit the number of substances which can be separated on a sheet, mixtures of five components have been readily handled.

In operating this container with highly volatile solvents such as petroleum ether, the sides and ends of the tank are lined with heavy blotting paper so that the bottom edge dips into the same mobile solvent that is used in the troughs. Ordinary

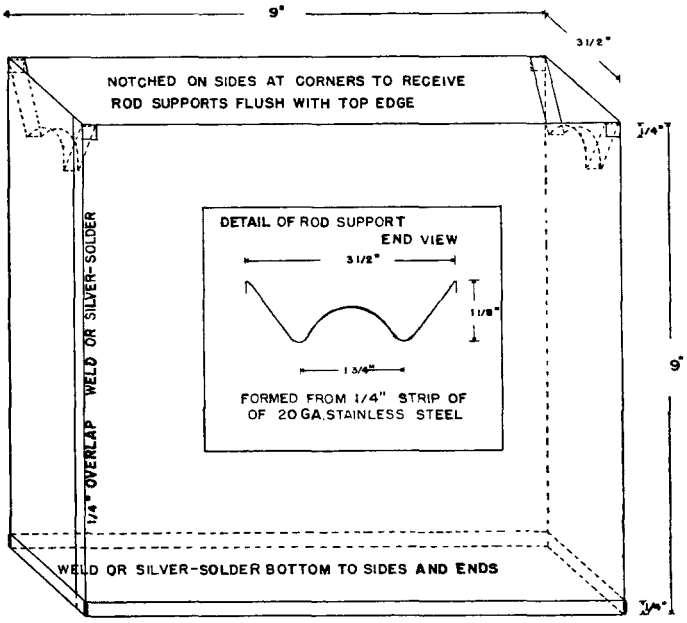

FIG. 1.—Chromatographic tank.

desk blotters, white or colored, cut to about $8\frac{3}{4}\times12\frac{1}{2}$ inches and bent into two L-shape pieces, require no fastening to remain in position and can be used repeatedly, with suitable drying between runs. An additional 50 ml or so of the volatile solvent is added directly to the bottom of the tank *just prior* to putting the papers into the troughs. Use of blotting paper gives better spots and a sharper solvent front, since the solvent front in the blotters moves just ahead of the front on the papers; this induces an atmosphere uniformly saturated with solvent vapor and allows minimum solvent loss from the chromatograms. (On the other hand if the solvent front on the blotters is too far ahead of that on the chromatograms, the solvent may condense on the upper portions of the papers and obscure the true front.) McFarren achieved similar conditions with double layers of filter paper (1).

REFERENCE

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ANALYSIS OF SICILIAN LEMON OILS

By J. W. Sale (Division of Food, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.)

In February of this year, this writer reported (1) the ultraviolet absorbancies and the chemical analyses of 12 samples of Italian lemon oils known to be pure. Those samples were collected at their source by an inspector of the Food and Drug Administration in the latter part of November, 1951. The ultraviolet absorbancies of 34 samples of Italian oils collected during their commercial production had been reported by Cultrera, *et al.*, and their results were also summarized.

It was hoped that a series of samples could be collected in Sicily during the 1952–1953 season with the cooperation of the Consulate General at Palermo. However, we have been able to obtain only four such samples because last year's drought, coupled with high lemon exports, held down essential oil production and rendered processing sporadic.

The four samples were air-expressed to the Food and Drug Administration and examined promptly. Collection and analytical data on these samples are given in Table 1.

TABLE 1.—*Sicilian lemon oils*[a]

SAMPLE NO.	DATE OF COLLECTION	MGFR'S. NO.[b]	BASE LINE ABSORPTION (LINE CD)	TOTAL ABSORPTION AT PEAK (CA 315 mμ)	STEAM DISTILLATION RESIDUE, PER CENT
INV 69–111 L	3/25/53	23	0.68	1.24	2.55
INV 69–112 L	3/25/53	15	0.70	1.28	2.61
INV 69–113 L	4/ 7/53	15	0.70	1.30	2.39
INV 69–114 L	4/28/53	23	0.73	1.37	3.35

[a] Collectors: E. Jan Nadelman and Giacomo Sausele of the American Consulate, Palermo, Italy; Analyst: W. O. Winkler, Division of Food, Food and Drug Administration, Washington, D. C.

[b] For methods used and meaning of line CD see Reference (1). Mfgr. No. 15 is the same as No. 15 in that reference. Mfgr. No. 23 was not represented in the previous investigation.

REMARKS

INV 69-111 L and INV 69-114 L.—The lemons were grown in the area of San Lorenzo Colli and were good winter lemons of second quality, not suitable for export. After the juice was expressed, the rind passed to an "Alfa Laval" separator

where the oil was separated. Samples were collected while the oil was being decanted from the oil and water mixture.

