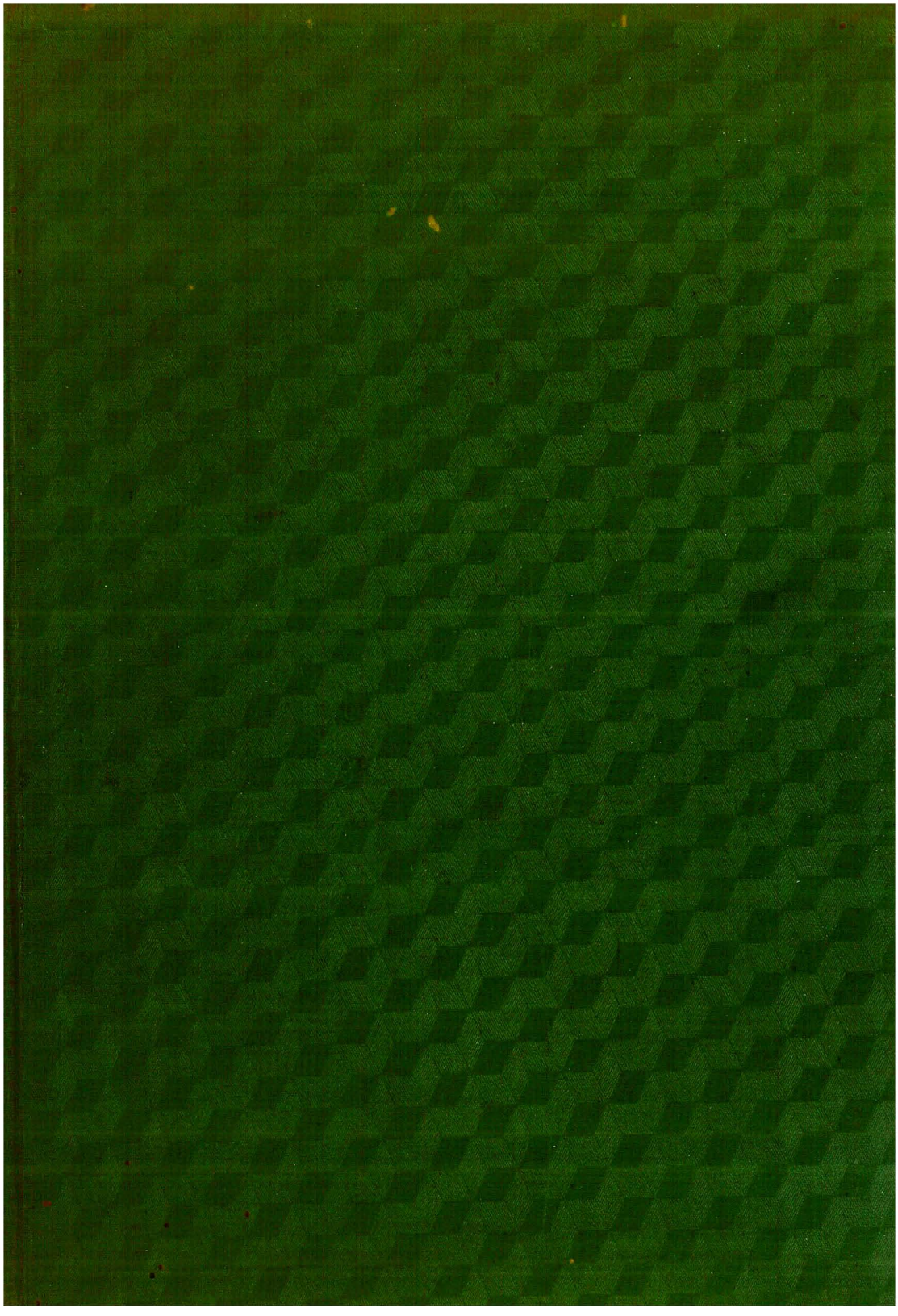

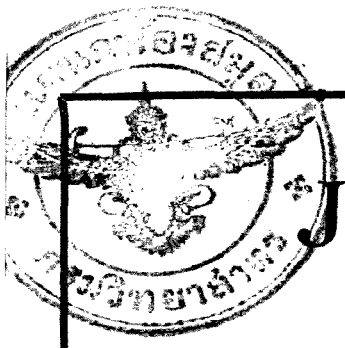

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OF THE

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VOL. 32 FEBRUARY 15, 1949 No. 1

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OF THE

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Vol. 32 FEBRUARY, 1949 No. 1

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Reprints will be furnished at cost to contributors of articles printed in *The Journal.* A table showing cost, with an order slip, is sent by the printer with proof.

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ANNOUNCEMENT

SIXTY-THIRD ANNUAL MEETING, 1949

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

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Hotel reservations should be arranged for directly with the hotel, and well in advance of the meeting.

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The Association urgently needs a substantial number of copies of Vol. 29, No. 1, the Feb. 15, 1946, issue of its Journal. Those having copies not intended as permanent files will favor the Association by returning them. Payment of $1.60 per copy will be refunded to cover their cost and postage as second class matter.

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PROCEEDINGS OF THE SIXTY-SECOND ANNUAL MEETING OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1948

The sixty-second annual meeting of the Association of Official Agricultural Chemists was held at the Shoreham Hotel, Washington, D. C., October 11, 12, and 13, 1948.

The meeting was called to order by the President, G. H. Marsh, on the morning of October 11, at 10:00 o'clock.

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LACTOSE IN MIXED FEEDS:
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FAT IN FISH MEAL:
Maurice E. Stansby, Fish and Wildlife Service, Seattle 2, Wash.

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CRUDE FAT OR ETHER EXTRACT:
H. H. Hoffman, Department of Agriculture, Tallahassee, Fla.

MICROSCOPIC EXAMINATION:
J. A. Schrader, Agricultural Experiment Sta., Lexington 29, Ky.

FLUORINE:
D. M. Doty, Purdue University, Lafayette, Ind.

MINERAL CONSTITUENTS OF MIXED FEEDS:
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CRUDE FIBER:
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HYDROCYANIC ACID GLUCOSIDES:
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SAMPLING AND ANALYSIS OF CONDENSED BUTTERMILK:
R. E. Bergman, State Department of Agriculture, St. Paul, Minn.

TANKAGE (HIDE, HOOF, HORN, AND HAIR CONTENT):
A. T. Perkins

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† Referees appointed during the year for unassigned subjects will be announced in the *Journal*.

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OIL EMULSIONS:
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SAMPLING:

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SUGAR:

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ZINC:

E. J. Benne, Agricultural Experiment Station, East Lansing, Mich.

COPPER AND COBALT:

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CAROTENE:

E. J. Benne

SODIUM:

Ray L. Shirley, Agricultural Experiment Sta., East Lansing, Mich.

CELLULOSE AND LIGNIN

Gordon H. Ellis, U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, N. Y.

PECTIN:

C. O. Willits, Eastern Regional Research Laboratory, Philadelphia, Pa.

STARCH:

Carroll L. Hoffpauir, Southern Regional Research Laboratory, New Orleans, La.

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COFFEE AND TEA
Referee:

COLORING MATTERS IN FOODS
Referee: C. F. Jablonski, Food and Drug Administration, New York 14, N. Y.

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ACIDITY OF MILK:
Guy G. Frary
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H. Van Dame, Food and Drug Administration, Cincinnati 2, Ohio

FAT:
L. C. Mitchell, Food and Drug Administration, Minneapolis, Minn.
AMMONIA NITROGEN:
E. B. Boyce, State Dept. Public Health, Boston 33, Mass.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS:
Referee: K. L. Harris, Food and Drug Administration, Washington 25, D. C.
DRUGS, SPICES, AND MISCELLANEOUS MATERIALS:
W. V. Eisenberg, Food and Drug Administration, Washington 25, D. C.
DAIRY AND EGG PRODUCTS:
K. L. Harris
NUT PRODUCTS:
Maryvee G. Yakowitz, Food and Drug Administration, Washington 25, D. C.
BAKED PRODUCTS, CEREALS, AND CONFECTIONERY:
J. F. Nicholson, Food and Drug Administration, Washington 25, D. C.
BEVERAGE MATERIALS:
F. A. Hodges, Food and Drug Administration, Washington 25, D. C.
FRUIT PRODUCTS:
W. G. Helsel, Food and Drug Administration, Washington 25, D. C.
VEGETABLE PRODUCTS:
F. R. Smith, Food and Drug Administration, Washington 25, D. C.
SEDIMENT TESTS (MILK AND CREAM):
C. R. Joiner, Food and Drug Administration, St. Louis, Mo.

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL INDICES):
Referee: W. I. Patterson, Food and Drug Administration, Washington 25, D. C.
FISH PRODUCTS:
Fred Hillig, Food and Drug Administration, Washington 25, D. C.
DAIRY PRODUCTS:
Fred Hillig
SHELLFISH:
R. E. Duggan, Food and Drug Administration, New Orleans 16, La.
APPLE PRODUCTS:

EGG PRODUCTS:
Fred Hillig
BLACKHEART IN PINEAPPLE:
W. O. Winkler, Food and Drug Administration, Washington 25, D. C.

GELATINE, DESSERT PREPARATIONS, AND MIXES:
Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.
JELLY STRENGTH:
Paul A. Kind, Kind-Knox Gelatine Co., Camden, N. J.
GELATINE AND GELATINE DESSERTS (COMPOSITION):
Joseph H. Cohen, General Foods Corporation, Woburn, Mass.

FISH AND OTHER MARINE PRODUCTS:
Referee: A. M. Allison, Food and Drug Administration, Boston 10, Mass.
TOTAL SOLIDS AND ETHER EXTRACT:
Menno D. Voth, Food and Drug Administration, Boston 10, Mass.

GUMS IN FOODS:
Referee: F. Leslie Hart, Food and Drug Administration, Los Angeles 15, Calif.
CHEESE:
M. J. Gnagy, Food and Drug Administration, Los Angeles, Calif.
FROZEN DESSERTS:
F. Leslie Hart
CACAO PRODUCTS:
Flora G. Mendelsohn, Food and Drug Administration, Los Angeles 15, Calif.
CATSUP AND RELATED TOMATO PRODUCTS:
E. W. Coulter, Food and Drug Administration, Chicago 7, Ill.

MEAT AND MEAT PRODUCTS:
Referee: Roger M. Mehurin, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.
SOYBEAN FLOUR IN MEAT PRODUCTS:
O. L. Bennett, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.
DEFATTED MILK SOLIDS IN MEAT PRODUCTS:

CREATIN IN MEAT PRODUCTS:
J. M. McCoy, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.
HORSEMEAT IN GROUND MEAT:
C. E. Hynds, State Food Laboratory, Albany, N. Y.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS:
Referee: H. J. Wichmann, Food and Drug Administration, Washington 25, D. C..
CADMIUM:
A. K. Klein, Food and Drug Administration, Washington 25, D. C.
COPPER:
W. C. Stammer, Continental Can Company, Inc., Chicago 39, Ill.
ZINC:
O. R. Alexander, American Can Company, Maywood, Ill.
MERCURY:
A. K. Klein
DDT AS SPRAY RESIDUE ON FOODS:
R. H. Carter, Bureau of Entomology and Plant Quarantine, Beltsville, Md.
INSECTICIDES IN CANNED FOODS:
W. A. Britton, Beechnut Packing Co., Canajoharie, N. Y.
PARATHION:
P. A. Clifford, Food and Drug Administration, Washington 25, D. C.

MICROBIOLOGICAL METHODS:
Referee: G. G. Slocum, Food and Drug Administration, Washington 25, D. C.
CANNED MEATS:
M. L. Laing, Armour & Company, Chicago 9, Ill.
CANNED ACID FOODS:
A. P. Dunningan, Food and Drug Administration, Washington 25, D. C.
CANNED VEGETABLES:
C. W. Bohrer, Natl. Canners Assn., Washington, D. C.
EGGS AND EGG PRODUCTS:
M. T. Bartram, Food and Drug Administration, Washington 25, D. C.

NUTS AND NUT PRODUCTS:
William R. North, Food and Drug Administration, Washington 25, D. C.
FROZEN FRUITS AND VEGETABLES:
H. E. Goresline, Production and Marketing Administration, Poultry Division, Washington 25, D. C.
SUGAR:
E. J. Cameron, Natl. Canners Assn., Washington, D. C.
CANNED FISHERY PRODUCTS:
L. R. Shelton, Jr., Food and Drug Administration, Washington 25, D. C.

MICROCHEMICAL METHODS:
Referee: C. O. Willits, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa.
ELEMENTAL ANALYSIS:
C. L. Ogg, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa.

NUTS AND NUT PRODUCTS:
Referee: A. M. Henry, Food and Drug Administration, Atlanta 3, Ga.

OILS, FATS, AND WAXES:
Referee: J. Fitelson, Food and Drug Administration, New York 14, N. Y.
UNSAPONIFIABLE MATTER:
Gardner Kirsten, Food and Drug Administration, New York 14, N. Y.
PEANUT OIL:
Gardner Kirsten
ANTIOXIDANTS:
S. Kahan, Food and Drug Administration, New York 14, N. Y.

PRESERVATIVES AND ARTIFICIAL SWEETENERS:
Referee: Margarethe Oakley, State Department of Health, Baltimore 18, Md.
QUARTERNARY AMMONIUM COMPOUNDS:
John B. Wilson, Food and Drug Administration, Washington 25, D. C.
MONOCHLORACETIC ACID:
JOHN B. WILSON
FORMALDEHYDE:
Howard Bennett, Food and Drug Administration, New Orleans, La.
MOLD-INHIBITORS, PROPIONATES:
L. H. McRoberts, Food and Drug Administration, San Francisco 2, Calif.
THIOUREA:
W. O. Winkler, Food and Drug Administration, Washington 25, D. C.
ARTIFICIAL SWEETENERS:
William S. Cox, Food and Drug Administration, Atlanta, Ga.

SPICES AND OTHER CONDIMENTS:
Referee: S. Alfend, Food and Drug Administration, St. Louis 1, Mo.
VINEGAR:
J. H. C. Loughrey, Food and Drug Administration, Boston, Mass.
VOLATILE OIL IN SPICES:
N. A. Carson, Food and Drug Administration, St. Louis, Mo.
SUGAR, ASH, AND PUNGENT PRINCIPLES IN MUSTARDS:
F. M. Garfield, Food and Drug Administration, St. Louis 1, Mo.
PREPARATION OF SAMPLE, AND FAT IN MAYONNAISE AND SALAD DRESSING:
Juanita E. Breit, Food and Drug Administration, Cincinnati 2, Ohio

SUBCOMMITTEE D: KENNETH L. MILSTEAD (1950) (Food and Drug Administration, Cincinnati, Ohio), *Chairman;* J. Walter Sale (1952); and C. S. Ferguson (1954)

ALCOHOLIC BEVERAGES
Referee: J. Walter Sale, Food and Drug Administration, Washington 25, D. C.
YEAST (TOTAL AND YEAST SOLIDS):
Robert I. Tenney, Wahl-Henius Inst., 64 E. Lake St., Chicago, Ill.
HOPS:
D. E. Bullis, Oregon State College, Corvallis, Oreg.
INORGANIC ELEMENTS IN BEER:
W. C. Stammer, Continental Can Company, Inc., Chicago, Ill.
COLOR AND TURBIDITY IN BEER:
B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Mo.
DISTILLED SPIRITS (OBSCURATION METHOD FOR PROOF AND TEST FOR AGING):
G. F. Beyer, Bureau of Internal Revenue, Washington 25, D. C.
CHROMATOGRAPHIC ABSORPTION OF WINES:
Peter Valaer, Bur. Internal Revenue, Washington 25, D. C.
CARAMEL IN ALCOHOLIC BEVERAGES:
Peter Valaer
CORDIALS AND LIQUEURS:
John B. Wilson, Food and Drug Administration, Washington 25, D. C.
FUSEL OIL IN DISTILLED SPIRITS (OFFICIAL METHOD):
G. F. Beyer
CARBON DIOXIDE IN BEER:
Irwin Stone, Wallerstein Laboratories, New York 16, N. Y.
METHANOL:
J. F. Guymon, Agr. Expt. Station, College of Agriculture, Davis, Calif.

CACAO PRODUCTS:
Referee: W. O. Winkler, Food and Drug Administration, Washington 25, D. C.
LECITHIN:
J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.
MALT SOLIDS:
E. W. Meyers, Hershey Chocolate Company, Hershey, Pa.
PECTIC ACID:
W. O. Winkler
CACAO INGREDIENTS:
W. O. Winkler
LACTOSE:
Donald G. Mitchell, Walter Baker Co., Dorchester 24, Mass.
FAT:
Carl Stone, Food and Drug Administration, Cincinnati, Ohio

CEREAL FOODS:
Referee: V. E. Munsey, Food and Drug Administration, Washington 25, D. C.
STARCH IN RAW AND COOKED CEREALS:

FAT ACIDITY IN GRAIN, FLOUR, CORN MEAL, AND WHOLE WHEAT FLOUR
Lawrence Zeleny, Agricultural Research Center, Beltsville, Md.
BENZOIC ACID IN FLOUR:
V. E. Munsey
MILK SOLIDS AND BUTTERFAT IN BREAD:
V. E. Munsey

PROTEOLYTIC ACTIVITY OF FLOUR:
Byron S. Miller, Federal Hard Wheat Quality Laboratory, Manhattan, Kans.
SOYBEAN FLOUR:
W. L. Taylor, General Mills, Inc., Minneapolis, Minn.
PHOSPHATED FLOUR:
Frank H. Collins, Food and Drug Administration, Cincinnati 2, Ohio
BAKED PRODUCTS (MOISTURE, ASH, PROTEIN, FAT, AND CRUDE FIBER):
R. P. Smith, National Biscuit Co., 449 W. 14th St., New York, N. Y.
MOISTURE IN SELF-RISING FLOUR AND IN PANCAKE, WAFFLE, AND DOUGHNUT MIXES:
S. Williams, Food and Drug Administration, Cincinnati, Ohio
BROMATES IN FLOUR:
W. L. Rainey, Commander-Larabee Milling Co., Minneapolis, Minn.
UNSAPONIFIABLE MATTER AND STEROLS IN NOODLES AND BAKERY PRODUCTS:
V. E. Munsey
ALBUMEN IN NOODLES AND MACARONI PRODUCTS:
V. E. Munsey

BAKING POWDERS AND BAKING CHEMICALS:
Referee: V. E. Munsey, Food and Drug Administration, Washington 25, D. C.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
Referee: John B. Wilson, Food and Drug Administration, Washington 25, D. C.
BETA-IONONE:
John B. Wilson
LEMON OILS AND EXTRACTS:
John B. Wilson
ORGANIC SOLVENTS IN FLAVORS:
R. D. Stanley, Food and Drug Administration, Chicago 7, Ill.
EMULSION FLAVORS:
John B. Wilson
MAPLE FLAVOR CONCENTRATES AND IMITATIONS:
J. H. Bornmann, Food and Drug Administration, Chicago 7, Ill.
DIACETYL:
John B. Wilson
VANILLA EXTRACTS AND IMITATIONS:

FRUITS AND FRUIT PRODUCTS:
Referee: R. A. Osborn, Food and Drug Administration, Washington 25, D. C.
TITRATION OF ACIDS:
H. M. Bollinger, Food and Drug Administration, Los Angeles 15, Calif.
FRUIT ACIDS:
L. W. Ferris, Food and Drug Administration, Buffalo, N. Y.
FRUIT AND SUGAR IN FROZEN FRUIT:
C. G. Hatmaker, Food and Drug Administration, Washington 25, D. C.
WATER-INSOLUBLE SOLIDS:
R. A. Osborn

SUGARS AND SUGAR PRODUCTS:
Referee: C. F. Snyder, National Bureau of Standards, Washington 25, D. C.

DRYING METHODS:
Lester D. Hammond, National Bureau of Standards, Washington 25, D. C.
DENSIMETRIC AND REFRACTOMETRIC METHODS:
C. F. Snyder
HONEY (FREE ACID AND COMMERCIAL SYRUP ADULTERANTS):
George P. Walton, Eastern Regional Research Laboratory, U. S. Dept. Agriculture, Philadelphia, Pa.
CONFECTIONERY:
C. A. Wood, Food and Drug Administration, New York 14, N. Y.
REDUCING SUGARS:
Emma J. McDonald, National Bureau of Standards, Washington 25, D. C.
CORN SIRUP AND CORN SUGAR:
G. T. Peckham, Jr., Clinton Company, Clinton, Iowa
COLOR AND TURBIDITY IN SUGAR PRODUCTS:
J. F. Brewster, National Bureau of Standards, Washington 25, D. C.
MICRO-SUGAR METHODS:
Betty L. Kostakos, National Bureau of Standards, Washington 25, D. C.
STARCH CONVERSION PRODUCTS:

WATERS, BRINE, AND SALT:
Referee:
BORON IN WATER:

FLUORINE IN SALT:

MEMBERS AND VISITORS PRESENT, 1948 MEETING

Adams, J. Richard, Spencer Chemical Co., Baltimore St., Kansas City 2, Mo.
Alexander, Lyle T., Bur. Plant Industry, Soils, & Agr. Engineering, Beltsville, Md.
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Allen, Raymond N., Dir. Biological Dept., Gorton Pew Fisheries Co., Ltd., Gloucester, Mass.
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Amick, C. Harold, Dir. Food & Drug Div., State Dept. of Agriculture, Charleston, W. Va.
Anderson, Myron S., Bur. Plant Industry, Soils, & Agr. Engineering, Beltsville, Md.
Anwar, M. H., Research Dir., Coca-Cola Expt. Corp., 515 Madison Ave., New York, N. Y.
Austin, W. R., Armour Test Works, Nashville 2, Tenn.
Axley, John, University of Maryland, College Park, Md.

Bacher, A. A., 735 N. Water St., Milwaukee, Wis.
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Banes, Daniel, Food & Drug Administration, Washington 25, D. C.
Barnhart, G. M., Chief Chemist, Mo. Dept. of Agriculture, Jefferson City, Mo.
Bartlett, L. E., Park & Pollard Co., 356 Hertel Ave., Buffalo 7, N. Y.
Bates, D. B., Smith-Douglas Co., Board of Trade Building, Norfolk 1, Va.
Batton, H. C., Swift & Co., Plant Food Div., Norfolk, Va.
Beacham, C. M., Food & Drug Administration, Washington 25, D. C.
Benne, Erwin J., Agricultural Expt. Station, State College, East Lansing, Mich.
Berry, Rodney C., State Chemist, Va. Dept. of Agr., State Office Bldg., Richmond 19, Va.
Beyer, G. F., Bureau of Internal Revenue, Washington 1, D. C.
Bidez, P. R., State Chemical Laboratory, 325 Magnolia St., Auburn, Ala.
Black, A., E. R. Squibb & Sons, New Brunswick, N. J.
Blackwell, A. T., Davison Chemical Corp., Baltimore, Md.
Blanchard, J. F., Supt. Lab. Food & Drugs, National Health & Welfare, Winnepeg, Canada
Bogge, Hugh M., Food & Drug Administration, Philadelphia 6, Pa.
Bogusky, John, Production & Marketing Adm., Insecticide Div., Beltsville, Md.
Bonney, V. B., Food & Drug Administration, Washington 25, D. C.
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Bowen, C. Verne, Bur. Entomology & Plant Quarantine, Beltsville, Md.
Bowling, J. D., Bur. Plant Industry, Soils, & Agr. Engineering, Beltsville, Md.
Bradford, Z. B., N. C. Dept. of Agriculture, Raleigh, N. C.
Bradley, Frank D., Chemicals Div., O.D.C., Dept. of Commerce, Washington, D. C.
Brock, F. D., Feed Control Service, Tex. Agr. Expt. Station, College Station, Tex.
Brooke, Richard O., Wirthmore Laboratory, Malden, Mass.
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Byers, C. R., Armour Fertilizer Works, Carteret, N. J.

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Chaney, Howard E., Branch Lab., State Dept. of Health, Cambridge, Md.
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Chapman, R. A., Natl. Health & Welfare, Foods & Drugs Div., Ottawa, Can.
Chapman, William, Consolidated Rendering Co., 178 Atlantic Ave., Boston 10, Mass.
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Gaddy, V. Lessesne, Bureau of Plant Industry, Beltsville, Md.
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Goulden, H. D., Dir. Research, Toilet Goods Assn., 9 Rockefeller Plaza, New York, N. Y.
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Green, Edmund L., Insecticide Div., Livestock Branch, Washington 25, D. C.
Grewe, Emily, 1621 T Street, Washington, D. C.
Gribbins, M. F., DuPont Co., Wilmington, Del.
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Grieve, A. D., Dir. Control Lab., Ayerst, McKenna, & Harrison, Ltd., Montreal, Canada
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Gutterman, Benjamin M., Food & Drug Administration, Washington 25, D. C.

Haenni, E. O., Food & Drug Administration, Washington 25, D. C.
Hagerty, James Francis, Food & Drug Administration, Washington 25, D. C.
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Halvorson, H. A., Feed & Fertilizer Control, Minn. Dept. of Agriculture, St. Paul, Minn.
Hammond, L. D., Bureau of Standards, Washington 25, D. C.
Hanson, H. H., 340 N. Bradford St., Dover, Del.
Hardesty, John O., Bur. Plant Industry, Beltsville, Md.
Hardin, L. J., Univ. of Tenn. Agricultural Expt. Station, Knoxville, Tenn.
Hardy, Paul W., American Can Co., 11th Ave., & St. Charles Road, Maywood, Ill.
Harris, Thomas H., Production & Marketing Adm., Insecticide Div., Beltsville, Md.
Harris, Kenton L., Food & Drug Administration, Washington 25, D. C.
Harrow, Lee S., Food & Drug Administration, Washington 25, D. C.
Hart, Gordon, Florida Dept. of Agriculture, Box 408, Tallahassee, Fla.
Hart, F. Leslie, Food & Drug Administration, Louisiana Station, La.
Hayward, J. W., Dir. Nutrition Research, Archer, Daniels, Midland Co., Minneapolis, Minn.
Heagy, A. B., Inspection Service, University of Maryland, College Park, Md.
Hege, Roy C., N. C. Board of Alcoholic Control, Raleigh, N. C.
Hein, Lloyd, Wisconsin Alumni Research Foundation, 506 N. Walnut St., Madison 5, Wis.
Helsel, W. E., Food & Drug Administration, Washington 25, D. C.
Henry, Arthur M., Food & Drug Administration, 416 Federal Annex, Atlanta, Ga.
Hess, Sidney M., Food & Drug Administration, Washington 25, D. C.
Hewett, B. H., Pres. Kans. State Board of Agriculture, Coldwater, Kans.
Hewston, Elizabeth M., Agricultural Research Center, Beltsville, Md.
Hicks, Rosalind K., Assoc. Official Agricultural Chemists, Box 540, Benjamin Franklin Station, Washington 4, D. C.
Hillig, Fred, Food & Drug Administration, Washington 25, D. C.
Hobbs, Norman L., Gelatin Products Div., R. P. Scherer Corp., Detroit 13, Mich.

Hodges, F. A., Food & Drug Administration, Washington 25, D. C.
Hoffman, Harold H., Florida Dept. of Agriculture, Tallahassee, Fla.
Hoffman, Henry J., Minn. Dept. of Agri., Dairy & Food, St. Paul, Minn.
Hoffpauir, Carroll L., Southern Reg. Research Lab., New Orleans 19, La.
Holtzman, H., Anspiecher-Siegle Corp., Rosebank 5, Staten Island, N. Y.
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PRESIDENT'S ADDRESS*

COMPOSITION FOUND BY ANALYSIS WITH THERAPEUTICAL CLAIMS FOR SOME PROPRIETARY MEDICINES

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The compounding of medicine is an old art. The people of ancient times had a meager knowledge of the medicinal properties of herbs and roots and their therapeutical action upon the human body. They gathered certain herbs and roots and made decoctions and infusions from some of them, chewed some, smoked some, and took some of them in powdered form. At the same time there existed somewhat of a halo of mystery about the action of medicine on the human body. That same idea which originated with the art of compounding of medicine still prevails in the minds of many people. They believe that there is some mysterious something in certain medicines. They cannot explain it to you, but still it exists in their minds.

This halo of mystery, or conception of medicinal values, has been a retarding factor through the years in getting proper medicinal aid to many people and it continues to be a hindrance even today. It is the controlling reason why many people do not heed the advice for correct and proper medication, but rather follow the mystic view they have of medicines and self medication, to the detriment of their health and even sometimes to the loss of life itself. This idea of the mystery of medicines and their healing power on the human body makes it profitable for those who are inclined to make use of the traffic in medicines for profit and, unfortunately, many are willing to take advantage of their fellow man's weakness: all for the sake of personal material gain. This is so well illustrated by the type of advertising, and the extensive therapeutical claims made so often.

The analysis for composition of many of the medicines which have been found on sale is a very broad and difficult field, of course, for the Drug Chemist in control work whose task it is to ascertain the composition of many of them. This is especially true in the case of medicines made up by the itinerant drug or medicine man, as they are called. It is likewise true in the case of the medicines put up in many cases by the small manufacturer, who puts up a medicine and jobs it out through small grocery stores, etc.

The seriousness of the situation is that in so many of the cases the party making the medicine is one who knows nothing about drugs, chemistry, physiology, or therapeutics. Even if he is honest in purpose, still he

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knows nothing about what value the medicine may or may not have. The person's mind purchasing the medicine is often filled with the idea of mystery, and he believes in some way it will alleviate his pain and suffering and per chance relieve him of his ailment. It is surely a case of the blind leading the blind as they grope around in darkness.

It is no easy task to cope with this situation in drug control, which we have briefly described. If there was the requirement that certain information be filed with the Drug Control Official, such as the composition of the medicine, copy of the label, and a copy of the proposed advertising matter, *before* the medicine is offered for sale, it would be very valuable in the control in the traffic of these medicines and in rendering of far greater protection of the health of the public. There are many of such medicines being sold. The business of concocting of them seems to be a never ending business; for as soon as one has been apprehended and put out of business, others seem to spring up in his place. It seems that those who know the least about medicine, pharmacy, chemistry, physiology, or therapeutics are the ones who manufacture and sell such medicines.

I shall refer to some specific cases where claims were made or implied as to the value of a remedy for certain diseases, and then give the composition of the medicine as was found by analysis in the laboratory.

(1) "TU-BU-KU"

This medicine was a colorless liquid sold in 6 oz. bottles, bearing directions on the label to take as a relief for all coughs. The name might imply that the product is to be used as a remedy for tuberculosis, since in most lung or throat infections of this disease a cough usually accompanies the disease. A sample consisting of two 6 oz. bottles was collected and analyzed in the laboratory. The Drug Chemist had no knowledge of the composition of the medicine since no ingredients were listed on the label, and further he had no knowledge of a medicine or any medicinal ingredient that was a remedy for tuberculosis. He began his search for the composition of the medicine, and his first clue was the odor of peppermint. A test showed that the medicine contained menthol in a very small amount. In carrying out the test for menthol, an indication was noticed of the presence of alcohol in the medicine. Tests were made and the presence of alcohol confirmed. A quantitative determination was made which showed that the medicine contained 10 per cent by volume of alcohol. In the test for alcohol an indication was noticed that the medicine contained a trihydroxy alcohol, and confirmatory tests showed this alcohol to be glycerine. A quantitative determination proved the presence of 3 per cent by volume of glycerine. The only other ingredient found present was water: 87 per cent by volume. The medicine actually consisted of an aqueous solution containing 10 per cent by volume of ethyl alcohol and 3 per cent by volume of glycerine, flavored with menthol. Fortunately, soon after

the analysis was completed and reported, the product was withdrawn from the market and so far as I know never came back on the market in our section. It was not possible to correlate any relation between the therapeutical implication in the name of the product on the label, and the composition found in the laboratory analysis of the product.

(2) "PELLAGRA REMEDY"

A sample of 4 oz. of this medicine was collected. It was a grayish powder intermingled throughout with a coarser black powder. The Drug Chemist, not having a knowledge of any medicine that was a specific remedy for pellegra, it was difficult for him to decide just what to test for in the medicine. It was decided to test first to ascertain what the coarser black powder scattered throughout the samples was. A portion was taken and sieved, and it was found not difficult to separate a portion of the black particles out from the grayish powder. Some of the separated coarser black particles were placed in a crucible, and heat applied. These particles proved to be charcoal not very finely pulverized. A qualitative test was carried out on a portion of the sample for heavy metals, and it was soon found that the medicine contained iron. A quantitative test was run to find the total iron content. It was then decided that, since the powder was water soluble, it was a salt; so tests were made to ascertain what salt or salts were present. It was found that it was a sulphate, and that the iron was in a ferrous form with only a trace of ferric iron present. No other salt was found to be present in the medicine. The composition of the product was calculated from the percentage of ferrous iron and the sulphate radical, and the product was found to be practically pure exsiccated ferrous sulphate, containing less than $\frac{1}{2}$ of 1 per cent of charcoal in the original medicine. It was concluded that the exsiccated ferrous sulphate was mixed with the small amount of charcoal to mask the appearance of the pure salt and carry that halo of mystery, in order to sell the medicine for extensive profits. The 4 oz. package sold for $1.00 a box. Pellagra was a rather common disease at the time among the people where this product was being sold. It was natural that the people were grabbing at something they hoped would be a relief for their ailment. There was little knowledge at the time of a scientific remedy for the treatment of the disease, pellagra, since it had not yet been found that it was a deficiency disease. The remedy was sold by an itinerant medicine man who was doing quite a profitable business until his product was seized; and so far as I know he discontinued the medicine business.

(3) "CANCER REMEDY"

The medicine was labeled "Rock of All Ages." A sample was collected consisting of two 4 oz. boxes. The article was found to be a dark granular powder, almost black. The label did not show the composition, as is so

generally the case with most of such medicines. It was extensively labeled as to what the medicine would do. It also bore labeling as to how to prepare the medicine for use. The powder itself was not to be taken, but the contents of the 4 oz. box were to be macerated in a quart of water for several hours, or overnight, and then the liquid poured off into another quart bottle, stoppered, and kept for administering as a remedy for cancer and almost any other disease one might become afflicted with. The directions for dosage, but no time limit as to when to stop taking the medicine when once begun, were mentioned on the label. An analysis of the solution, obtained according to directions of preparation, showed but one ingredient in any appreciable quantity, and that was ferric sulphate. There was a trace of another substance, namely arsenic, but not in sufficient quantities to be of material consequence. An analysis of the original solid substance, or so-called "Mineral Rock of All Ages," showed that it contained sand and clay and a small amount of ferrous sulphate and ferric sulphate. The seller of this product was a local itinerant medicine man; however, he jobbed some of this medicine to grocery stores and occasionally to a country drug store. He was convicted three different times for selling the medicine. He was put in jail overnight, and fined $50.00 and court costs, the first time. For the second offence he was convicted and fined $100.00 and court costs, and given a 6-month's prison sentence, which was suspended. The third time he was convicted and fined $100.00 and court costs, and given a 6-month's prison sentence. The case was appealed to the Supreme Court, where the lower court was upheld. The medicine man died about the time the Supreme Court's decision was handed down. Each trial was in a separate county.

(4) "LINIMENT ANTISEPTIC"

This medicine was put up in 3 oz. bottles and bore the following statement on its label: "Liniment Antiseptic will relieve rheumatic pains, blood poison, toothache, does relieve pyorrhea, good for tonsillitis, asthma, relieves backache, sprains, soreness of body, hard of hearing, boils, bunions, corns, sore feet, good for colitis, fine for weak eyes, for colds, catarrh of the head, headache and flu. It kills all germs, etc." Two bottles were collected for a sample. The Drug Chemist, having no knowledge of a specific remedy for relief of all rheumatic pains, pyorrhea, flu, asthma, colitic, or all the other diseases enumerated on the label of this medicine, found it all the more difficult to know where to begin analysis or what to look for in the medicine. The first indication that was observed was the odor of camphor contained in the product: the article being a solution, also indicated that it contained alcohol. Tests were made which proved the presence of both camphor and alcohol in the medicine. The color of the liquid was amber, so that indicated the presence of still other substances. A quantitative test was made for the percentage of alcohol,

and also for the percentage of camphor; and qualitative tests were made for iodine which proved the presence of iodine. There was about 84 per cent by volume of alcohol present in the article and, from the amount of iodine present, the indication was that about 3 per cent of tincture of iodine was added to spirits of camphor to make up the remedy. It was not possible to correlate the therapeutical claims of the label with the composition found by analysis of the medicine. The label of this medicine has been corrected to such an extent that one would hardly recognize the product except by its color and the name of the manufacturer.

(5) "HIGH POWER MAJIC INHALER"

This was a medicine put up in $\frac{1}{2}$ oz. bottles. Two bottles were collected for samples. The following statement appeared on the label: "High Power Majic Inhaler. Try Majic for headaches, head colds, sinus troubles, hay fever, asthma. Directions—Just inhale the fumes from the cork, for headaches rub the cork over the forehead. Keep corked when not in use." The analysis of the contents of this medicine showed it to consist of a mixture of powdered leaves, cinnamon bark, calamus root, hulls of anise, caraway, corriander seed and bay leaves, saturated with volatile oil of mustard. It was a powerful remedy. It required only a small portion to be very irritating. It was a dangerous medicine being sold by an itinerant medicine man. He was apprehended but received only a moderate fine; however he discontinued business in the section where the sample was collected.

(6) "LONESOME PINE HEALING OIL"

The following statement appeared on the label: "In case you have kidney trouble or brights disease write in for treatment with Lonesome Pine Healing Oil." Another statement on the label was as follows: "For cuts, colds, ulcers, burns, bruises, toothaches will relieve pyorrhea, also for flu, asthma, hay fever, poison oak, ring worms, athletes foot, etc." Three 2 oz. bottles were collected as a sample. Examination of these bottles showed them to contain a straw-colored oil. Further analysis showed it to be an impure rosin oil, or a partially resinified pine oil. No other substances were found to be present in the product. It was not possible to correlate the claims on the label for the therapeutical value of this product, with the composition found by analysis in the laboratory. The analysis of this product was not as difficult as many others, since it was indicated in the name of the product that it contained some form of pine oil, and that proved to be true.

(7) "A MALARIA REMEDY"

This was a liquid medicine, colored. Claims were made that the article contained quinine sulphate as active ingredient, with other ingredients

having tonic properties. The claim on the label was that the dosage recommended, which was a tablespoonful, contained 5 grains of quinine sulphate. The article was sampled and examined in the laboratory. Quinine sulphate was found to be present. No other active ingredient, except a small amount of gentian, was found in the medicine. A quantitative determination revealed the presence of only 2.50 grains of quinine sulphate per tablespoonful of the medicine, instead of the 5 grains claimed to be present. It is rather generally accepted that quinine sulphate is a specific for Malaria Plasmosis if given in certain recognized dosage. But a person who knew that fact and knew the correct dosage, and who depended upon expected results after taking this remedy, would have been sadly disappointed. The article contained the declared active ingredient, but not in the amount claimed. A case of misbranding, also a subtle type of declaration of the dosage, made the article deceptive, since 5 grains is the common accepted amount given each time to adults until an ample amount has been administered to give the desired effect in allaying malaria.