INV 69-112 L and INV 69-113 L.—The manufacturing procedure was the same as that of manufacturers numbered 14 and 15 (1).

SUMMARY

It is noted that the ultraviolet data average close to the values previously reported for Italian oils. The average residue by steam distillation for the four samples, viz., 2.72 per cent, is considerably higher than the value (1.87 per cent) previously reported.

REFERENCE

(1) SALE, J. W., *et al.*, *This Journal*, **36**, 112 (1953).

BOOK REVIEWS

Plant Anatomy. By KATHERINE ESAU. John Wiley and Sons, Inc., New York, 1953. 735 pp. Illus. Price $9.00.

Teacher, investigator, and student have in this volume a thoroughly up-to-date and lucidly written account of the basic subject matter of developmental plant anatomy. The topics are arranged in a logical manner and are so systematically treated that the unity of the organism is kept prominent in the reader's mind. A feature of special value is the devotion of a few paragraphs at the beginning of each topical discussion to a simple statement of the modern concepts, an evaluation of the classical concepts, and a clarification of problems of terminology. Discussions of the various cell types, tissues, and organs are developmental in viewpoint, but pertinent references are made to the phylogenetic and physiologic aspects of the subject. The results of modern research in cell wall structure and chemistry and in the experimental-morphological field are integrated with the best of the older data. The well-written text is supported by an abundance of superior line drawings and half-tones and excellently selected references to the classical and modern literature which will have real value to all workers in the plant sciences. Finally, a word of commendation must be said for the publisher who, in these days, has been able to print this book for a modest nine dollars.

J. E. ADAMS

Encyclopedia of Chemical Technology. Vol. X. By RAYMOND E. KIRK and DONALD F. OTHMER, Editors. The Interscience Publishers, Inc., New York, 1953. xvi + 976 pp. Illus. Price $25.00.

The 976 pages of Volume X contain 47 articles which vary in length from a few pages to the 140 pages devoted to three articles on petroleum, petroleum chemicals, and petroleum waxes. Each article has one or more bibliographies, and there are numerous cross references to subjects described in previous volumes and in the volumes still to be published.

The major emphasis in Volume X is, naturally, upon technology. For the technologist there are sections on perfumes, petroleum, petroleum chemicals, petroleum waxes, pharmaceuticals, phenolic resins and plastics, photoelectric cells, photography, pigments (inorganic, organic, pulp, and flush colors), pilot plant, pipes and valves, plant layout, plant location, plasticizers, plastics, plywood, poisons (economic), polishing materials, polyamides, and polyethylene.

Of particular interest to the organic chemist are articles on pentaerythritol, peroxides (organic), phenanthrene, phenol and phenols, phenolic aldehydes, phenolic ethers, phenolphthalein, phenolsulfonic acids, phenylenediamines and tolylenediamines, phloroglucinol, phosgene, phosphorus compounds (organic), phthalic acids, phthalocyanine dyes, and polymethine dyes. The inorganic chemist will pay special attention to the sections on peroxides (inorganic), phosphoric acid and phosphates, phosphorus and phosphorus alloys, phosphorus compounds (inorganic), and the platinum group metals. Articles of analytical interest deal with polarimetry and polarography. Physical chemistry is represented by sections on the phase rule, photochemistry, and polymers. The biochemist will note the sections on plant growth substances and plasma expanders.

The "Health and Safety Factors" subheadings in many of the articles merit the attention of all chemists; the material concerning the legal requirements for pharmaceuticals and other chemical products will be welcome to the technologist.

This volume has no index. The eventual publication of a general index will greatly increase the value of the work.

The "Encyclopedia of Chemical Technology," with its extensive coverage, should be a valuable addition to all technical libraries.

SYLVAN H. NEWBURGER

Fertilizer Technology and Resources in the United States. Agronomy: A Series of Monographs, Vol. III. Edited by K. D. JACOB. Academic Press, Inc., New York, 1953. 454 pages. Illus., index. Price $8.50.

The past century has seen the fertilizer industry develop from that of a scavenger to one of the largest units of our present-day heavy chemical industry. This monograph, a compilation of papers delivered at a Short Course in Fertilizer Technology held at the University of Maryland in August, 1950, under the sponsorship of the Fertilizer Committee of the Soil Science Society of America, covers the technological developments which were so intimately associated with this transformation. It well fulfills its stated objective of assembling concise and authoritative information on the resources, chemistry, technology, and manufacture of fertilizers and fertilizer raw materials.

The 14 chapers, each authored by a prominent authority in his field, contain a wealth of information which should prove useful to the student, technologist, fertilizer manufacturer, and others interested in fertilizer manufacturing technology.

The first chapter, which is somewhat brief, deals with topics such as the importance of fertilizers in food production, the major technological developments in fertilizer processing, and consumption trends. The next three chapters are devoted to the technology of nitrogen compounds. The first of these discusses consumption of nitrogen compounds, natural organic and inorganic nitrogen products, nitrogen from coal, and synthetic nitrogen fixation processes and products. This chapter closes with an interesting discussion of the present supply-demand relationship and probable trends in future demand for nitrogen fertilizers. A rather short but informative review of the principal processes for utilizing anhydrous ammonia in the production of solid nitrogen compounds is contained in the chapter on conversion of ammonia to fertilizer materials. Flowsheets are used to great advantage in describing these processes, since it is hardly possible for the author to treat in detail each product and process in the narrow confines of a single chapter. The chemical and physical properties of nitrogen compounds and their sphere of usefulness is well covered in the concluding chapter on nitrogen.

The three chapters devoted to fertilizer phosphates include discussions of phosphate resources and facilities, chemistry and manufacture of superphosphates and phosphoric acid, and chemistry and technology of new phosphatic materials. Although the first and third chapters of this group are particularly detailed in treatment, each chapter includes numerous references.

The first of two chapters dealing with potassium materials concerns potash resources in the United States in relation to world supplies and is essentially a summary of the available literature. As such, the information is well presented and documented. The chapter on production and processing of potassium materials covers the mining and refining of the various potash compounds.

The single chapter devoted to often-called "secondary nutrient elements" deals primarily with the resources and processing of materials carrying calcium, magnesium, and sulfur.

Probably one of the most difficult tasks an author may be assigned is that of describing the mechanics of mixed fertilizer production because of the wide variance in processes and products involved. Taking this into consideration, the author of the chapter dealing with this topic is deserving of much credit. The same applied to the chapter which discusses physical and chemical problems in mixed-fertilizer produc-

tion. The final chapter dealing with mixed fertilizers treats the subject of high-analysis mixtures. Included in this discussion are developments in high-analysis fertilizers, cost factors in mixed fertilizers, and some problems encountered in the formulation and use of high-analysis mixtures. The concluding chapter contains information on a wide variety of subjects such as special fertilizers, special uses for fertilizers, and nonfertilizer sources of plant nutrients. For the sake of completeness, it is regrettable that circumstances did not permit the publication in this volume of those papers dealing with the resources and processing of trace-element materials, and fertilizer grades and ratios.

Without doubt, this text is by far the most complete work of its kind available at the present time.

EDWIN C. KAPUSTA

Methods in Food Analysis Applied to Plant Products. By MAYNARD A. JOSLYN. Academic Press, Inc., New York, N. Y., 1952. 525 pp. Price $8.50.

The author has emphasized the principles on which many of the common determinations depend, with special reference to their adaptability for analyzing fruit and vegetable products. This objective contrasts sharply with aims which in descriptions of precise methods are often designed to show reproducibility of analytical values rather than their accuracy or correctness. The writer briefly explains the suitability and limitations of certain procedures for determining different properties and constituents in plant products, with a detailed description of at least one method. Each chapter is replete with documented references to basic research on the methods discussed.

After a review of procedures for sampling and sample preparation and preservation, 16 pages are devoted to interpretation of results based upon a statistical analysis thereof. Succeeding chapters deal with moisture determination, ashing, separation, and purification of constituents by selective solvents, densitometry, colorimetry, polarimetry, and spectrophotometry. The remaining chapters are devoted to the principles of potentiometry, *p*H, and buffer capacity of foods, viscometry, acidimetry, alcoholometry, and in determinations of reducing and nonreducing sugars and complex carbohydrates, pectins, pentosans, tannins, and of total and separable organic nitrogen-bearing complexes.

Application of the principles of analysis for plant products may be extended with little or no change to other types of products. Although intended as a text, the book furnishes explanations of procedures commonly used both in research and in routine analysis. The variability which characterizes biological growth phenomena is a challenge to critical consideration of representative sampling and statistical interpretation of analytical values, exclusive of errors inherent in the method. The author has recognized this need. The book will find a suitable place in laboratories engaged in both routine and research activities.

A. H. ROBERTSON

Richmond's Dairy Chemistry. 5th Ed. Revised by J. G. DAVIS and F. J. MACDONALD. Charles Griffin & Co., Ltd., London, 1953. 603 pp. Illus., index. Price 60*s* plus 1*s* 6*d* postage ($8.52 plus 21¢).