(8) "U. S. TONIC"

This was a medicine put up in 12-ounce bottles, labeled as "U. S. Tonic" and bearing a small flag on the bottle. The flag was a paper design of the stars and stripes. The name "Washington D. C." was also on the label. The dosage to be given was about the only other information on the label. It was not possible to collect an unopened bottle of this medicine, but two bottles which had been opened were collected from two different persons who had purchased a bottle at the price of $5.00. The examination revealed that the article was principally water with a little gentian and fluid extract of cascara sagrada. The reason, probably, for leaving the claims for use of the medicine off of the label was because that information was given orally, according to the purchasers' statements, by the itinerant medicine man. He traveled in a rather nice Buick Coupe Automobile, dressed quite nicely and wore a white coat similar to that worn by doctors in their offices. He also carried a hand bag very similar to those commonly used by doctors for carrying certain equipment and supplies in their professional calls. He carried a stethoscope in this bag; also a few bottles of the U. S. Tonic when he approached the patient in his home. His first approach was that he was a Government agent sent out to make check-ups on the people as to their state of health. He quickly told the prospective patient that there was no charge for the examination. With this approach, it was not difficult to obtain permission to proceed with the check-up on the patient. The agent opened up the hand bag, took out the stethoscope and began the examination of the patient as to state of health and general condition. When the check-up was completed he informed the patient what he had found wrong. He then reiterated his statement that there was no charge for the examination, but that the patient should have some

medicine. He then took out a bottle of the U. S. Tonic with the U. S. Flag on the bottle and announced that was the remedy, or medicine, needed by the patient in order to correct his or her trouble. The patient was informed that the price was $5.00 per bottle; also that there was only a limited amount of it available for sale. The examination in the laboratory by the Drug Chemist showed the article to contain a very small amount of active ingredients. The trouble was, the procedure used made it difficult for the type of people who were approached to refuse to purchase the medicine. This agent traveled rapidly, and we found only two places in the state where he operated. His visits at these two sections were in rural communities during harvest time. It was also during the last war and we have not been able to find where he has been in the state since.

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will follow in their usual order.

Third Day

WEDNESDAY—AFTERNOON SESSION

REPORT OF COMMITTEE ON CLASSIFICATION OF METHODS

Your committee has received much helpful comment on the feasibility of the six recommendations made at the 1947 meeting.* It appears that many tentative methods are well on their way toward either adoption as official or deletion, depending on their worth. Accordingly, one proposed amendment to the by-laws abolishes the "tentative" classification. This covers recommendations (1) and (2).

Recommendation (3) provided for a "pending official" classification. This, in the light of the past year's experience, seems unnecessary, and indeed undesirable. It is convenient to discuss (3) in connection with (4), which dealt with methods that have long been in the "official, first action" classification. An amendment to the by-laws is proposed providing adoption of methods as "first action" and "official." Those tentative methods not deleted or not adopted as official at the 1949 meeting could be adopted as "first action" at that time on recommendation of the appropriate referee, provided they had received collaborative study. All "official, first action" methods remaining in that status at the close of the 1949 meeting would automatically become "first action" methods.

Recommendation (5) dealt with methods of a screening or sorting nature, and (6) with suggestions as to other useful examinations, manipulations, tests, and the like. These two recommendations are covered by a proposed amendment providing for the adoption by the Association, on recommendation by the appropriate referee, of recommended procedures. This term is broad enough to cover all operations where there are impediments to rigid collaborative study, yet whose adequacy has been established by long experience. Such operations would include screening tests to determine whether a precise analysis needs to be made, for making a representative selection of unit packages from a lot, drawing a repre-

* *This Journal*, 31, 63 (1948).

sentative sample from bulk shipments or large units, handling and storing the sample, as well as others of any type designed to guide or facilitate analysis. Not a few of such procedures are of universal application regardless of circumstances or objectives, and we recommend that the 1950 edition of the Book of Methods carry a prefatory note touching on their purpose and proven usefulness, but also pointing out that there may be situations where modifications are called for which, in the judgement and experience of the operator, will better accomplish his purpose.

We recommend further that the title of the Book of Methods be changed to "Official Methods of Analysis of the Association of Official Agricultural Chemists."

PROPOSED AMENDMENTS TO BY-LAWS

(A) Delete paragraphs 5, 6, and 8 and add the following paragraphs:

"5. Methods shall be adopted as 'first action' and 'official.' No method shall be adopted by first action until it has received collaborative study and has been recommended by the appropriate referee at an annual meeting. No method shall be adopted as official except on recommendation of the appropriate referee at an annual meeting following the adoption of such method by first action. No change in an official method shall be adopted until after the changed method has received collaborative study and has been recommended by the appropriate referree at two annual meetings. No official method shall be repealed until such action has been recommended by the appropriate referee at two annual meetings. Such adoption, change, or repeal of methods shall become effective on the thirtieth day after publication of the record of such action in the *Journal* of the Association.

"6. Upon the recommendation by the appropriate referee, the Association may adopt well-established procedures for examination or treatment of a mechanical, microscopic, physical, chemical, or other nature."

W. B. WHITE, *Chairman*
W. F. REINDOLLAR
K. D. JACOBS

Approved.

AMENDMENTS TO CONSTITUTION AND BY-LAWS*

The following amendments to the constitution were approved by vote of the Association, having been presented and read on the first day, at the Monday-morning session.

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* The constitution and by-laws reprinted to include the revisions made at the 1948 meeting appear on pages 36–38.

Change Article III by deleting that portion beginning with the word "On," line 19 of the second paragraph, and substituting the following:

"The President and Secretary shall appoint referees, from the active members of the Association, on the general subjects designated by the Committee on Recommendations of Referees, and shall also appoint associate referees on subjects designated by that Committee as supplemental to the general subjects assigned to referees. It shall be the duty of referees and associate referees:

(1) To direct and conduct research on the methods and subjects assigned;

(2) To prepare and distribute samples, and direct and conduct collaborative studies of methods; and

(3) To present at the annual meeting the results of the work done and recommendations for methods to be based thereon."

The amendments to the by-laws proposed in the report of the committee on Classification of Methods were approved by vote of the Association. By-laws 11 and 12 were deleted by action of the Association in the approval of the report of the committee on Definitions of Terms and Interpretation of Results on Fertilizers and Liming Materials.

CONSTITUTION

The Constitution is reprinted to show the changes that were made in 1931, 1934, 1935, 1937, 1941, and 1948.

Article I

NAME AND OBJECT

This Association shall be known as the Association of Official Agricultural Chemists of North America,[1] Inc.

The objects of the Association shall be:

1. To secure, device, test, and adopt uniform and accurate methods for the analysis of fertilizers, soils, foods, feeding stuffs, dairy products, economic poisons, and other materials relating to agricultural pursuits; also medicinal products; cosmetics; and caustic poisons;

2. To secure uniformity in the statement of analytical results;

3. To conduct, promote, and encourage research in chemistry in its relation to agriculture;

4. To afford opportunity for the discussion of matters of interest to agricultural chemists.

Article II

MEMBERSHIP

Active Members

Chemists and other workers along analytical and research lines in the subjects mentioned in Article I, Section 1, connected with the following institutions of North America, shall alone be eligible *ex officio* to active membership:

1. The United States Department of Agriculture;

2. Any national, state, or provincial experiment station, college, or body engaged in research in agricultural chemistry; and

3. Any national, state, or provincial institution or body charged with official control of any of the materials named in Article I.

Associate Members

Chemists and other workers along analytical and research lines in the subjects mentioned in Article I, Section 1, connected with municipal laboratories in North America charged with control of any of the materials or subjects named in Article I are eligible *ex officio* to associate membership.

Chemists engaged in research in agricultural chemistry who are not eligible to active membership and active members of the Association who lose their right to such membership by retiring from the positions indicated above as requisite for eligibility to active membership may be elected to associate membership upon recommendation of the Executive Committee.

Honorary Members

Upon recommendation of the Executive Committee, persons may be elected to honorary membership by the two-thirds vote of those present at any regular meeting of the Association.

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[1] NOTE: The term North America is intended to include the United States and its colonial possessions, Canada, Cuba, the British West Indies, Haiti, Santo Domingo, Mexico, and the republics south of Mexico as far as the Panama Canal. This excludes the French West Indian Islands of Martinique and Guadeloupe.

Article III

OFFICERS AND COMMITTEES

The officers of the Association shall consist of a president, a vice-president, and a secretary who shall also act as treasurer.

These officers shall be elected annually from and by the active members and they shall perform the usual duties of their respective positions. These officers, the immediate past president, and three other active members to be elected by the Association shall constitute the Executive Committee. In case of the inability or disability of any one of the additional members of the Executive Committee, any past president in active membership may be designated to serve in his stead. The special duties of the officers of the Association shall be further defined, when necessary, by the Executive Committee. The elected officers of this Association shall constitute the Board of Directors of the Corporation, With the concurrence of the Executive Committee, the president shall appoint an Editorial Board, and the Executive Committee shall determine the membership and tenure of office of the members of this Board. The secretary-treasurer shall be *ex-officio* chairman and executive officer of the Editorial Board. With the concurrence of the Executive Committee, the president shall appoint a chairman and a committee of twelve other members, which shall be designated a Committee on Recommendations of Referees, one-third of the membership of which shall be appointed at intervals of two years to serve six years, the chairman to be appointed annually. The chairman shall assign the twelve members to subcommittees (A, B, C, and D) and shall assign to each subcommittee the reports and subjects which it shall consider. The President and Secretary shall appoint referees, from the active members of the Association, on the general subjects designated by the Committee on Recommendations of Referees, and shall also appoint associate referees on subjects designated by the Committee as supplemental to the general subjects assigned to referees. It shall be the duty of referees and associate referees:

1. To direct and conduct research on the methods and subjects assigned;
2. To prepare and distribute samples, and direct and conduct collaborative studies of methods; and
3. To present at the annual meeting the results of the work done and recommendations for methods to be based thereon.

Article IV

MEETINGS

The annual meeting of the Association shall be held at such place as shall be decided by the Association, and at such time as shall be decided by the Executive Committee. Announcement thereof shall be made, if possible, three months prior to the time of said meeting. Special meetings shall be called by the Executive Committee when in its judgment it may be necessary.

Article V

CHANGES IN CONSTITUTION

All proposed changes or amendments to this constitution shall be presented in writing and read in full to the Association not later than the second day of the regular annual meeting, shall be referred to the Executive Committee, and after a report from this Committee may be adopted as the first order of business on the third day by a vote of three-fourths of the active members present.

BY-LAWS

1. Any amendment to these by-laws or changes therein may be proposed in the same manner and adopted by the same procedure as amendments to the constitution, but only a two-thirds vote of the active members present shall be required for their adoption.

2. These by-laws or any portion of them may be suspended at any regular meeting of the Association without previous notice, by a vote of three-fourths of the active members present.

3. Only one qualified active member of a department, college, experiment station, board, or other institution shall be entitled to vote on general questions before the whole Association. At the discretion of the Chair, any institutional vote upon which there does not seem to be adequate representation may be conducted by letter ballot.

4. In voting upon questions involving methods of analysis, definitions, nomenclature, and laws or regulations relating to materials mentioned in Article I of the constitution, each of the said institutions shall be entitled to vote only upon questions relating to those materials over which said institution exercises official control.

5. Methods shall be adopted as "first action" and "official." No method shall be adopted by first action until it has received collaborative study and has been recommended by the appropriate referee at an annual meeting. No method shall be adopted as official except on recommendation of the appropriate referee at an annual meeting following the adoption of such method by first action. No change in an official method shall be adopted until after the changed method has received collaborative study and has been recommended by the appropriate referee at two annual meetings. No official method shall be repealed until such action has been recommended by the appropriate referee at two annual meetings. Such adoption, change, or repeal of methods shall become effective on the thirtieth day after publication of the record of such action in the *Journal* of the Association.

6. Upon the recommendation by the appropriate referee, the Association may adopt well established procedures for examination or treatment of a mechanical, microscopic, physical, chemical or other nature.

7. No changes shall be made in the methods of analysis used in official inspection until an opportunity shall have been given all active members having charge of the particular inspection affected to test the proposed changes.

8. When any officer ceases to be eligible for membership in the Association, his position shall be considered vacant, and a successor may be appointed by the Executive Committee to continue in office until the next regular meeting. Should any referee or associate referee resign or cease to be eligible for membership in the Association, his office shall be considered vacant and a successor shall be appointed as prescribed in Article III of the constitution. Should a vacancy occur in the Executive Committee, such vacancy may be filled by the action of the other members.

9. Chemists connected with commercial firms or institutions and others interested in the objectives of the Association who are not eligible to either active or associate membership may attend its meetings, take part in the discussions, and, if permission is obtained from the Executive Committee, may present papers.

REPORT OF THE EDITORIAL BOARD

By H. A. LEPPER, *Chairman*

Since the report at the last meeting, the first printing of 9,000 copies of *Methods of Analysis* has been sold. To meet the continuing demand, an issue of 1,000 copies of an electrolytic reproduction of the book was purchased from the College Offset Press of Philadelphia. The reproduction was bound in green cloth and lettered in gold identically as the original and it is difficult, even on close scrutiny, to detect any distinction between the two. A second reissue of 1,000 copies followed the sale of the first, and as of September 30 we had 450 copies on hand. It is hardly likely that this demand will be maintained with the approach of the publication of the seventh edition in 1950. During the coming year a new committee on revision of methods must be appointed to undertake the preparation of the seventh edition immediately after the 1949 meeting. This year will require more than the usual activity on the part of the referees to bring the respective chapters into conformity with the newly adopted classification of methods.

The financial status of our *Journal* will be discussed in the Secretary's report. Dr. White will present the Editor's report on the *Journal.*

Approved.

REPORT OF THE EDITORIAL COMMITTEE OF THE *JOURNAL*

W. B. WHITE, *Editor and Chairman*

Again there were annoying delays in the printing of the *Journal*, due to production difficulties, so that all issues were late in getting to our subscribers. At the time this report was prepared we did not have the page proof of the November issue. and thus must estimate our increase in numbers of pages by the first three issues only. Last year there were 594 pages; this year 800 pages.

In other respects we can measure our growth for the entire year. Last year there were 38 contributed papers, 3 notes, and 7 book reviews; this year there will be 43 papers, 3 notes and 9 book reviews.

Despite the unsettled conditions abroad, involving shortage of dollar exchange and even stoppage of the mails, there was a gratifying increase in the number of subscribers from 1800 to 1917. You will note, however, from the Treasurer's report that the *Journal* is very far from being self-supporting. All members are urged to take every opportunity to point out to educational institutions, experimental stations, and others the value of the *Journal* as a source of up-to-date regulatory methods and as a record

of the genesis and testing of new methods. The committee appointed to publicize the value of the *Journal* to potential subscribers will welcome suggestions along this line as to advertising media, lists of prospects, and the like. These may be sent to W. A. Queen, H. A. Lepper, or W. B. White, all of the U. S. Food and Drug Administration, Washington 25, D. C.

Again we are indebted to our contributors for the high caliber of the papers submitted, and for their unfailing courtesy in considering editorial suggestions. Our able reviewing staff has been invaluable in reaching decisions on what papers would be of interest to our readers, what revisions should be made in manuscripts, and what book reviews should be published. The Committee takes this opportunity of extending thanks both to contributors and reviewers.

Approved.

REPORT OF COMMITTEE TO CONFER WITH AMERICAN PUBLIC HEALTH ASSOCIATION ON STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS

Your Chairman is happy to report that copies of the Ninth Edition of *Standard Methods for the Examination of Dairy Products* became available late in August. The introduction of a simple cross reference system for the thirteen chapters and the carefully organized arrangement of the applications and interpretations of quality tests in Chapter 1 makes this edition of *Standard Methods* distinctively more useful than previous editions both to administrators and to laboratory workers.

In order that certain laboratories engaged in the examination of milk and cream, by one or more of the four routine methods described, may have several copies of the directions in which they are most interested without buying the complete edition, paper bound separates of Chapter 2 will be available. Chapter 2 describes the Agar Plate Method, the Direct Microscopic Method, the Methylene Blue Reduction Method, and the Resazurin Reduction Method. Many teachers of dairy sanitation will want their students to have copies of the separate as a reminder after graduation that there is a *Standard Methods,* and an American Public Health Association.

In addition to the separate referred to above, a "cook-book" style revision of Chapter 2 is in process. The intent is that this style of presentation will provide teachers of bacteriology with an abridged laboratory guide, which will in turn establish greater uniformity of instruction among students.

Action taken by this Association in 1947 relative to the deletion of all previously recognized tests for residual phosphatase in heat treated dairy products, and the adoption in place thereof of the Sanders and Sager method, has created a problem. In the Ninth Edition of *Standard Meth-*

ods, description of the Sanders and Sager method is limited to its application to hard type cheese. The New York State Department of Health method and the New York City Department of Health Laboratory Method No. 1 have been included among the official procedures for application to milk.

Soon after the Boston meeting it is expected that the details of the complete Sanders and Sager procedure will be printed in the American Journal of Public Health. Reprints of this procedure will then be made available to all laboratories who wish to compare the Sanders and Sager procedure with the methods which have been officially recognized in the Ninth Edition of *Standard Methods for the Examination of Dairy Products*. Before the next edition of *Methods of Analysis* is printed, it is hoped that the differences can be reconciled.

A. H. ROBERTSON, *Chairman*
GUY G. FRARY
J. O. CLARKE

Approved.

REPORT OF COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

The following definition was proposed at the last meeting and should be made tentative.

Fused Calcium-Magnesium Phosphate is a product derived from the fusion of rock phosphate with approximately thirty per cent (30%) of magnesium oxide (MgO), as such or as a mineral silicate. Its fineness and content of available phosphoric acid (P_2O_5) shall be stipulated. Example: Fused Calcium-Magnesium phosphate, twenty per cent (20%) available phosphoric acid (P_2O_5).

It is the unanimous vote of the Committee that the work on Definition of Terms and Interpretation of Results on Fertilizers and Liming Materials be discontinued and the Committee be discharged. Such action obviates the need for by-laws 11 and 12, and their deletion is recommended.

Approved.

L. S. WALKER, *Chairman*
F. W. QUACKENBUSH
M. ELMER CHRISTENSEN
L. E. BOPST
JOHN B. SMITH
W. H. MACINTIRE

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

WM. F. REINDOLLAR, *Chairman*

In the 1947 report of this Committee a plan to simplify the handling and distribution of referee reports and to relieve referees and associate referees of the requirement of sending reports to several individuals was recommended. This proposal, in brief, was to have each associate referee mail six copies of his report to the Secretary-Treasurer who in turn forwarded them to the chairman of the committee, the general referee, and the members of the appropriate subcommittee. A similar system applied to general referees except that they send five copies to the Secretary-Treasurer and retain one for themselves. This plan was put into operation during the past year and appears to be generally satisfactory. Early in the year referees and associate referees were sent an abstract of the 1947 Committee report together with the "Guiding Considerations Relative to Collaborative Work." This was followed by another letter in June, again stressing the new procedure for handling reports, and a copy of the report of the Committee on the Classification of Methods. Finally, each member of the group received a return postcard asking whether or not he would have a report and advising him when it was due. As a result of these efforts, a majority of the reports were sent to the proper source, the Association's Secretary was informed regarding papers to be presented, and the formulation of the program materially facilitated.

The chairman wishes to express his appreciation to the several subcommittees, referees, associate referees, and collaborators whose efforts have contributed so materially to the success of this Conference.

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

By H. A. HALVORSON, Department of Agriculture, Dairy and Foods, St. Paul, Minn., *Chairman:* E. L. GRIFFIN, and J. B. SMITH

FEEDING STUFFS

It is recommended—

(1) That further study be made on the following:

(a) Calcium and iodine in mineral mixed feeds.
(b) Lactose in mixed feeds.
(c) Adulteration of condensed milk products.
(d) Crude fat or ether extract.

* These recommendations submitted by Subcommittee A were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

(e) Fluorine.
(f) Protein evaluations in fish and animal products.
(g) Hydrocyanic acid glucosides.
(h) Sampling and analysis of condensed buttermilk.
(i) Microscopic examination of feeds.
(j) Tankage (hide, hoof, horn, and hair content).

(2) That the tentative method for calcium and phosphorus, *This Journal* **31,** 98 (1948), be made official, first action.

(3) That the tentative acetone method for fat in fish meal, *This Journal,* **31,** 98 (1948), be adopted as official, first action.

FERTILIZERS

It is recommended—

(1) That study of sampling equipment and method of sampling be continued.

(2) That preparation of sample for analysis be studied.

(3) That the formaldehyde titration method, as recommended by the Associate Referee be adopted as official, first action for determining nitrogen in ammonium nitrate.

(4) That the study of determining nitrogen in high nitrate-chloride mixtures be continued.

(5) That a survey be made of the different types of mills being used for the preparation of samples.

(6) That collaborative work on determining potash in samples prepared by different mills be continued on a greater variety of samples.

(7) That the changes in methods for phosphoric acid described in the first, second, third, and fourth recommendations of the Associate Referee be made.

(8) That sec. **2.16** be altered (first action) by

(a) Changing the phrase, in sec. **2.16(a)** first line, "*Acidulated samples*" to "*Acidulated samples and mixed fertilizers.*"

(b) Deleting the words, in sec. **2.16(b)** first line, "*other than basic slag.*"

(9) That sec. **2.17** be changed by deleting the words, in second sentence, "in acidulated samples, dicalcium phosphate, precipitated bone phosphate and precipitated bone" (first action).

(10) That the methods for citric acid-soluble phosphoric acid in basic slag, sec. **2.18, 2.19,** and 2.20, be deleted (first action).

(11) That work on methods for phosphoric acid be continued, with emphasis on

(a) Evaluation of sintered, fused, and calcined alpha phosphates as fertilizers.

(b) Aging of the molybdate solution used in the volumetric method to determine if a time limit should be put on its use or an addition made to preserve it.

(12) That the air-flow method be made official for determining free water in fertilizers, first action.

(13) That the vacuum-desiccation method with a drying period of 16 to 18 hours be made official for determining free water in fertilizers, first action.

(14) That the official procedure for oven drying be modified to state only one drying temperature, the selection of the temperature to be determined by study, not necessarily collaborative, during the coming year.

(15) That further study be made on the applicability of the forementioned three methods.

(16) That the tentative method for determining moisture in fertilizers with the use of distillation with toluene be deleted from the "Methods of Analysis."

(17) That the phraseology of the present official procedure for oven drying be modified as follows:

(a) Change parenthetical remark now worded "Not applicable to samples containing compounds other than H_2O that are volatilized at the temp. of drying." to read "Not applicable to samples that yield volatile substances other than H_2O at the temp. of drying."

(b) Change first sentence of text now worded "Heat 2 g of prepared sample, **2.2,** for 5 hours in water oven at temp. of boiling H_2O (98–100°) to read "Heat 2 g of prepared sample, **2.2,** for 5 hours in oven at temp. of 99–101°."

(c) Change second sentence of text now worded "In case of potash salts, $NaNO_3$, and $(NH_4)_2SO_4$, heat at ca 130° to constant weight." to read "In case of $NaNO_3$, $(NH_4)_2SO_4$, and potash salts heat to constant weight in oven at temp. of 129–131°."

(d) Change section heading to read *Water* instead of *Moisture.*

(18) That the work of the other Associate Referees on fertilizers be continued.

ECONOMIC POISONS

It is recommended—

(1) That a study be made of the determinations of rotenone in the presence of other insecticidal and fungicidal ingredients or of diluents.

(2) That work be continued on the analysis of oil emulsions that are prepared with non-soap emulsifiers, giving consideration to the use of chromatography for determination of the oil.

(3) That work on methods for the analysis of rodenticides containing alpha naphthyl thiourea (ANTU) and sodium fluoroacetate (1080) be continued.

(4) That method No. 20 for the determination of 2, 4–dichlorophenoxyacetic acid, and method No. 21 for the determination of salts of 2, 4-dichlorophenoxyacetic acid as described in the report of the Associate Referee be continued, giving consideration to the amount and manner

of adding the indicator so as to improve the end-point in the titration.

(5) That method No. 23, as described in the report of the Associate Referee for determination of esters of 2, 4-dichlorophenoxyacetic acid in the presence of soap, acids, alcohols, and oils, be further studied with emphasis placed upon a broader survey of ester mixtures and upon methods of breaking emulsions formed by this type of product.

(6) That the study of methods for the analysis of tetraethyl pyrophosphate preparations be continued.

(7) That the methods for determination of DDT, based on determination of the total chlorine content, which were adopted as tentative last year, be adopted as official methods, first action.

(8) That work on the determination of benzene hexachloride be continued.

(9) That study be started on organic thiocyanates and dimethyl dithio carbonate.

DISINFECTANTS

The Referee and his Associates have presented before the Association two papers relative to semi-micro methods of testing disinfectants and factors affecting the resistance of test organism. It is recommended that the work be continued.

LEATHER AND TANNING MATERIALS

No report was received. It is recommended that these chapters be dropped and the work be discontinued.

PLANTS

It is recommended—

(1) That the study of methods for zinc in plants be continued.

(2) That the magnesium uranyl acetate method for sodium in plants be kept tentative and that the study be continued, especially in respect to tolerance of the procedure to various levels of interfering ions which occur in plant materials.

(3) That collaborative work on the lignin method be continued for another year, using other samples of plant material.

(4) That the cellulose method be studied to determine the causes for the variations in results which have been found.

(5) That the spectrophotometric measurement of starch-iodine dispersions be investigated as a possible method for the determination of starch in samples low in starch.

(6) That the "Chromotrope B" method for boron be further investigated and that collaborative work on it be undertaken this year.

(7) That the other Associate Referees retain their assignments and continue their studies.

SPECTROGRAPHIC METHODS

No report has been received. It is recommended that work on this subject be continued.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That studies on the "combination dithizone-spectrographic method" and on the polarographic procedure for the determination of zinc in soils be continued.

(2) That the study of the determination of copper in soils be continued.

(3) That the utilization of carmin as an indicator in the determination of boron content of soils be studied further and that p-nitrobenzeneazo-1, 8-dihydroxy napthalene-3, 6-disulphonic acid, or "chromotrope-B" be studied as a suitable reagent for the determination of boron in soils.

(4) That further studies of *p*H in soils of arid and semi-arid regions be based upon soil systems of moisture content representative of an air-dry soil.

(5) That the analytical technic previously proposed for ignition of charge and distillation of fluorine therefrom be studied collaboratively.

(6) That a study be made as to the adequacy of calcium hydroxide as a fixative for fluorine in soil charges of 1 to 1 proportion with calcination at 500°C. in 5 to 60 minute periods.

(7) That the direct distillation of unignited soil with H_2SO_4 at 165°C., followed by distillation of an aliquot at 135°C. be studied collaboratively.

(8) That the "2-point" barium hydroxide-barium acetate titration procedure for the determination of exchangeable hydrogen in soils, as reported upon at this meeting, be studied further in relation to calcite equilibria in a variety of soils.

(9) That the survey and comparison of methods for the determination of phosphorus, (a) that fraction in "available" state and (b) the proporrions of organic-inorganic forms therein, be continued (*This Journal*, **30**, 43).

(10) That the survey and comparisons of methods for the determination of exchangeable potassium in soils be continued (*This Journal*, **30**, 44).

(11) That the tentative procedures for neutralization value of calcium silicate slags, **3.11**(**a**), be annotated by the statement "without correction for sulphide content." This constitutes a clarification of (13), *This Journal*, **31**, 44.

(12) That the procedure for the determination of sulfide sulfur content of calcium silicate slags, as reported by the Associate Referee, be adopted as tentative.

(13) That the Associate Refereeship on Exchangeable Calcium and Magnesium be maintained.

STANDARD SOLUTIONS

It is recommended—

(1) That method II for titanium trichloride solutions, **21.37** (p. 290),

substituting potassium dichromate for potassium permanganate be made official, first action.

(2) That preparation and standardization of potassium dichromate solutions be studied further, giving consideration to at least one recrystallization of potassium dichromate to insure uniformity.

(3) That the method of standardization of hydrochloric acid with standard sodium hydroxide (which was adopted as official, first action, in 1938) be adopted as official, final action. (A.O.A.C. Methods **43.7** and **43.8**).

(4) That the method for standardization of sulfuric acid by the standard borax method (which was adopted as tentative in 1939) be adopted as official, first action. (A.O.A.C. Methods **43.14** and **43.15**)

VITAMINS

It is recommended—

(1) That, with the following minor corrections, the present tentative method for vitamin A in fish liver oils be made official, first action:

The ether in **36.2** (page 599), should be specified as "U.S.P. ethyl ether in ½ pound cans, anesthesia grade, free from peroxides."

In the second sentence of **36.3** the "ground glass joint" should be changed to "glass joint." In the seventh line of **36.3**, change of the time from "2 minutes" to "allow the mixture to stand (about 2 minuter) until separation is visible complete, as determined by the absence of refraction streaming and the presence of distinct layers." In **36.4** (first line, p. 600) change "0.4" to "0.398." In **36.6** (p. 601) density values in the table are to be corrected to agree with the following—

T.	D.
0.7	2.16
7.0	1.16
11.0	0.959
26.5	0.577
27.5	0.561
29.5	0.530
32.5	0.488
55.5	0.256
63.0	0.201
71.5	0.146

(2) That the growth method for thiamine (vitamin B_1), (**36.16–36.23**), be made official, first action.

(3) That the fermentation method for thiamine (**36.27–36.31**) be made official, first action.

(4) That the fluorometric (thiochrome) method for thiamine (**36.24–36.26**) be changed as recommended in the report of the Associate Referee

and that it remain official, first action, and be studied further during the coming year.

(5) That the tentative microbiological method for the determination of riboflavin (*This Journal*, **30**, 79) be dropped and the directions as revised by the Associate Referee be made official, first action.

(6) That the fluorometric method for the assay of riboflavin, as described by the Associate Referee, be made official, first action.

(7) That the method for vitamin C (**36.47–36.48**) be revised to include the minor corrections and additions suggested by the Associate Referee and that it then be made official, final action.

(8) That the tentative method for determining vitamin D in milk (**36.49–36.60**) be made official, first action.

(9) That the Associate Referee on folic acid, on the basis of this year's results, select a single procedure and subject it to intensive collaborative study during the coming year.

(10) That the alternative tentative method (*This Journal*, **31**, 111, 1948) be adopted as official, first action, for carotene in hays and dried plants.

(11) That the method for carotene (*This Journal* **30**, 84, 1947) be discontinued for hays and dried plants but that it be continued as a tentative method of analysis for other materials.

(12) That the official, first action, method, *This Journal*, **30**, 82–84 (1947), for nicotinic acid be revised as recommended by the Associate Referee.

(13) That studies on the analysis of carotene be continued.

(14) That the work of the Associate Referees on other vitamins be continued.

(15) That the method for vitamin A in animal feeds be studied.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

By H. J. Fisher (Agricultural Experiment Station, New Haven, Connecticut), *Chairman;* G. R. Clark, and F. H. Wiley

NAVAL STORES

No report was received. It is recommended that the subject be continued.

RADIOACTIVITY

No report was received. It is recommended that the subject be continued.

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* These recommendations submitted by Subcommittee B were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

VEGETABLE DRUGS AND THEIR DERIVATIVES

It is recommended—

(1) That the study of chemical methods for ergot alkaloids be discontinued.

(2) That the proposed method for physostigmine in ointments be adopted as official, first action, and the topic closed.

(3) That the subject of theobromine and phenobarbital be studied further.

(4) That the study of the separation of aminopyrine, ephedrine, and phenobarbital be reassigned.

(5) That the subject of quinine be continued for the purpose of submitting the Herd procedure to collaborative study to determine if it should be made official.

(6) That the subject of chemical methods for penicillin be dropped.

(7) That the subject of rutin in tablets be continued.

(8) That the subject of ethylmorphine in syrups be continued.

(9) That method **39.73** for arecoline hydrobromide be made official (first action).

SYNTHETIC DRUGS

It is recommended—

(1) That the subject of methylene blue be continued.

(2) That the subject of sulfanilamide derivatives be continued.

(3) That the subject of propadrine hydrochloride be continued.

(4) That the subject of carbromal be continued.

(5) That the subject of butacaine sulfate be continued.

(6) That the subject of spectrophotometric methods be continued.

(7) That the proposed method for trichlorethylene be adopted as official (first action), and the subject be closed.

(8) That the subject of propyl thiouracil be reassigned.

(9) That the subject of phenolphthalein in chocolate preparations be continued.

(10) That the subject of pyribenzamine and benadryl be continued.

(11) That the subject of synthetic estrogens be reassigned.

MISCELLANEOUS DRUGS

It is recommended—

(1) That the following topics on which no reports have been received be continued:

Microscopic tests for alkaloids and synthetics
Alkali metals
Glycols and related compounds
Preservatives and bacteriostatic agents in ampul solutions
Estrone and estradiol

(2) That the subject of chromatographic separation of drugs be dropped.
(3) That a collaborative study be made of the application of the Rotondaro method to drugs containing mercury as a major ingredient.
(4) That the subject of the separation of bromides, chlorides, and iodides be continued.
(5) That the proposed method for calcium, phosphorus and iron in vitamin preparations be adopted as official (first action) and the subject closed.
(6) That method **39.202** for iodine be reworded as recommended by the Associate Referee (first action).
(7) That the subject of methyl alcohol be continued for the specific purpose of restudying the accuracy of method **39.162.**

COSMETICS

It is recommended—

(1) That the following topics be continued:
Cosmetic creams
Deodorants and anti-perspirants
Depilatories
Hair dyes and rinses
Moisture in cosmetics
Mascaras, eyebrow pencils, and eye shadow
Cosmetic skin lotions
(2) That the following topics be discontinued:
Alkalies in cuticle removers
Mercury salts in cosmetics
Hair straighteners
(3) That the proposed methods for pyrogallol in hair dyes be adopted as official, first action, and the topic closed.
(4) That the proposed methods for the analysis of face powder be adopted as official, first action, with the following changes:
(a) That the methods be adopted individually, as methods for the various constituents of face powder rather than as an entire method for the analysis of face powder. This will provide for the addition of methods for other constituents, not included in the study to date, should the necessity for them appear.
(b) The proposed method for stearate be designated "Fats and Fatty Acids as Stearic Acid."
(5) That the subject of sun tan preparations be studied.

COAL-TAR COLORS

It is recommended—

(1) That the following topics be continued:
Acetates, carbonates, halides, and sulfates in coal-tar colors

Buffers and solvents in titanium trichloride titration
Ether extract in coal-tar colors
Identification of coal-tar colors
Volatile amine intermediates
Non-volatile unsulfonated amine intermediates in coal-tar colors
Sulfonated amine intermediates in coal-tar colors
Unsulfonated phenolic intermediates in coal-tar colors
Sulfonated phenolic intermediates in coal-tar colors
Intermediates derived from phthalic acid
Mixtures of coal-tar colors for drug and cosmetic use
Spectrophotometric testing of coal-tar colors
Subsidiary dyes in D&C colors
Subsidiary dyes in FD&C colors
Hygroscopic properties of coal-tar colors

(2) That the proposed method for the determination of pure dye in D&C Reds Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 31, and 34 and Ext. D&C Red No. 2 be adopted as official (first action) and the subject continued.

(3) That the proposed method for lead in coal-tar colors not containing calcium, barium, or strontium be adopted as official (first action), and the subject closed.

(4) That the proposed method for lead in lakes of coal-tar colors be adopted as official (first action), and the subject closed.

(5) That the following new topics be studied:

Arsenic in coal-tar colors
Heavy metals in coal-tar colors

(6) That the boiling range of pseudocumidine and xylidine in certified coal-tar colors be studied.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By J. O. CLARKE, Food and Drug Administration, Washington, D. C., *Chairman;* PAUL A. CLIFFORD; and A. H. ROBERTSON

PROCESSED VEGETABLE PRODUCTS

It is recommended—

(1) That studies of methods for determining quality factors in canned and frozen fruits and vegetables be continued.

(2) That studies of the determination of moisture in dried vegetables be continued.

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* These recommendations submitted by Subcommittee C were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

(3) That studies of methods for the estimation of enzymatic activity of frozen fruits and vegetables be continued.

FILL OF CONTAINER METHODS

It is recommended—

(1) That studies of methods for determining fill of container for foods, drugs, and cosmetics be continued.

COFFEE AND TEA

It is recommended—

(1) That a Referee be appointed to review the chapter on coffee and tea and make recommendations for additional work where this appears desirable.

(2) That the Fendler-Stüber method (Modified; **18.15**) be made official, final action.

COLORING MATTERS IN FOODS

It is recommended—

(1) That the rapid method of detection of small amounts of tartrazine (FD&C Yellow No. 5), as published in *Methods of Analysis*, **20.125**, be made official, final action with slight modification adopted last year, *This Journal*, **31**, 82 (1948).

(2) That the method outlined by the Referee for the quantitative estimation of FD&C Yellow No. 5 (tartrazine) in the presence of FD&C Yellow No. 6 (Sunset Yellow F.C.F.) be tested collaboratively.

(3) That investigational work be undertaken to separate and determine quantitatively FD&C Green No. 2 (Light Green S.F. Yellowish), FD&C Green No. 3 (Fast Green F.C.F.), and FD&C Blue No. 1 (Brilliant Blue F.C.F.).

(4) That investigational work be undertaken to separate and estimate quantitatively FD&C Yellow No. 3 (Yellow AB), FD&C Yellow No. 4 (Yellow OB), FD&C Orange No. 2 (Orange SS), and FD&C Red No. 32 (Oil Red XO).

(5) That investigational work on analytical methods for coal-tar colors certifiable for use in foods be conducted.

DAIRY PRODUCTS

It is recommended—

(1) That the Sanders-Sager method for phosphatase in dairy products be further studied; and that the Associate Referee conduct collaborative work on milk or cream, comparing results by this method with those of the phosphatase tests given in **22.43** to **22.57.**

(2) That methods for the determination of ash in milk and in evaporated milk be further studied.

(3) That the statement in the method for fat in cheese, **22.130,** "Add ca 0.5 g of sand, previously digested with HCl, to prevent bumping . . . " be changed to read, "Add a few glass beads or other inert material, pre-

viously digested with HCl, to prevent bumping, ..."; and that study of methods for sampling, fat, and moisture in cheese be continued.

(4) That studies of methods for the detection of chlorine added to milk be discontinued.

(5) That studies of methods for the determination of the acidity of milk be continued.

(6) That the use of mechanical stirring or shaking devices and of artificial methods of cooling, suitable for use in the present official method for the preparation of butter samples, be further studied.

(7) That studies of methods for the detection of reconstituted milks be continued.

(8) That the Associate Referee conduct fundamental studies of the acetic serum method (**22.28**) and the copper serum method (**22.30**); and that the sour serum method (**22.29**) be dropped, first action.

(9) That studies be continued to ascertain whether the present Babcock method for fat in milk should be modified when used for the determination of fat in homogenized milk; and that the Roese-Gottlieb method for fat in dairy products be further studied.

(10) That studies be undertaken on sampling and preparation of sample of soft cheese.

(11) That studies be continued on methods for frozen desserts.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That an Associate Referee be appointed to study the present tentative method for fat by acid hydrolysis (**23.8**).

(2) That the Referee study paragraphs **23.29** and **23.30** (Acidity of Ether Extract, Official) and **23.31** and **23.32** (Tentative, Rapid Method) with a view towards formulation of a consolidated procedure retaining the best features of each, and that collaborative studies be undertaken.

(3) That the tentative qualitative method for glycerol (**23.25**), and the quantitative method as outlined by the Associate Referee, be tested collaboratively with a view towards official adoption next year.

(4) That an Associate Referee be appointed to investigate the value of the method for ammonia nitrogen (**23.33**) in the estimation of the age of shell eggs.

MICROANALYTICAL METHODS FOR EXTRANEOUS MATERIALS IN FOODS AND DRUGS

It is recommended—

(1) That the method for rodent excreta, in corn meal, **42.32,** be made official final action.

(2) That the methods for mold in cranberry sauces, *This Journal,* **31,** 783 (1948), for fruit products and beverage materials be adopted as tentative, and that the suggested methods for strawberries be further studied.

(3) That the method for extraneous materials in starch, **42.38**, as modified by the Associate Referee, be adopted as tentative; that work on flour, **42.29–42.31**, be continued; that the suggested methods for filth in popcorn be made tentative; and that collaborative work on baked products and prepared cereals be undertaken.

(4) That the new methods for pepper and prepared mustard, as devised by the Associate Referee, be subjected to collaborative study.