This book, originally published in 1899, is now in its fifth edition, and has been ably revised by experienced British dairy chemists. It deals primarily with the major constituents of milk and dairy products—their normal and abnormal range of values and the available methods of analysis—frequently with a critical appraisal of the latter, and discussion of the various factors involved.

The first part, entitled "General and Theoretical," which comprises somewhat

more than half the book, covers the composition and variation in composition of milk with particular emphasis on total fat, solids not fat, and freezing point; other constituents of milk: proteins, carbohydrates, minerals, fat components, and minor constituents (enzymes and oxygen; vitamins are deliberately omitted); milks from animals other than the cow; physical examination (specific gravity, refractometric methods, freezing point, and *p*H); formulas for milk calculations (relationships between specific gravity, fat, and solids not fat); detection of adulteration due to added water or abstraction of fat; cream; condensed and dried milk; butter and examination of butterfat; margarine and examination of margarine fat; cheese and fermented milk products; rennet and annatto; and legislation. The sections on freezing point and examination of butterfat by presently available methods are especially notable, the former for the detailed analysis of the various factors involved in the determination. Most interesting is the statement " . . . it is very unusual for duplicate determinations of the freezing point of milk to differ by more than 0.001° . . . " in view of statements made by several laboratories in this country regarding the non-reproducibility of their measurements.

The second part of the book is a recitation of methods or references, with pertinent comments and notes. Many A.O.A.C. methods are quoted or referred to. One major dairy product has been omitted—ice cream and related frozen desserts.

In the way of methods, there is little concession to modernity. Volatile acids (in connection with sour milk and cheese) are determined by modifications of the Duclaux distillation method. Even here the more recent work of Hillig is not mentioned. Chromatographic procedures for organic acids are covered by a single sentence. The method for lead in dairy products utilizes sulfide as the reagent. There is, however, a discussion of glyceride analysis by the Hilditch methods.

This volume is primarily a text dealing with methods for the detection of economic adulteration of dairy products, although one chapter is devoted to dirt in milk. One can speculate that the British do not have a problem involving decomposition, since this analytical phase is conspicuous by its absence.

It is not difficult to see why this volume has enjoyed great popularity in Great Britain, since the numerous tables and the discussions of the interpretation of methods make the book an invaluable aid to the diary chemist, despite the fact that much of the data are more than twenty years old. Unfortunately, these data cannot be applied to dairy products of this country without verification and, therefore the chief value of the book here is in the comments on, and interpretations of, methods. The fundamental data compiled here are rarely published these days, although they are undoubtedly available in the files of industrial and regulatory laboratories. A future edition could well stand a drastic pruning of older methods and data.

WILLIAM HORWITZ

Technics of Plant Histochemistry and Virology. By T. E. RAWLINS and W. N. TAKAHASHI. National Press, Millbrae, Calif., 1952. ix+125 pp. Illus., index. Price $3.50.

This text deals essentially with a number of isolated subjects of interest to the research worker in the botanical sciences. Each subject is worthy of a complete text and has been dealt with in that manner by other writers. The present treatment of each subject thus necessarily is brief and becomes primarily a survey course serving to introduce the student to a number of applied sciences such as optical crystallography, chemical microscopy of plant tissues, X-ray crystallography, statistics, photography, and virus technics. The application to plant pathology is stressed.

It should be pointed out, however, that several of the topics are treated in a

very sketchy manner. Thus, the material in Chapter II, titled "Use of X-rays in Determination of Structure," is presented in two and one-half pages. It would have been more appropriate to have included this material as a subsection in the chapter on virus technics, since the application of this technic in characterizing the crystalline structure of plant viruses is mentioned briefly. One also wonders what purpose is served in a text of this name by Chapter IV which outlines several chemical methods for the determination of copper in spray residues. Chapter VII is limited to one-half page in which is discussed a statistical analysis of toxicity testing by referring to only two papers on the subject; Chapter VIII, titled "Use of the Microscope," merely outlines, in less than half a page, six steps on how to secure proper illumination. Thus, it becomes apparent that the book wavers between being a manual of laboratory exercises and a syllabus of topics for classroom discussion and reading elsewhere.

The chapter on "The Polarizing Microscope" is an excellent summary of the use of this instrument in determining structure and chemical constituents of plant tissues. The chapter on "Identification of Plant Constituents" presents a very complete tabulation of histochemical tests for plant constituents. These two chapters comprise more than half the volume of the text.

This text in reality is a revision of the senior author's book "Phytopathological and Botanical Research Methods," (John Wiley & Sons, 1933), which title, this reviewer believes, more aptly describes the contents of the present volume. It is hoped that in the second edition of this book the authors will achieve the arrangement and coverage of subject matter and the excellent references to citations which characterized the earlier work.

W. V. EISENBERG

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