(5) That collaborative studies on filth in nuts and nut products, confectionery, and eggs and egg products be undertaken.

(6) That the wording of **42.61** (**b**) and **42.75** be changed as recommended by the Associate Referee.

SEDIMENT TEST (MILK AND CREAM)

It is recommended—

(1) That the method outlined by the Referee for the preparation of standard sediment discs be subjected to collaborative study.

(2) That further work be done on the preparation and use of standard sediment discs rendered permanent by treatment with a transparent plastic or by other means.

(3) That this topic be properly assigned to the chapter "Extraneous Materials in Foods and Drugs."

DECOMPOSITION IN FOODS

It is recommended—

(1) That the revised method for the study of volatile acids in fish be studied collaboratively.

(2) That the method for water-insoluble acids in butter and cream be adopted as official, first action.

(3) That the study of chemical means for detecting decomposition in fruits, in particular, "blackheart" in pineapple, be developed further.

(4) That the study of chemical means for detection of decomposition in dairy products be continued.

(5) That an Associate Referee be appointed to study succinic acid and water-insoluble acids as indices of decomposition in eggs and egg products and in fish.

(6) That the title of this topic be revised to: "Chemical Indices of Filth and Decomposition in Foods."

GELATINE, DESSERT PREPARATIONS, AND MIXES

It is recommended—

(1) That work in this field be continued, as recommended by the Referee.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the extract obtained by using a mixture of ethyl ether and

petroleum ether, *This Journal*, **31**, 334 (1948), be designated "Crude Fat (Acid Hydrolysis Method)" and that the method so designated be adopted as official, first action.

(2) That work be continued on methods for the determination of total solids in fish.

(3) That a study be made of methods for the determination of drained liquid in clams.

GUMS IN FOOD

It is recommended—

(1) That the method for detection of gums in mayonnaise and French dressing, 33.57, be amended by substituting "50 ml" for "1.5 oz." in line 3, paragraph 2, and that the method, as amended, be adopted as official, first action.

(2) That studies be continued on the detection of gums in soft curd cheeses.

(3) That studies be continued on the detection of gums in cacao products.

(4) That studies be continued on the detection of gums and other stabilizers in frozen desserts.

(5) That an Associate Referee be appointed to study detection of gums in catsup and related tomato products.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That work be continued for the development of methods for the determination of soya flour in meat products.

(2) That work be initiated on the development of a method for the determination of non-fat dry milk solids in meat products.

(3) That studies be made of additional methods for the determination of creatin in meat products.

(4) That methods for the detection of horse-meat in ground meat be developed and that an Associate Referee be appointed for this purpose.

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

It is recommended—

(1) That the study of methods for the determination of cadmium, copper, lead, mercury, and zinc in foods be continued with collaboration where necessary.

(2) That the study on the determination of DDT in foods in general be continued with sufficient collaboration to support adoption and inclusion of a DDT method in the next edition of *Methods of Analysis*.

(3) That the effect of the canning process on the decomposition of the newer insecticides be studies, with respect to the nature of possible decomposition products and their effects on their methods of analysis.

(4) That a new Associate Referee on the determination of parathion be appointed for study of that subject.

MICROBIOLOGICAL METHODS

It is recommended—

(1) That work be continued on canned meats, canned acid foods, canned vegetables, eggs and egg products, nuts and nut products, frozen fruits and vegetables, sugar, and canned fish.

MICROCHEMICAL METHODS

It is recommended—

(1) That a collaborative study of microchemical methods for carbon, hydrogen, and nitrogen be undertaken.

(2) That the chapter be expanded by the inclusion of other valuable methods of elemental microanalysis.

NUTS AND NUT PRODUCTS

It is recommended—

(1) That methods suggested by the Referee for the microscopic examination of nuts and nut products be referred to the Referee on Chapter 42 of the *Methods of Analysis*.

(2) That the methods for preservation and preparation of sample be adopted as tentative.

(3) That methods for moisture, crude fat, crude protein, crude fiber, ash, reducing sugars, sucrose, and salt, as suggested by the Referee, be adopted as tentative and further studied with a view towards their official adoption.

(4) That paragraphs **30.1** through **30.10**, **30.13** through **30.17**, be dropped. (Recommendation 3, above, substitutes more suitable methods.)

(5) That methods for added coloring materials, preservatives and artificial sweeteners, and metals, other elements, and residues, be adopted as tentative by reference to the appropriate chapter.

(6) That sorting methods for moisture and fat, and methods for added starch in nut butters and pastes, and added glycerol and glycols be studied.

OILS, FATS, AND WAXES

It is recommended—

(1) That a chromatographic procedure for the purification of unsaponifiable matter be further studied.

(2) That the modified Bellier Test, **31.47–31.48**, as revised be adopted as official, first action, and further efforts be directed towards making the test quantitative.

(3) That studies of methods for the determination of antioxidants in fats be continued.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That work on the esters of benzoic and vanillic acids be discontinued for the present.

(2) That methods for the quantitative determination of saccharin be further studied and that the method described by the Associate Referee, *This Journal*, **30**, 492 (1947), be tested collaboratively.

(3) That the ferricyanide method for quaternary ammonium compounds in commercial preservatives, *This Journal* **31**, 105 (1948), be made official, final action; that the method for table sirup, *ibid.*, **31**, 108, be made official, final action; that the method for bottled beverages containing fruit juice, *ibid.*, **31**, 106, be made official, final action; that the method for beer, *ibid.*, **31**, 108, be made official, final action.

That collaborative study be continued on methods for the determination of quaternary ammonium compounds in fruit juices, bottled sodas, milk, mayonnaise, salad dressings, and sandwich spreads and pickles and relishes.

(4) That the method for the determination of monochloracetic acid in carbonated beverages, *This Journal*, **31**, 104, with the changes suggested by the Associate Referee, be adopted as official, final action.

That the method for the determination of monochloracetic acid in beer and wine, with the changes suggested by the Associate Referee, be made official, first action.

That the method for monochloracetic acid in commercial preservatives, as recommended by the Associate Referee, be made official, first action.

That the barium test, barium-indigo test, indigo test, and pyridine tests for qualitative identification of monochloracetic acid in commercial preservatives be adopted as official, first action.

That the qualitative indigo test and pyridine tests for monochloracetic acid in carbonated beverages, orange juice, beer, and wine be adopted as official, first action.

That further work be done on fruit juices containing orange juice.

That further work be done on qualitative tests for monochloracetic acid.

(5) That work on dichloracetic acid be discontinued for the present.

(6) That work be continued on the development of improved methods for the detection of formaldehyde in foods.

That the modified methods selected by the Associate Referee be submitted to collaborative study and that unnecessary and unused methods be deleted.

(7) That use of an alternative chromatographic technique for the identification and estimation of volatile acids in baked products be further investigated.

(8) That the rapid oxidation method for thiourea in oranges and orange juice outlined by the Associate Referee be adopted as tentative and that the present tentative method for thiourea in orange juice be dropped.

That work on the determination of thiourea in foods be continued.

(9) That work be continued on methods for the estimation of dulcin in foods.

That work be initiated on methods for the detection and estimation of the artificial sweetener having the composition, propoxy, 2-amino, 4-nitrobenzene.

SPICES AND OTHER CONDIMENTS

It is recommended—

(1) That studies of methods for the detection of caramel in vinegar be continued.

(2) That studies of the determination of tartrates in vinegar be continued.

(3) That the permanganate oxidation method, **33.91,** as modified by the Associate Referee, be made official, first action.

(4) That methods for the determination of free mineral acids in vinegar be further studied.

(5) That methods for determining sorbitol, and the usefulness of this value in detecting cider vinegar in wine vinegar, be studied.

(6) That the official, first action, method for starch in mayonnaise and salad dressing be made official, final action.

(7) That work be carried out looking toward the adoption, as official, of methods for the preparation of samples and determination of total fat in mayonnaise and salad dressing, particularly as regards determination of fat in starchy salad dressings low in fat.

(8) That the efficiency of the preliminary removal of fat in the official method for nitrogen in mayonnaise be studied.

(9) That the official, first action, method for starch in prepared mustard and mustard flour be made official final action.

(10) That studies be initiated looking toward adoption of a method for the determination of ash in prepared mustard.

(11) That studies be continued on the determination of sugars in prepared mustard.

(12) That studies be continued of methods for the determination of volatile oils and other pungent principles in prepared mustard and mustard flour.

(13) That studies be continued for methods for the determination of volatile oil in spices.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

By C. S. FERGUSON, State Department of Public Health, Boston, Mass., *Chairman;* KENNETH L. MILSTEAD, AND J. WALTER SALE.

ALCOHOLIC BEVERAGES

Malt Beverages, Brewing Materials, and Allied Products:

It is recommended—

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

(2) That the dye color method for the estimation of color in wort and beer, described in the Associate Referee's report for 1948, be adopted as official, first action.

(3) That the tentative method for color in beer and wort, *This Journal*, **30**, 68 (1947), be dropped.

(4) That work on photometric beer color evaluations be continued.

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

(6) That the tentative method, **14.112–14.115**, for total solids in yeast, be adopted as official, first action, including the revision of **14.114**, preparation of sample, for total solids as described in the report of the Associate Referee.

(7) That the Milos test for caramel, **14.35**, be deleted, final action.

(8) That the Mathers test for caramel, *This Journal*, **31**, 76 (1948), be studied collaboratively with respect to its application to beer.

(9) That the study of carbon dioxide in beer be continued.

(10) That the direct (non-ashing) orthophenanthroline method, described in Proceedings of the Eleventh Annual Meeting of the American Society of Brewing Chemists, pages 32 and 37, for the determination of iron in beer, be studied further (a) with a view to eliminating the use of the reducing agent, hydroxylamine hydrochloride, and (b) use of crystalline ferrous ammonium sulfate in place of metallic iron for standardization.

(11) That further work on copper be postponed, pending outcome of proposed work by the Referee on metals in foods.

(12) That work be continued on polarographic and spectrographic methods for tin in beer.

Wines:

It is recommended—

(1) That chromatographic studies of wine be continued.

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* These recommendations of Subcommittee D were approved by the Association. Unless otherwise given all references are to *Methods of Analysis, A.O.A.C.*, 1945.

(2) That the official Milos test for caramel (**15.38**) be deleted, final action.

(3) That the official, first action, Mathers test, *This Journal*, **31**, 76 (1948), be adopted as official, final action.

Distilled Liquors:

It is recommended—

(1) That the study of methods of analysis with reference to the aging or maturing of whisky in laminated (plywood) barrels be continued.

(2) That the Mathers test for caramel, *This Journal*, **31**, 76 (1948), be adopted as official, final action, for distilled liquors and that it be studied collaboratively, with respect to its application to cordials and liqueurs, before adoption as final action.

(3) That the official modified Marsh test and the official Milos test, **16.39** and **16.41** for caramel, be deleted, final action.

(4) That the study of colorimetric methods for fusel oil be continued.

(5) That the study of the method **16.22–16.23** for fusel oil be continued.

(6) That the official method, **16.29**, for methanol in distilled liquors by the immersion refractometer method be studied in the light of the findings of Beyer and Reeves, *This Journal*, **28**, 800 (1945).

(7) That an Associate Referee be appointed to determine if the immersion refractometer method for alcohol in **15.4(c)** should be incorporated in Chapters 14 and 16.

(8) That the rapid method for proof of distilled spirits, as recommended in the report of the Associate Referee on obscuration test for proof of distilled spirits be adopted as official, first action.

(9) That study be continued of the official Denigès method for methanol, **16.25**, and the tentative method for methanol in **39.161** and **39.162** to bring about uniformity in these procedures so far as possible.

Cordials and Liqueurs:

It is recommended—

(1) That section "**16.45** Specific Gravity, see **14.3**" be changed to read "**16.45** Specific Gravity, proceed as under **16.2**."

(2) That a collaborative study be made of methods for caramel in cordials and liqueurs.

(3) That a collaborative study be made of the two tentative methods for total solids, *i.e.*, **16.51(a)** From sp. gr. of dealcoholized sample, and **16.51(b)** By evaporation.

(4) That a collaborative study be made of the tentative method for total acidity, **16.62.**

CACAO PRODUCTS

It is recommended—

(1) That the method for lecithin in cacao products be further studied.

(2) That the method for lactose in cacao products, reported this year, be studied to increase the rapidity of the method and to correct for effect of the presence of higher sugars in corn sirup solids, and that collaborative work be conducted.

(3) That the study of methods for maltose and cacao ingredient be continued.

(4) That the tentative method for pectic acid, **19.16,** be revised as recommended by the Referee.

(5) That the method for pectic acid in milk chocolate be further studied.

(6) That the method of separation of fat, **19.25,** when used on milk chocolate, be studied and compared with the method for refractory sample proposed by Ferris, *This Journal,* **31,** 728 (1948).

CEREAL FOODS

It is recommended—

(1) That procedures adopted as official, first action, last year, *This Journal,* **31,** 79, for the determination of phosphorus in cereals and cereal products be adopted as official, final action, and the study be discontinued.

(2) That the studies on determination of starch in raw and cooked cereals be continued.

(3) That the tentative method for the determination of fat acidity in grain and flour (**20.18–20.21,** incl., and **20.76**) be adopted as official, first action, and the study continued.

(4) That a study of the application of the method for reducing and nonreducing sugar in flour, **20.28–20.30,** incl., be made to the determination of sugars in bread and other bakery products, with special consideration given to the article on this subject published by R. M. Sandstedt and G. C. Fleming, *This Journal,* **30,** 550–552.

(5) That the tentative method for benzoic acid in flour (**20.53**) be replaced by the method proposed by the Associate Referee for wheat flour and adopted as official, first action, as a qualitative test and the study continued.

(6) That work on methods for determination of available CO_2 in self-rising flour, containing added $CaCO_3$, be discontinued.

(7) That the methods for the determination of lactose in bread be further studied.

(8) That the method for the determination of fat and fat number in bread as proposed by the Associate Referee in this year's report, replace **20.86** and be adopted as official, first action.

(9) That the method proposed by the Associate Referee in this year's report for the determination of proteolytic activity of flour and malted wheat flour he adopted as official, first action, and that the work be continued.

(10) That the methods for soybean flour, *This Journal,* **31,** 81 (1948) for moisture, deleting "**20.2,**" ash, nitrogen, and oil or petroleum benzine

extract, be adopted as official, first action, and the study be continued.

(11) That the study on the detection and determination of soybean flour in cereal products be discontinued.

(12) That the method proposed by the Associate Referee in this year's report for the determination of added inorganic material in phosphated flour be adopted as official, first action, and the study continued with elimination of self-rising flour.

(13) That the method referred to in *This Journal*, **25**, 83–84, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicability to bakery products containing eggs.

(14) That the study of methods for the determination of albumen in noodles and macaroni products be conducted.

(15) That the study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(16) That the study on the determination of moisture in flour products containing sodium bicarbonate as one of its constituents be continued.

(17) That the study on the determination of bromates in flour be continued.

(18) That the tentative methods **20.32** to **20.38**, incl., and **20.60**, be dropped.

BAKING POWDERS

It is recommended—

(1) That the tentative, modified McGill method, **17.21**, be dropped.

(2) That the tentative qualitative test for phosphoric acid, **17.31**, be adopted as official, first action.

(3) That the tentative methods and the oven modification No. 1 as set forth in the Associate Referee report for this year on residual CO_2 in baking powder be adopted as official, first action.

(4) That the HCl (1+2), be adopted as alternate for H_2SO_4 (1+5) in the official method for total CO_2, **17.4** and **17.6**.

(5) That the available CO_2, **17.9**, be determined by subtraction of residual CO_2 (see recommendation 3) from the total CO_2, **17.6**.

(6) That the official gasometric method **17.8** be dropped, final action.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That the collaborative study of the reflux method for determination of peel oil in citrus fruit juices and the use of the modified oil separation trap be continued.

(2) That collaborative work be continued on the method for determination of beta-ionone where small amounts are present.

(3) That collaborative studies on the Ripper method for determination of aldehydes in spirits as applied to lemon oils and extracts be continued.

(4) That collaborative studies of the methods proposed by the Referee for determination of esters in lemon extract be continued.

(5) That collaborative studies on the Seeker-Kirby method for determination of esters in lemon and orange oils (Dept. of Agri. bull. 241) be continued.

(6) That collaborative studies of extract methods containing both isopropyl alcohol and acetone be continued.

(7) That collaborative study of the photometric method for determination of vanillin and coumarin be continued.

(8) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts with special reference to the automatic extraction of vanilla and coumarin.

(9) That the study of emulsion flavors be continued.

(10) That studies on maple concentrates and imitations be continued.

(11) That study of the method for determination of diacetyl, published in *This Journal*, **25**, 255, be continued.

(12) That the Referee study collaboratively the modification of **25.23**, in lemon, orange, and lime extracts, as given in last year's report, *This Journal*, **31**, 202 (1948).

(13) That the Referee study collaboratively the modifications of **25.54** for alcohol in almond extract as given in last year's report, *This Journal*, **31**, 203 (1948).

(14) That the methods for vanilla resins in vanilla extract, **25.15** and **25.16**, be studied collaboratively.

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That study of methods for determining fruit and sugar content of frozen fruits be continued.

(2) That further collaborative study be made of the method for the electrometric titration of acidity.

(3) That further study be made of methods for separating and determining fruit acids.

(4) That procedures as given by the Associate Referee in his 1948 report for the rapid determination of water-insoluble solids and for the determination of seeds of berry fruits be adopted as tentative and that the procedures be subjected to collaborative study.

(5) That **26.18(a)** be changed as recommended by the Referee (first recommendation).

(6) That the sentence beginning "If recoveries are low . . . " in **26.19**, Note (3), be deleted.

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the method for the determination of shellac on confectionery *This Journal*, **31**, 196 (1948) modified as described in this year's report of the Associate Referee, be adopted as official, first action, and that further collaborative work be conducted on it.

(2) That the method of the Java Sugar Experiment Station for unfermented reducing substances in molasses, adopted as a tentative method last year, be made official, first action, with the changes recommended by the Referee.

(3) That the study for the determination of moisture be continued.

(4) That study be continued on tables of density of solutions of sugar at various temperatures.

(5) That the Zerban and Martin values for refractive indices of dextrose and invert sugar solutions (*This Journal*, **27**, 295) be subjected to collaborative study.

(6) That the official method for the determination of free acid in honey, **34.99**, be further studied collaboratively with a view to establishing the end point more accurately.

(7) That study be made of methods for the detection of adulterants in honey, particularly commercial sirups.

(8) That study be continued on the determination of dextrose, maltose, and dextrins, by copper reduction methods in carbohydrate mixtures.

(9) That the tentative methods, **34.133–34.155**, inclusive, be subjected to collaborative study.

(10) That the procedures in N.B.S. Circular C440, pp. 324–334, for measurement of transmittancy of solutions of commercial sugar products, be study collaboratively.

(11) That the Somogyi Modification (*J. Biol. Chem.*, V. **160**, p. 61, 1945) of the tentative micro method for reducing sugars (**34.63**) be studied.

(12) That Ofner's method for the determination of invert sugar in the presence of sucrose be made official, first action.

(13) That the method **34.8** for solids by refractometer be amended by adding: "In liquid products containing invert sugar, correct the per cent solids obtained from 44.7 by adding 0.022 for each per cent invert sugar present in the sample." (First recommendation.)

REPORT BY REPRESENTATIVES OF THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL

By W. H. MacIntire

This report is being presented in behalf of our beloved and lamented confrere, Dr. H. J. Patterson, of whom the Chairman of the Board of Governors of the Institute wrote so recently—

"I was tremendously sorry to learn of the death of Dr. Patterson. He was a great character and a man of profound accomplishment."

As though there he had a premonition of his demise, our beloved Past

President had suggested that he be succeeded in his assignment as representative from this Association. Because of his sustained interest in the objectives of the Institute, however, he acceded to the urgent request that he continue to serve under designation of this Association. Through his advice, counsel, and interest Dr. Patterson contributed much to the success of this Institute.

The activities of the Institute during the past year have covered a wide range of research. The program included exploratory studies of new chemical compounds as insecticides and fungicides; the influence of inert extenders on the behavior of insecticidal dusts; development of formulae for aerosols; exploration of mildew-proofing agents; studies on moth proofers; research in chemotherapy and in analytical procedures for organic phosphates.

The Institute has continued to function upon basis of cooperation and collaboration between the research chemists and the biological experimenters of the academic institutions and those workers of like designation among the industrial organizations. Such joint efforts have led to the discovery of new and better materials for plant protection and have pointed the way to profitable manufacture and effective use of the various new compounds. During the past year guidance and direction by the Institute have been sought by 19 collaborating agencies, laboratories, and corporations.

The policies and functions of the Institute during the past year were directed by its Board, which was comprised of Dr. W. C. O'Kane (Chairman) of the New Hampshire Experiment Station, Dr. D. M. DeLong, Ohio State University, and. A M. Boyce of the Riverside unit of the California Experiment Station, as representatives of Entomology; Dr. H. W. Thurston (Vice-Chairman) of the Pennsylvania Experiment Station, Dr. J. G. Horsfall, Director of the Connecticut Experiment Station, and Director C. R. Orton of the West Virginia Experiment Station representing Plant Pathology; Dr. S. E. A. McCallan of Boyce Thompson Institute for National Research Council, and H. J. Patterson and W. H. MacIntire as designates of the Association of Official Agricultural Chemists.

The Institute continues to serve as a liaison between the research worker and the user of the worker's findings. Its usefulness has been recognized by those industrial agencies that need factual information and scientific guidance. To be on its Board has been an educational and inspirational experience to your remaining representative.

Approved.

REPORT OF THE SECRETARY-TREASURER

By HENRY A. LEPPER

The meeting of the executive committee was held Sunday, October 10, 1948, at 10 A.M., in the Board Room of the Cosmos Club. All members of the committee were in attendance. The audit of the firm of Snyder, Farr, and Company was presented and accepted.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, INC.

BALANCE SHEET—SEPTEMBER 30, 1948

ASSETS

Current Assets:		
Cash, Lincoln National Bank	$20,964.29	
Office cash fund	56.14	$21,020.43
Accounts receivable	$ 4,632.46	
Less reserve for doubtful accounts	163.90	4,468.56
Accrued interest receivable, Government bonds		250.00
Inventories		6,161.37
Total Current Assets		$31,900.36
Fixed Assets:		
Furniture and fixtures		756.01
Investments		42,121.00
Total Assets		$74,777.37

SURPLUS

Balance, October 1, 1947		$72,408.75
Add: Net profit for the fiscal year ended September 30, 1948	$ 2,379.92	
Recreation fund transferred from old account	39.00	
		2,418.92
		$74,827.67
Less: Old returned checks written off		50.30
Balance, September 30, 1948		$74,777.37

It has been our custom through the years in evaluating the financial status of our *Journal* to balance the income from subscriptions and advertising against printing and mailing costs. On this basis we have considered *The Journal* to be self-sustaining. This year we had the auditor estimate the cost of publishing *The Journal* by allocating administrative expenses to gross profits on sales of *Methods of Analysis* and *The Journal* based on the ratio of sales of each to total sales. Such expense is properly chargeable to *The Journal*, and with this addition we find that it has cost

the Association $1,851.36 from our general operating fund to publish and distribute this year's volume of *The Journal*. Your executive committee recognizes that scientific journals generally do not balance their budgets by depending solely on subscription returns. The committee further regards our *Journal* as a contribution to scientific literature and supplemental to our publication of the *Methods of Analysis*. To serve such interests it is therefore recommended that the Association contribute from its general fund the support necessary for *The Journal* in lieu of an increase in the subscription price with the possible decrease in the number of subscribers. It further suggests the appointment of a subcommittee of three of its members to consider and put into effect steps which might be taken looking toward an increase in the number of subscribers. To expedite the work, members located in Washington should constitute the subcommittee, and Messrs. Queen, White, and Lepper are suggested.

The association has received word from G. E. Gratton of the Department of agriculture of Canada that through a change of position he will no longer be able to attend our meetings and continue his activities in the Association. The vacancy thus created on Subcommittee A has been filled by the appointment of John B. Smith of Rhode Island. The terms of C. S. Ferguson on Committee D and H. J. Fisher on Committee B expire this year and their reappointment is recommended. The present chairman of the Committee, W. F. Reindollar, was reappointed.

During the past year the addressing machine authorized for purchase at the last meeting and an adding machine authorized during the year by the executive committee were purchased at a total cost of $475.01. These have expedited the clerical and routine work of the business office in complete fulfillment of our expectations. The committee also authorized the purchase of two additional projectors and screens costing $261.00, and all in attendance at this meeting, when they are available for the first time, appreciate their worth in the presentation of reports at the several sectional meetings. Their usefulness should increase as the members now know of their availability.

Approved.

REPORT OF COMMITTEE ON NECROLOGY

JOSEPH A. AMBLER

Dr. Ambler was a native of Danbury, Connecticut, born June 30, 1889; he died October 6, 1948, in New Orleans, La. He was chief organic chemist with the Department of Agriculture Research Division in New Orleans. Dr. Ambler received his doctor's degree in organic chemistry from Yale University in 1913. He joined the Department of Agriculture as a chemist in 1917. In 1937 he was cited by the department for explaining the effects of contaminants on the production of sugar.

HORACE T. HERRICK

Dr. Herrick was born April 22, 1887, a native of Brooklyn, N. Y. and died October 7, 1948, in Washington, D. C. He was graduated from Lawrenceville School and Princeton University and received a degree in chemical engineering from Columbia University. One of his outstanding accomplishments in 22 years with the U. S. Department of Agriculture was his assistance in setting up four regional research laboratories to search for new industrial outlets and markets for farm crops. Prior to his appointment in 1946 as Special Assistant to the Chief of the Bureau of Agricultural and Industrial Chemistry, he was successively head of the Bureau's Color and Farm Waste Division; head of the Industrial Farm Products Research Division; and special assistant on the four regional research laboratories project.

WILLIAM F. HAND

Dr. Hand was born December 1, 1873, in Shubuta, Mississippi, and died September 25, 1948. Teacher and scientist for 53 years, and vice president of Mississippi State College for 13 years, Dr. Hand designed and built the Mississippi State Chemical Laboratory which is dedicated to him. He was graduated from Mississippi Agricultural and Mechanical College in 1895, and from Columbia University in 1903, with the degree of Ph.D. He was President of the Association in 1921 and President of the American Society of Feed Control Officials. In recent years he prepared all regulatory feed, fertilizer, paint, food, drug, and petroleum laws for the State of Mississippi.

HARRY J. PATTERSON

Dr. Patterson was born in Yellow Springs, Pa., December 17, 1866, and died on September 11, 1948, in Boston, Mass. He was dean emeritus of the University of Maryland, College of Agriculture and Experiment Station. Before coming to the University of Maryland, Dr. Patterson held positions at Pennsylvania State College. He was Secretary of the Maryland State Board of Agriculture from 1908 to 1916. His work was in the field of chemistry, feeding and fertilizing problems; methods of rendering phosphates available to crops, etc.

ARTHUR E. PAUL

Dr. Paul was born in Germania, Pa., September 29, 1874, and died on January 1, 1948. He was originally appointed as an assistant chemist at the Chicago Station of the Food and Drug Administration in 1909. He served successively as Chief Chemist of the Chicago Station, Chief of the Cincinnati Station, Chief of the Chicago Station, and Assistant Chief of the Central District. Dr. Paul was particularly interested in the scientific aspects of food and drug work. He served faithfully in the work of the

Association of Official Agricultural Chemists and was president of that body in 1932.

ALFRED E. TAYLOR

Dr. Taylor was born in Portland, Connecticut, October 16, 1871, and died in Washington, D. C., on May 30, 1948. Dr. Taylor reported to the old Bureau of Chemistry in April, 1907, following seven years as a chemist in the Customs Service. From 1918 until his retirement in October, 1941, he supervised and coordinated the work on imports in the Food and Drug Administration. Dr. Taylor was graduated from Harvard University in 1894 with an A.M. Degree, and from Cornell University in 1896, with a Ph.D. degree. At the time of his retirement in 1941, Dr. Taylor had served the Federal government for 41 years.

E. PECK GREENE

Mr. Greene was born in Clarksville, Arkansas, July 15, 1884, and died at Tallahassee, Florida, April 4, 1948. He was educated in the public schools of Florida and at the University of Florida at Lake City. In 1909 he joined the State Department of Agriculture and at the time of his death was one of the oldest state employees in point of service, having served for more than 35 years. Mr. Greene was greatly interested in plants, shrubs, and flowers, and the E. Peck Greene Park in Tallahassee, Florida, has been named by the city in his honor.

ARTHUR L. SULLIVAN

Mr. Sullivan was born in Suncook, New Hampshire, in 1881, and died August 16, 1948, in Baltimore, Md. He was graduated from the University of New Hampshire in 1902, with the degree of B.S. He worked as an assistant chemist with the U. S. Bureau of Internal Revenue from 1903 to 1907, when he transferred to the U. S. Bureau of Chemistry as a food and drug inspection chemist. In 1912 he was made Chief of the Boston Station of the Food and Drug Administration. In 1915 he returned to Washington to be in charge of the Washington laboratory. He was made Chief of the Baltimore Station in 1917, serving until 1920, when he was made Food and Drug Commissioner for the State of Maryland. As a State food official Mr. Sullivan cooperated in the enforcement of the Federal law. The improvement of the sea-food industry in Maryland is due in no small part to his interest in that industry and his enforcement of laws, especially those covering sanitation.

J. W. SALE, *Chairman*
W. L. HILL
JOHN B. SMITH

REPORT OF THE COMMITTEE ON NOMINATIONS

Your committee presents the following designations and recommends their election to the offices indicated:

President: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

Vice-President: W. A. Queen, Food & Drug Administration, Washington 25, D. C.

Secretary-Treasurer Emeritus: W. W. Skinner, Kensington, Md.

Secretary-Treasurer: Henry A. Lepper, Food & Drug Administration, Washington 25, D. C.

Additional Members of the Executive Committee: H. A. Halvorson, State Chemist, St. Paul, Minn.; W. B. White, Washington, D. C.; H. J. Fisher, Conn. Agricultural Experiment Station, New Haven, Conn; G. H. Marsh, Dir. Chemistry Division, Montgomery, Ala.

Approved.

W. H. MacIntire, *Chairman*
J. J. T. Graham
K. L. Milstead

REPORT OF COMMITTEE ON RESOLUTIONS

Acknowledging our indebtedness to the officers and members of the Association who were responsible for making this annual meeting a useful and profitable gathering, we hereby extend to all and to each of them our sincere thanks for their able performance of duties and our congratulations upon the success of this 62nd annual meeting; and

Whereas, the management and employees of the Shoreham Hotel have afforded us a most convenient and delightful meeting place and most courteous treatment, therefore be it

Resolved, that we extend to the hotel our thanks and this expression of appreciation of their effective aid in making our stay pleasant in all respects; and

Whereas, we recognize the value of publicity to the best functioning of the Association, therefore, be it

Resolved, that we extend our thanks hereby to the press and their representatives who have covered this meeting; and

Whereas, we all acknowledge our debt of gratitude of the Referees and Associate Referees, and those who have collaborated with them in carrying on during the past year the real work of the Association, therefore be it

Resolved, that we by this expression assure them of our real appreciation of their efforts and of our earnest intention to assist them in the future.

Approved.

H. J. Wichmann, *Chairman*
J. L. St. John
Guy G. Frary

CHANGES IN OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE SIXTY-SECOND ANNUAL MEETING, OCTOBER 11, 12, AND 13, 1948*

The changes in the methods of the Association recorded below become effective March 17, 1949, the thirtieth day after the date of publication of this Report, Feb. 15, 1949, as provided in section 5 of the by-laws as amended at the 1948 convention (see page 38).

The classification of methods employed in this report follows that of previous years. The classification provided by the by-laws as amended this year (see p. 33) will be applied to revisions made at the 1949 meeting for publication in the contemplated seventh revision of *Methods of Analysis*, in 1950.

1. SOILS

No additions, deletions, or other changes.

2. FERTILIZERS

(1) The following was adopted as official, first action.

NITROGEN IN AMMONIUM NITRATE

Formaldehyde Titration Method

(May also be adapted to Ammonium Sulfate)

Weigh out 7.004 or 14.008 g of sample and make up to 250 or 500 ml. Pull off 25 or 50 ml and put into a 300–500 ml Erlenmeyer (ca. 1.5 g may be rapidly weighed and washed directly into flask). Add ca. 1 ml of reagent formaldehyde for each 0.1 g of sample in aliquot. Make total volume 150–200 ml and allow 5 min. before titrating with 0.25–0.50 *N* sodium hydroxide, using 5 drops of phenolphthalein as indicator. Titrate until there is no perceptible color change at the point of contact, or until the proper color of pink persists. Run a blank on the formaldehyde.

$$\% \text{ Nitrogen} = \frac{\text{Net ml of NaOH} \times \text{Normality} \times 2.8016}{\text{Wt. of Sample}}.$$

(2) The official, first action, changes reported in *This Journal*, **31**, 71, under fertilizers (2) (a), (b), (c), (d), (e), and (f) were made official, final action.

(3) The title "Acidulated samples" of **2.16(a)** first line was changed to "Acidulated samples and mixed fertilizers," and lines 1 to 11 beginning with "after" and ending with "retentiveness" were replaced with the following, official, first action.

"After removing water-soluble P_2O_5, **2.13**, transfer the filter and residue, within a period not to exceed 1 hour, to 200 or 250 ml flask containing 100 ml NH_4 citrate soln previously heated to 65°. Close flask tightly with a smooth rubber stopper, shake vigorously until filter paper is reduced to pulp, relieve pressure by momentarily removing stopper, and proceed by one of the following methods: (1) Loosely stopper flask to prevent evaporation, place in water bath regulated to maintain contents of flask at exactly 65°, keep level of H_2O in bath above that of citrate soln in flask, and

* Unless otherwise given all references in this Report are to *Methods of Analysis*, *A.O.A.C.*, 1945.

shake every 5 min; (2) continuously agitate contents of stoppered flask by means of apparatus equipped to maintain contents of flask at exactly 65°. At expiration of exactly 1 hour from time filter and residue were introduced, remove flask from bath or apparatus and immediately filter contents as rapidly as possible thru Whatman filter paper No. 5 or other paper of equal speed and retentiveness."

(4) The words "other than basic slag" **2.16(b)**, first line, were deleted, first action.

(5) That part of **2.17**, line 3, beginning "in acidulated samples" to the end of the section, was deleted, first action.

(6) Methods **2.18**, **2.19**, and **2.20** for citric acid-soluble phosphoric acid in basic slag were deleted, first action.

(7) The following methods were adopted as official, first action.

FREE WATER IN FERTILIZERS

Air-flow Method

APPARATUS

(A) *Manifold Assembly.*—A metal box $10\frac{1}{2} \times 2\frac{1}{2} \times 1\frac{1}{4}$ inches in size† is equipped with a $\frac{1}{4}$-inch nipple centrally located on one side for attachment to a vacuum line, and 6 $1\frac{1}{4}$-inch tapered stopper seats evenly spaced along the top to accommodate No. 6, one-hole, rubber stoppers. A $1\frac{1}{4}$-inch length of light metal tubing, $\frac{1}{2}$ inch in diameter, extends thru each rubber stopper to a height of $\frac{1}{4}$ inch above the surface for the purpose of centering a fritted glass crucible over the hole in the stopper. Since the crucible is held in place by suction, it is necessary to grind a smooth surface on the lower edge of each fritted glass crucible and on the surface of the stopper in order to insure an air-tight connection between the edge of the crucible and the stopper when air is being drawn thru the sample in the crucible.

(B) *Crucibles.*—Pyrex glass, approximately $1\frac{3}{4}$ inches tall, $1\frac{3}{16}$ inches in diameter and having a $\frac{7}{8}$-inch fine-porosity fritted glass plate. Individual crucibles of a set should all have approximately the same porosity. A matched set may be obtained by selecting several that pass a given quantity of air at constant pressure in approximately the same length of time.

(C) *Vacuum Gage.*—A standard instrument for insertion in the rubber vacuum line between the source of suction and the manifold.

(D) *Constant Temperature Oven.*—A standard laboratory oven, preferably of the type vented so that incoming air passes directly over the heating coils.

DETERMINATION

Weigh 2 g of prepared sample, **2.2**, in a tared, fritted glass crucible. (Extremely hygroscopic or damp materials should be weighed out of difference in covered crucibles.) Place crucible on manifold in the oven at 60°. Aspirate for 2 hours under 15 in. of vacuum. Cool in desiccator, for 30 min. and reweigh. Calculate percentage loss in weight.

Vacuum-drying Method

DETERMINATION

Place 4 g of prepared sample, **2.2**, in a short-type, tared weighing dish of a size not less than 2 in. in diameter. (Extremely hygroscopic or damp materials should

† An illustration of this apparatus is given in *This Journal*, 31, p. 235 (1948).

be weighed out of difference in covered dishes.) Place in a vacuum desiccator over anhydrous magnesium perchlorate for 16 hours under not less than 25 in. of vacuum. Reweigh and calculate percentage loss in weight.

NOTE: A drying period of 16 hrs. represents over-night drying. The average type of fertilizer will release its free moisture in 3–6 hrs. However, over-night drying insures best results, especially on very damp materials and on samples containing high amounts of adsorbed water.

Vacuum-Desiccation Method

Place 2 grams of sample in a tared low-form weighing dish (4 grams of sample may be used with large weighing dishes, 1.5–2 inches in diameter) and place it in a vacuum desiccator at 25–30°C. over anhydrous magnesium perchlorate (or equivalent desiccant) for 16–18 hours under not less than 20 inches of vacuum. Report percentage loss in weight as moisture.

(8) The tentative method for moisture by distillation with toluene, **2.5** and **2.6**, was deleted.

(9) The parenthetical statement in the method for moisture, **2.4** "(Not applicable to samples containing compounds other than H_2O that are volatilized at the temp. of drying)" was changed to "(Not applicable to samples that yield volatile substances other than H_2O at the temp. of drying)."

(10) The wording of the directions in the official method for moisture—by drying, was changed to read "Heat 2 g of prepared sample, **2.2**, for 5 hours in oven at temp. of 99–101°. In case of $NaNO_3$, $(NH_4)_2SO_4$ and potash salts heat to constant weight in an oven at temp. of 129–131°"; and the heading "Moisture" was changed to "Water."

3. AGRICULTURAL LIMING MATERIALS

(1) The method for neutralization value of calcium silicate slags **3.11(a)**, (p. 44) was annotated by the statement "without correction for sulfide content."

(2) The following method was adopted as tentative.

SULFIDE SULFUR IN CALCIUM SILICATE SLAGS

REAGENTS

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

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

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

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

(e) *Standard acid.*—0.1 *N* HCl.

APPARATUS

A 250-ml Erlenmeyer evolution flask to fit No. 5.5 stopper, a 60-ml separatory funnel fitted into the stopper and the lower third of its stem drawn to a 2 mm open-

ing and bent upwards at its extremity. The funnel in the stopper is adjusted so that its stem is ¼ inch from the bottom of the evolution flask. Two 25×150 mm test tubes are fitted with No. 4 two-hole stoppers and inlet and outlet of 6 mm tubing, which serve to contain jointly the 50 ml of absorbent. The tubes are in series and should be placed in a 600-ml beaker filled with cold water, and the beaker placed upon a tripod on level with the evolution flask. Another test tube of similar size is half-filled with H_2O and clamped in position between evolution flask and absorbent solution to provide trap for HCl vapor.

DETERMINATION

Weigh 1 g of minus 80-mesh slag into the evolution flask; add 1 g of Zn dust and wash down sides with 5–10 ml H_2O; mix contents by means of flattened end of stirring rod and connect the flask. Introduce 50 ml of HCl (1+4) into separatory funnel; open stopcock to allow acid to flow into reaction flask, swirling the contents meanwhile. If necessary, apply pressure to effect complete transfer of acid from funnel. Close stopcock and continue the swirling 5 min. at brief intervals. Apply heat; continue the swirling until boiling starts and then regulate flame to maintain active boiling, altho not too vigorous, thruout 10 min. Keep the contents of the HCl vapor-trap near the boiling point thruout dissolution of the slag, then snap off the rubber tubing at the $CdSO_4$ intake quickly, taking care not to allow a backing-up of the contents. Cut off heat, discard contents of the evolution flask and of the scrubber tube, and set up apparatus for the next determination.

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

4. COSMETICS

(1) The following method was adopted as official, first action.

PYROGALLOL IN HAIR DYES

Qualitative test

Add 5–10 ml of sample to separatory funnel containing ca 0.5 g of $NaHSO_3$ and extract with 2 or 3 successive 30-ml volumes of ether. Filter ether extracts thru cotton and evaporate to dryness on steam bath. Dry in oven at 100°C for 30–60 min. Pulverize residue, mix well, and take melting point. If it does not melt between 131° and 134°C, sublime and again take melting point, which should fall within above range. Mix small portion of residue with equal quantity of sublimed pyrogallol and determine the melting point; it should not change.

Quantitative Determination

REAGENTS

Ferrous Tartrate Reagent.—Dissolve 1.00 g of sodium potassium tartrate (Rochelle salt) and 0.200 g of $FeDO_4 \cdot 7\,H_2O$ in water and dilute to 100 ml in a volumetric flask. PREPARE FRESH DAILY.

Sodium Acetate Solutions.—Dissolve 15.00 g $NaC_2H_3O_2 \cdot 3H_2O$ in water, bring to room temp. and dilute to 100 ml.

Aluminum Cream—**34.19(b).**

Standardization.—To six 100 ml volumetric flasks add from buret 2.50, 5.00, 7.50, 10.00, 12.50, and 15.00 ml of standard pyrogallol solution (Reagent Grade, 0.2000 g/500 ml). Develop color as follows on not more than three standards at a time, and make readings within 15 min. after color is developed. Pipet into flasks 10 ml each of Na acetate soln and ferrous tartrate reagent, dilute to volume and mix. Using 1 cm or ½ inch cells, measure optical density of solutions with photometer or spectrophotometer at 540 mμ. With neutral wedge filter photometer, filter designated No. 56 (5.8 mm of Corning didymium #512, 2.0 mm of Jena VG 3, 2.0 mm of Jena BG 18, and 4.5 mm of Corning yellow shade yellow #351) is more suitable than filter No. 54. With filter photometers obtain zero point by reading "blank" soln containing 10 ml of Na acetate and ferrous tartrate reagents in 100 ml. Draw standard curve, plotting concentration of pyrogallol against photometer readings, on large-scale graph paper so that pyrogallol can be read to 0.01 mg. A straight line should be obtained between concentrations of 1 mg and 6 mg per 100 ml. With spectrophotometer use freshly prepared "blank" as reference soln. Draw standard curve as directed above, or, if straight line passing thru origin is obtained, the average value of k may be calculated from the formula

$$k = \frac{D}{c}$$

where k is extinction coefficient
D is measured optical density of solution
c is concentration of pyrogallol in mg per 100 ml

This value of k may be used to calculate the concentration of unknowns directly from optical density readings.

Liquid Dyes

Extract a convenient aliquot of sample (usually 10 ml are sufficient) by one of the following methods. In handling sample give it a minimum of exposure to air, as pyrogallol is readily oxidized.

1. *Continuous Extraction.*—Pipet sample aliquot into suitable continuous extractor containing ca 0.3 g of $NaHSO_3$. Extract with ether until pyrogallol is completely removed (3–7 hours, depending upon efficiency of extractor). Determine time required for each extractor under a certain set of conditions by extracting an aqueous soln of known pyrogallol content or by testing for complete extraction as follows: After the extraction is thought to be complete, remove flask containing ether and replace it with one containing fresh volume of ether and continue extraction for 30–60 min. Treat this extract as directed below, and use 50 ml aliquot of filtrate to develop color. Evaporate ether extract on steam bath to volume of 8–10 ml and continue evaporation at temp. not exceeding 40° until odor of ether is completely gone. Dissolve residue in 20 ml of water and wash completely in 100 ml volumetric flask. Dilute to volume and mix. (If the liquid sample contained chlorophyll, treat residue from ether extraction as described for residue obtained from evaporation of ethyl acetate extract in method for henna powder dyes, beginning, "Add ca 10 ml water to beaker and loosen the residue. . . . "). Filter thru dry paper and discard first 20 ml of filtrate. (If determination cannot be completed same day extraction is made, let ether extract stand overnight, preferably in refrigerator, before ether is evaporated. Do not let aqueous soln stand overnight.) Use suitable aliquots of filtrate to develop color as directed under "Standardization," beginning "Pipet

into flasks. . . . " If 5 ml aliquot contains more than 6 mg of pyrogallol, make suitable dilution in volumetric flask and use aliquots of diluted soln to develop color. For final calculation use average of results obtained on at least two aliquots of different sizes, preferably containing between 2 mg and 5.5 mg of pyrogallol. Calculate to g/100 ml in original sample.

2. *Extraction in Separatory Funnels.*—Pipet sample into 125 ml separatory funnel containing ca 0.3 g of $NaHSO_3$ and extract 6 times with ether. For each extraction use volume of ether equal to 3 or 4 times volume of sample and shake vigorously for one min. Filter ether extracts successively thru cotton wet with ether. (Six extractions carefully made will completely remove the pyrogallol. If desired, a 7th may be made and used to test for complete extraction as described under "Continuous Extraction.") Evaporate the combined ether extracts as directed under "Continuous Extraction.

Henna Powder Mixture

Weigh 0.9 to 1.1 of thoroughly mixed sample in paper extraction thimble. Cover sample with small piece of cotton and place thimble in Soxhlet extractor. If temperature and humidity conditions are such that water will condense on condenser, connect tube containing drying agent to outlet of condenser. Extract 5 hours with ethyl acetate having minimum purity of 99% (N.F. VIII or better quality). Boil at such rate that solvent siphons off 15 to 20 times per hour. If ethyl acetate extract is clear, evaporate to dryness as directed below. If extract contains any sediment, evaporate to ca 75 ml if necessary, cool to room temperature, and completely transfer to 110 ml glass-stoppered volumetric flask. Dilute to volume and mix. Filter thru dry paper, taking precautions to prevent evaporation of solvent. Pipet 100 ml of filtrate into 250 ml beaker and evaporate to volume of ca 5 ml on hot plate or steam bath. Continue evaporation to complete dryness at temperature not exceeding 40°. Add 10 ml water and loosen residue with stirring rod. Pour into 50 ml volumetric flask. Rinse beaker 4 or 5 times with small volumes of water and add rinsings to flask. Add 1.2 ml alumina cream, dilute to volume, mix and filter thru dry paper. Extract 25 ml by one of methods given under "Liquid Dyes." (If extraction cannot be started immediately add ca 0.4 g of $NaHSO_3$ to filtrate and hold no longer than overnight.) Calculate to per cent pyrogallol in original sample.

(2) The following methods were adopted as official, first action.

CONSTITUENTS OF FACE POWDERS

Fats and Fatty Acids as Stearic Acid

Weigh about 2 g of the powder into glass-stoppered 250-ml Erlenmeyer flask. Add 30 ml of benzene and swirl to mix thoroly. Add 10 ml of HCl, and swirl, removing stopper frequently to allow escape of CO_2 from carbonates. When pressure has spent itself, add 50 ml of petroleum ether, and shake cautiously with periodic removal of stopper until pressure again subsides. Then shake vigorously about 50 times. Decant ether layer thru a pledget of cotton into flask containing a few glass beads, that has been weighed with a similar flask as a counterpoise. (This decantation involves no danger of loss, for the particles of powder are tenaciously retained in the acid layer.) Again add 50 ml of petroleum ether and repeat shaking and decantation. Repeat with a third 50-ml portion of petroleum ether. Evaporate to dryness on steam bath under hood. Place in a draft oven at 100 degrees C. for one hour, heating flask used as a counterpoise at the same time. Remove flasks, cool, and weigh as stearic acid.

Total Zinc

REAGENTS

(a) *Wulfing's precipitant.*—Dissolve 80 g of finely ground ammonium carbonate in a mixture of 90 ml of NH_4OH and 375 ml of H_2O, and add 475 ml of 95% ethyl alcohol, which may or may not cause precipitation, depending on the temperature. Let any precipitate settle, and use supernatant soln.

(b) *Wash soln.*—Mix equal volumes of Wulfing's precipitant and 95% ethyl alcohol.

DETERMINATION

Weigh ca 2 g of the powder into a platinum dish and ignite to light gray ash at 600–650°C. Do not heat longer than necessary. With the aid of a wide-mouth funnel, transfer ash to a 500-ml glass-stoppered Erlenmeyer flask. Add 100 ml of Wulfing's precipitant in such manner as to wash down funnel. Stopper flask, and shake vigorously for 1 min., pausing from time to time to remove stopper and relieve pressure. Let sit overnight. Filter contents thru 12½ cm medium quantitative paper. With wash soln from a wash bottle, wash out flask, pouring washings thru filter; but make no attempt completely to transfer residue. Reserve flask for later determination of acid-soluble constituents. Wash residue on paper thoroly with wash soln. Determine zinc in filtrate as follows: Exactly neutralize to methyl red with HCl, add 200 ml of H_2O, and bring nearly to boiling on hot plate. Add 60 ml of 10% $(NH_4)_2HPO_4$, and continue to heat at just below boiling for 30 min. Remove and allow to cool slowly to room temp. Filter thru Gooch crucible that has been tared after ignition for 10 min. at full heat of Fisher burner. Wash with freshly prepared 1% soln of $(NH_4)_2HPO_4$, and finally with 50 ml of 50% alcohol. Discard filtrate. Place Gooch crucible in porcelain crucible of suitable size, and dry over low flame. Increase temp. and ignite at full heat to constant weight.

$Zn_2P_2O_7 \times 0.534$ equals ZnO.

(Talcum powders often contain boric acid. When present, it will accompany ZnO, and must be separated before precipitation of the zinc.)

Acid-Soluble Calcium

Place paper containing residue from the zinc separation in a platinum dish and burn off paper at below 650°C. Transfer to a 250-ml beaker. Use 100 ml of (1+9) HCl to wash residue out of flask used in zinc separation, adding washings to beaker. (If some residue still clings to inside of flask, tilt up at steep angle over beaker, and wash out with stream of water from wash bottle.) Stir thoroly, allow to sit for 10 min and filter thru medium quantitative paper. Disregard turbidity in filtrate, since this will be recovered in the next step. Wash residue on paper three times with distilled water. Place in a platinum dish not less than 6 cm in diam. nor less than 2 cm high, and hold pending addition of recovered acid-soluble Fe, Al, and $BaSO_4$. With NH_4OH, nearly neutralize the filtrate to methyl red. Add 200 mg of $(NH_4)_2SO_4$ and enough Br_2 water to destroy indicator and distinctly color soln. Boil free of bromine, add more methyl red, and while still nearly boiling add NH_4OH dropwise to the first distinct yellow, avoiding any excess. Let sit for ca 3 min., and filter thru a medium quantitative paper. Wash with hot 2% soln of NH_4Cl. Transfer paper and residue to the platinum dish containing acid-insoluble constituents. Determine calcium in filtrate according to 6.48, beginning with "heat to boiling. . . . "

Acid-Soluble Magnesium

Determine in filtrate from acid-soluble calcium as directed in 37.64 (p. 642).

$Mg_2P_2O_7 \times 0.3621$ equals MgO

Decomposition of Silicates; Solution of Titanium; Estimation of Barium Sulfate

APPARATUS

Air bath.—On a tripod over Fisher burner place clay triangle having a per side length of about 3 inches. In this triangle set a nickel or iron crucible of about 125 ml capacity, and on top of the crucible set a second clay triangle having a per side length of about 2½ inches. Purpose is to supply controlled radiated heat to platinum dish resting on the top triangle.

METHOD

Ash residues reserved in platinum dish (acid-insoluble portion and materials recovered prior to calcium precipitation) at below 650°C. Pulverize ash with flattened glass rod, and moisten with 4 ml of water. Add 4 ml of H_2SO_4, place under a hood, and fill dish to ca one-fourth of its depth with HF (48%). Evaporate on the air bath, swirling occasionally to mix contents, until only the H_2SO_4 appears to remain; then cautiously heat over the low flame of a Fisher burner to a pasty consistency. (Do not take to complete dryness.) Add 15 g of pulverized potassium pyrosulfate, and heat to melting. Continue heating, gradually raising temp. until a clear melt is obtained. This will be achieved only when the dish glows red-hot and the melt orange-red. Too rapid heating will cause spattering. Foaming will occur but is not to be feared. At the completion of fusion, the clarity of the melt may be marred by bubbles and possibly by a few flakes of K_2SO_4 produced by the high temp., but these may be disregarded if the melt is generally clear. Set dish aside on an asbestos board and allow to cool. Melt will normally crack away from dish during cooling. Dislodge melt into a 600-ml beaker, wash dish with successive portions of hot (1+19) H_2SO_4 until a volume of about 150 ml is obtained, and boil until the melt goes into soln. If present, $BaSO_4$ comes down at this point. In this event, let digest on a steam bath for one hour, allow to cool, dilute to about 400 ml, stir well, and allow to sit for at least two more hours. Filter thru the finest available quantitative paper, catching filtrate in a 500-ml volumetric flask. Wash thoroly three times with water. Transfer residue to a tared porcelain crucible, burn off paper at low temp., and ignite at dull red heat. Weigh as $BaSO_4$. (Residues amounting to less than 0.5% should not be counted as $BaSO_4$. Where they occur, they represent HF-resistant silicate or quartz originally present in the talc or kaolin.)

Total Titanium and Iron

APPARATUS

Jones reductor.—Take a 50-ml pinchcock buret (without pinchcock attachment), and with a long glass rod ram down into its constricted lower end a pledget of glass wool. Fill buret to about the 15 ml mark with 20 or 30-mesh amalgamated Zn. (Zn may be amalgamated by letting fall into 200 ml of H_2O containing 4 g of dissolved $HgCl_2$ and 10 ml of H_2SO_4. It should be washed several times with distilled H_2O by decantation before being put into buret.) Fit constricted lower end of buret with a 4-inch piece of thick-walled rubber tubing bearing a screw-clamp about the middle and terminating in a glass tube thrust thru a one-hole #7 rubber stopper. The stopper should be fitted to a 500-ml vacuum flask and the glass tube should be of such length as to reach within about 2 inches of the bottom of the flask. When not in use, the Jones reductor should be kept filled with distilled water.

DETERMINATION

Make filtrate from $BaSO_4$ to volume, pipet into a beaker an aliquot of 100 ml, and add with stirring 5 ml of H_2SO_4. Place in vacuum flask 10 ml of 10% ferric alum

(free of ferrous Fe and other substances reducing $KMnO_4$). Fit flask to reductor, apply vacuum, and open screw clamp enough to permit controlled passage of liquid into flask. When meniscus in buret has sunk nearly to level of zinc, add more soln. (It is preferable never to expose amalgamated zinc to the air.) When all of soln has been added, add in the same manner about 100 ml of distilled H_2O. Close screw clamp just before meniscus of last washing reaches level of zinc, release vacuum, and disconnect flask. Transfer contents to 300-ml tall-form beaker and add 3 ml of syrupy phosphoric acid. Using a 10-ml microburet, titrate over a white surface with 0.1 *N* $KMnO_4$ to the first pink. Make up a blank containing 3 g of potassium pyrosulfate and 6.5 ml of H_2SO_4 in 100 ml of distilled H_2O. Put this thru identically the same treatment the sample received, finally titrating to the same shade of pink. Subtract titre of blank from that of sample. Corrected titre $\times .008$ equals total (TiO_2 plus Fe_2O_3) (these have practically the same equivalent weight).

Total Iron

REAGENT

Titanium trichloride, 0.05 *N* $TiCl_3$.—Make up according to directions in **21.36** (p. 290), but containing only half as much $TiCl_3$ as required for the 0.1 *N* soln. Standardize according to either of the methods listed under **21.37** except that standardization should be conducted using an ordinary micro-buret and titrating into an open beaker. The soln should be kept in an ordinary glass-stoppered bottle and restandardized immediately before each set of determinations.

DETERMINATION

Pipet an aliquot of 100 ml from volumetric flask into a 150-ml beaker. Add 1 g NH_4CNS. Slowly and with thoro stirring, titrate with 0.05 *N* $TiCl_3$ from a microburet to disappearance of the red color. Run a blank on 3 g of potassium pyrosulfate and 6.5 ml H_2SO_4 in 100 ml of distilled H_2O. (Blank is often nil.) Corrected titer $\times .004$ equals Fe_2O_3.

Total Titanium

Per cent total (TiO_2 plus Fe_2O_3) − per cent total Fe_2O_3 equals per cent total TiO_2.

Total Oxides of Iron, Titanium, and Aluminum

Pipet an aliquot of 250 ml from volumetric flask into a 600-ml beaker. Add a few drops of methyl red indicator and 5 g of NH_4Cl, and bring to boil. Neutralize by adding NH_4OH dropwise just to the first distinct yellow. Let sit for about 3 min., and filter thru a 12½ cm medium quantitative paper. Wash several times with hot 2% NH_4Cl. Place paper in a tared crucible and dry in an oven or an air bath. Transfer to a muffle furnace at room temp., and raise heat to about 1100 degrees. Ignite to constant weight. Result is total (Al_2O_3 plus Fe_2O_3 plus TiO_2).

Total Aluminum

Per cent total (Al_2O_3 plus Fe_2O_3 plus TiO_2) − per cent total (Fe_2O_3 plus TiO_2) equals per cent total Al_2O_3.

Acid-Insoluble Calcium

Determine calcium in the filtrate from the ammonium hydroxide precipitate according to directions in **6.48** (p. 66), beginning with "heat to boiling. . . . "

Acid-Insoluble Magnesium

Determine in filtrate from acid-insoluble calcium by **37.64** (p. 642). $Mg_2P_2O_7 \times 0.3621$ equals MgO.

Silica

Weigh about 1 g of the powder into a 250-ml beaker. Moisten with alcohol and add 100 ml of (1+9) HCl. Stir, and allow to stand for 10 min. Filter thru 12½ cm medium quantitative paper. Wash the residue 3 times with H_2O. Transfer paper to a platinum crucible and ash at below 650°C. Cool, and pulverize ash with a flattened glass rod. Add 6 g of Na_2CO_3 a portion at a time, intimately mixing with the same glass rod between additions. Use the last of the Na_2CO_3 to sprinkle over the top of the mixture. Place in a muffle furnace at below 800°C., and raise temp. to bring contents into fusion. Heat at ca 1000°C. for 15 min. Remove the crucible and let cool. Dislodge the melt into a dry 500-ml beaker. (Dislodging the melt is not always easy. It often helps to return the crucible to the hot furnace for ½ min., then remove it and immediately dip about two-thirds of its length in a beaker of H_2O. If repeated a sufficient number of times, this treatment causes the melt to crack away from the platinum so that it can be removed by simply upending the crucible over the beaker.) In a graduate mix 15 ml of HNO_3 with 5 ml of H_2O, and wash the crucible with small successive portions of the mixture, adding washings to the beaker. If soln of the melt becomes slow, hasten its disintegration by gentle pressure with a glass rod. When the Na_2CO_3 in the melt has dissolved, place the beaker under a hood and add, in the order named, 5 g of NH_4Cl and 25 ml of $HClO_4$ (60%). Cover the beaker with a watch glass, and boil over a moderate flame until oxides of nitrogen have passed off and the $HClO_4$ refluxes down the sides of the beaker. Cool the mixture slightly, add 150 ml of very hot water, stir, and let sit until silica settles to the bottom. Decant supernatant liquid thru a 12½ cm medium quantitative paper, and transfer residue to paper using hot water and policing out beaker. Wash thoroly five times with hot water. Transfer to a platinum dish, burn off paper, and ignite to constant weight at about 1100°C. Weigh as crude silica. To the residue in the dish add ca 2 ml of (1+9) H_2SO_4 and enough HF (48%) to cover the silica. Heat on a steam bath under the hood until silica and excess HF have passed off. Cautiously heat over the non-reducing flame of a Fisher burner until fumes of SO_3 have ceased to be evolved, and then heat strongly for several min. Cool and reweigh. The difference between this weight and the weight of crude silica is weight of SiO_2.

Starch

Weight ca 5 g of the powder into a 500-ml Florence flask (preferably standard taper). Moisten with 10 ml of alcohol. Acid-wash according to directions in **17.20** (p. 212), hydrolyze starch as directed under **27.33** (but filter hydrolyzed mixture before and not after making to volume), and determine dextrose by **34.39** and **34.40** (p. 572).

5. ENZYMES

No additions, deletions, or other changes.

6. ECONOMIC POISONS

(Formerly Insecticides and Fungicides)

(1) The tentative methods for DDT, *This Journal*, **30**, 64–66 (1947) as amended, and *Ibid.*, **31**, 73 (1948) were adopted as official, first action.

(2) The tentative method for DDT, *This Journal*, **31**, 72 (1948) entitled "Total chloride in emulsions containing DDT, Solvent, Emulsifying Agent, and Water" was adopted as official, first action.

7. CAUSTIC POISONS

No additions, deletions, or other changes.

8. NAVAL STORES

No additions, deletions, or other changes.

9. GELATINE, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or other changes.

10. LEATHERS, AND 11. TANNING MATERIALS

The methods in Chapters 10 and 11 were deleted.

12. PLANTS

No additions, deletions, or other changes.

13. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

14. MALT BEVERAGES, SIRUPS, AND EXTRACTS, AND BREWING MATERIALS

(1) The tentative method for color determination of beer and wort, *This Journal*, **30,** 68 (1947), was deleted.

(2) The following method was adopted as official, first action.

DYE COLOR METHOD*

REAGENTS

(a) A dye color stock soln is prepared by dissolving 0.2636 g Amaranth #184, FD&C Red #2; 0.6948 g Tartrazine #640, FD&C Yellow No. 5, and 0.0487 g Patent Blue, V.F., Extra Concentrated, in 200 ml of distilled water. Add 200 ml of methanol and make up to 1 liter in a volumetric flask. Transfer the soln to an amber, glass-stoppered bottle and store in a dark place. Under these conditions the soln is stable indefinitely.

(b) *Mercuric chloride*, saturated soln (ca 80 g per liter).

METHOD I

To prepare the various Dye Color Reference Solutions, add by buret the volume of stock soln in ml corresponding to the Dye Color Value (Lovibond Equivalent) desired. For the range 1° to 5° dye color values in 0.25° intervals, use 1 ml, 1.25 ml, 1.5 ml, etc., each diluted to 500 ml in a volumetric flask. Before the final volume is reached, add 10 ml of mercuric chloride soln to each flask as a preservative. After thoro mixing, pour these dye color solns into properly labeled 4 oz. comparison bottles (Owens-Illinois #A-4906 is suitable type) and keep in a closed box or covered comparison rack.

METHOD II

This alternate procedure may be used to advantage in most instances.

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* The complete apparatus, stock soln, bottles, and dyes can be obtained from the Fisher Scientific Co 2109 Locust St., St. Louis 3, Mo. The dyes can also be obtained from American Society of Brewing Chemists, 64 E. Lake St., Chicago, Ill. For complete description of the method and apparatus, see *This Journal* 29, 287 (1947).

By means of the buret, add 12 ml of the concentrated stock soln to a 1-liter volumetric flask and add distilled water to volume. This produces a 6° dye color stock soln which is further diluted as follows to make up the necessary lower values.

	°*Dye Color*								
Dye Color Reference Solutions.....	1,	1¼,	1½,	1¾,	2,	2¼,	2½,	2¾,	3,
Vol. (ml) 6° Stock soln/120 ml.....	20,	25,	30,	35,	40,	45,	50,	55,	60,
Dye Color Reference Solutions.....	3¼,	3½,	3¾,	4,	4¼,	4½,	4¾,	5	
Vol. (ml) 6° Stock soln,120 ml.....	65,	70,	75,	80,	85,	90,	95,	100	

(For the color of beer, the first four values are usually not required. It is customary, therefore, for beer color sets, to prepare the colors from 2° to 5°, instead of beginning with the 1° soln.)

Add, by buret, the above indicated volumes of 6° stock soln, to labeled 4 oz. comparison bottles. Add 5 ml of saturated mercuric chloride soln to each and make up to 120 ml by adding distilled water in appropriate volume from a buret. For example, the 3° dye color soln contains 60 ml of 6° stock soln, 5 ml of mercuric chloride soln, and 55 ml of distilled water. The finished dye color solns are kept in a closed box.

DETERMINATION

Prepare the sample by filtering thru Reeve Angel #202, 15 cm paper using a small amount of filter-cel. Occasionally a double filtration is necessary. Filtration does not affect the color.

Pour the prepared sample into a comparison bottle and match with the Dye Color Reference Solutions against a source of constant, diffused light. For example, a white blotter illuminated by a frosted light bulb or fluorescent light will serve. Matching should be done by placing the sample between consecutive dye color bottles and viewing them thru the longer axis of the bottle. Repeat until the proper pair is found. It is often possible to interpolate a value between consecutive reference solns. Report the color in terms of degrees dye color, which is equivalent to °Lovibond, ½″ Cell, Series 52.

(3) The tentative method for total solids in yeast, **14.112–14.115**, incl. (p. 178) was adopted as official, first action, with the following revision of **14.114.**

SAMPLING

14.114

(a) *Collection of primary sample*—

(1) *Liquid yeast.*—From small tanks and tubs mix contents well to uniform consistency, taking care to blend in the heavier deposit on bottom of vessel. Take ca 500 ml as sample in cold glass container which will hold at least twice actual sample volume.

From larger tanks or those whose contents may not be mixed, take at least a 4-liter composite sample of separate aliquots taken from hose line as yeast is transferred, or by use of a trier device which will take samples from all levels in the tank. Mix this composite sample to homogeneity, then take the smaller sample from this, as above.

(2) *Pressed yeast.*—Remove portions from different parts of surface as well as center of cake and collect ca 150 g in 1000 ml beaker. Weigh to nearest 0.1 g. Prepare slurry by adding H_2O at rate of ca 3 parts of H_2O/1 part of pressed yeast. Again weigh to nearest 0.1 g. With stirring rod break up yeast portions and stir until completely uniform liquid suspension is obtained.

(b) *Care of sample.*—To prevent changes in analytical results due to autolysis and fermentations, proceed with examination of yeast samples immediately after the samples have been obtained.

(c) *Preparation of sample.*—Mix well and stir until complete uniform suspension is obtained. If lumps or particles of trub are present, pass the entire bulk of the primary sample thru sieve of ca 100 mesh. Make sure that all lumps and particles, including trub, are broken up and forced thru the sieve.

Remove, by scraping, any liquid or solids adhering to the sieve and reincorporate with the sieved sample. Mix well by stirring, then withdraw aliquot for separate determinations.

(4) The official Milos test for caramel, **14.35** (p. 158) was deleted, final action (first action, *This Journal*, **31**, 75 (1948)).

15. WINES

(1) The official Milos test for caramel **15.38** (p. 188) was deleted, final action (first action, *This Journal*, **31**, 75 (1948)).

(2) The official, first action, Mathers test for caramel, *This Journal*, 31, 76–77 (1948) was made official, final action.

16. DISTILLED LIQUORS

(1) The official modified Marsh test for caramel **16.39** (p. 202) was deleted, final action (first action, *This Journal*, **31**, 77 (1948)).

(2) The official Milos test for caramel **16.41** (p. 202), was deleted, final action (first action, *This Journal*, **31**, 77 (1948)).

(3) The official, first action for caramel, *This Journal*, 31, 77(1948), was made official, final action for distilled liquors.

(4) The following method was adopted as official, first action.

PROOF OF DISTILLED SPIRITS (RAPID METHOD)

Determine the apparent proof of the distilled spirits with an accurately standardized hydrometer, preferably one graduated in ½ degree in proof. Determine the extract (solids) according to section **16.8** and for every 100 mg extract add 0.4° proof to the apparent proof. NOTE: If the extract amounts to more than 600 mg this method does not apply.

(5) In the method for specific gravity of Cordials and Liqueurs, **16.45** (p. 203), "proceed as under **16.2**" was substituted for "*see* 14.3."

17. BAKING POWDERS AND BAKING CHEMICALS

(1) The tentative modified McGill method for starch, **17.21** (p. 212) was deleted.

(2) The tentative qualitative test for phosphoric acid, **17.31** (p. 214) was adopted as official, first action.

(3) The tentative method for residual carbon dioxide, *This Journal*, 31, 78 (1948), was adopted as official, first action.

(4) In **17.4** and **17.6** (pp. 208 and 209), HCl (1+2) was adopted as alternate for H_2SO_4 (1+5).

(5) The following method was adopted as official, first action.

RESIDUAL CARBON DIOXIDE

DRYING OVEN METHOD

Place 1.7 g of baking powder in clean, dry 250 ml wide-mouthed Soxhlet extrac-

tion flask (flask **A 17.6**). Tap flask to spread sample evenly on bottom. Add 10 ml of H_2O with a pipet. Stir with a glass rod to break up powder which may have caked on bottom of flask. Wash down stirring rod and sides of flask with 10 ml H_2O. Place the flask into an air drying oven set to maintain a temp. of 100°C. ±2°, on a shelf near the center of the oven and evaporate to dryness. After 5 hours remove from the oven, add 10 ml H_2O and cool to the same temp. as the air surrounding the Chittick apparatus. Determine CO_2 present in residue with Chittick apparatus **17.6**, using correction factors in **44.30**. Shake the flask vigorously until further shaking produces no increment in reading.

(6) The official method for available carbon dioxide **17.9** (p. 210) as revised, *This Journal*, **31**, 78 (1948) was changed to read "Subtract residual CO_2, **17.7**, from total CO_2, **17.3**; or subtract residual CO_2 by gasometric method, *This Journal*, **31**, 78 (1948), or by the drying oven method (see change No. 5, above) from total CO_2, **17.6**."

(7) The official gasometric method for residual carbon dioxide, **17.8** (p. 210) was deleted, final action.

18. COFFEE AND TEA

(1) The Fendler-Stüber modified method for caffeine in coffee, **18.15** (p. 217) was adopted as official, final action (first action, *This Journal*, **30**, 70 (1947)).

19. CACAO BEAN AND ITS PRODUCTS

(1) In the second paragraph of **19.16** (p. 227) of the tentative method for pectic acid, line 5, beginning "Neutralize" to the end of the paragraph, was deleted and the following substituted:

"Neutralize to litmus with NH_4OH (1+1) (ca 1 ml), then make slightly acid with acetic acid and add 50 ml of 2% NH_4 oxalate soln. Place power driven glass rod stirrer with a vertical loop at the end in the flask with the shaft thru tube inserted in a No. 10 rubber stopper. Place flask in water bath held at 90–92°C. and stir contents gently and continuously for three hours."

20. CEREAL FOODS

(1) The official, first action, methods for phosphorus in cereals and cereal products, *This Journal*, **31**, 79 and 80 (1948) were adopted as official, final action.

(2) The tentative method for acidity in flour and grain, **20.18–20.21**, incl. (pp. 241 and 242) and **20.76** (p. 260) were adopted as official, first action.

(3) The tentative method for benzoyl peroxide bleach in flour, **20.53** (p. 253) was deleted. (Method retained for rye flour.)

(4) The following method was adopted as official, first action.

BENZOIC ACID IN FLOUR (WHEAT)

QUALITATIVE TEST

Place 50 g of flour in (preferably) 500 ml glass-stoppered flask, add 30–40 glass beads (about 6 mm diam.), 0.1 g powdered iron, 100 ml ether (isopropyl ether, which is cheaper and less volatile, may be used). Allow to stand few min., shake with rotary

motion and add slowly (preferably dropwise) 2.5 ml HCl from Mohr pipet. Allow to stand overnight. Shake well with rotary motion, allow flour to settle a few minutes and decant thru Büchner funnel (100 mm) fitted with filter paper moistened with ether, into 500 ml suction flask. Add 50 ml ether, shake and allow to settle a few min., decant as before, repeat twice more, transferring the whole contents to funnel following last addition. Transfer thru large funnel into 250 ml separatory funnel, add 20 ml 5% $NaHCO_3$ soln., mix without too much vigorous shaking, and draw off the lower clear layer into 125 ml Erlenmeyer flask, repeat with one more 20 ml portion and two 10 ml portions of 5% $NaHCO_3$. Add to this soln 0.3 g Nuchar W, shake and filter (11 cm 589 white ribbon, S&S or equivalent) into 200 ml Erlenmeyer flask, wash flask, and filter with about 20–25 ml H_2O, using fine stream from wash bottle, add 2.0 ml H_2SO_4 (1+1) dropwise to avoid foaming out of flask. Swirl contents gently to reduce foaming. (The soln should be definitely acid to litmus paper.) Transfer to 125 ml separatory funnel, rinse flask with 12 ml ether and add to funnel, shake gently with frequent release of pressure due to ether and CO_2. (During first extraction with ether, it is preferable to release pressure after each shake, to avoid possibility of loss.) Repeat with two more 12 ml extractions with ether. Rinse flask each time with ether. After each extraction draw off aqueous soln into the same 200 ml Erlenmeyer flask and transfer ether to a Pyrex test tube (ca 50 ml capacity, 25 mm diam. and 150 mm length). Add 2 ml 10% NaOH, hold top of tube firmly against the palm of the hand and shake vigorously, insert piece of copper wire (1 mm diam. ×200 mm) into tube, evaporate the ether very slowly on steam bath. Remove copper wire. Then place tubes into beaker of boiling water and evaporate to nearly dryness, add slowly up to 0.5 ml 30% H_2O_2, followed by another 0.5 ml as soon as foam condition permits. (Minimum frothing is desirable to permit better contact for nitration.) Continue evaporation until there is no apparent moisture in tube. (The introduction of a gentle air blast into the tube hastens evaporation.) Add from Mohr pipet 4 ml of (1+1) mixture of H_2SO_4 and fuming nitric acid, taking care to have it wash down the sides of tube, heat 20 min. in gently boiling water bath (occasionally rotate or mix to ensure contact with nitrating mixture) immediately cool under tap to below room temperature, add 6 ml water while continuing to keep cool. Then *slowly* add 5 ml conc. NH_4OH by means of a Mohr pipet with continuous shaking under the tap to keep soln cool. Add 10 ml more of conc. NH_4OH keeping soln continuously cool. Add 2 ml of 6% hydroxylamine hydrochloride soln, stir, place in 65°C. water bath 5–6 min., stirring occasionally. (Temperature of bath should be a few degrees above, since the cold tubes cause some decrease.) Cool to room temp. under tap, filter immediately thru folded filter into similar tube and observe color of filtrate. Red or definitely pink color indicates the presence of benzoic acid.

Immediately transfer this soln (within 30 min.) to a 2 in. glass cell and read in a neutral wedge photometer, using No. 51 filter or in other equally precise instrument at wave length 510. Standardize the instrument by placing in test tubes 0.0, 0.4, 0.8, 1.0 and 1.2 mg benzoic in acetone soln (0.5 mg to 1 ml). Add 2 ml 10% NaOH, shake to mix well, and proceed as above beginning "place tubes into beaker of boiling water." Report individual results in p.p.m. of benzoic acid.

(5) The tentative method for fat and fat number of bread **20.86** (p. 261) was deleted.

(6) The following method was adopted as official, first action.

FAT AND FAT NUMBER OF BREAD

Slice one loaf of bread, and allow to dry overnight, or until sufficiently dry to grind. Grind bread to ca size of openings on 20-mesh sieve, mix, sample, and transfer 50 g to 600 ml beaker. Add 100 ml of H_2O and mix. Add 100 ml of HCl, mix, cover and heat on steam bath 1 hour, stirring well 6 or 7 times. Cool in cold (15° or less)

water bath, and stir. Add 10 g of Filter Cel, or other similar adsorbents, stir, and mix completely. Prepare 90 mm Büchner funnel as follows:

Place two No. 590 S&S 9 cm filter papers (or equivalent) in funnel and apply suction. Mix 10 g of Filter Cel with 50 ml of H_2O and rapidly pour mixture into funnel. (This should make a smooth even layer of Filter Cel over filter paper, with no cracks or openings.) Filter sample immediately. Rinse out beaker several times with ice cold H_2O. Just before filtration is complete, wash down sides of Büchner with ca 100 ml of ice-cold H_2O (or until clear filtrate comes thru). Up to this point do not allow pad to suck dry. Continue with suction until Filter Cel pad seems dry. Transfer this mass, without filter paper, from Büchner to original beaker. Break up mass with rod, dry (overnight) on steam bath and then heat in oven at 100° ca ½ hour to remove all moisture (material must be dry or fat results will be low). Break up any lumps. Prepare large Knorr extraction tube of ca 200 ml capacity (glass tubing 5 cm in diameter with height of 12 cm from shoulder to top of tube). Pack tube with asbestos tamped tightly to form pad ca ⅜" thick. Insert stem of tube into 2-holed rubber stopper in filtering bell jar connected to suction thru 2-way stopcock. Place 500 ml Erlenmeyer flask within bell jar so that stem of tube passes thru neck of flask. To cool beaker and contents, add 100 ml of mixed ether and petroleum benzine (1+1) and macerate against sides of beaker with medium-sized stiff metal spatula 3–4 min. Decant into extraction tube. Suck dry. Add to beaker 80 ml of the mixed ethers. Work as before 2 min. Transfer contents of beaker to extraction tube, suck dry, and tamp with flattened stirring rod until all ether is removed. To material in tube add 80 ml of the mixed ethers that have just previously been used to rinse out beaker, mix thoroly with stirring rod a few min., allow to stand a min., then suck dry, and tamp material as before. Make two additional extractions, turning suction on and off carefully to avoid loss of sample in Erlenmeyer flask. Transfer to 1 liter beaker. Evaporate on steam bath, completely transfer fat with small amounts of petroleum benzine to tared 150 ml beaker, carefully evaporate benzine on steam bath, dry at 100° to constant weight (ca 30 min.), cool, and weigh. Calculate percentage of total fat on moisture-free basis.

Weigh duplicate samples of 1 g (within ±.03 g) of fat into 300 ml Florence flasks, add 4 ml of glycerol-soda solution, **31.28(c)**. Heat the flask carefully over asbestos gauze until bubbles start to appear, then hold the flask about an inch over the heated gauze until cloudiness or turbidity disappears, and a perfectly clear mixture is obtained. After the mixture first becomes clear, ½ or 1 min. more gentle heating insures complete saponification. Cool, add few pieces of previously ignited pumice stone, 138 ml of CO_2-free H_2O, and 3 ml of H_2SO_4 (1+4), and proceed as directed in **31.29**, using same apparatus. Use 0.02 *N* NaOH for titration. Multiply ml of 0.02 *N* NaOH used by 1.1 and divide by weight of fat used. Run blank determination and make correction. Report number of ml 0.02 *N* NaOH per 1 g of fat as "fat number."

(7) The following method was adopted as official, first action.

PROTEOLYTIC ACTIVITY OF FLOUR AND MALTED WHEAT FLOUR

(Applicable to slightly active materials such as patent flour or to diluted extracts of active proteolytic preparations.)

REAGENTS

(a) *Buffer stock soln.*—Make 120 ml of acetic acid and 164 g anhydrous sodium acetate up to 1000 ml with H_2O. Dilute 1:20 before using (*p*H 4.7).

(b) *Bacto-hemoglobin substrate.**

(c) *Trichloroacetic acid solns:*

* A suitable quality is obtainable from the Difco Laboratories, Detroit, Mich.

Soln (1).—Dissolve 36 g trichloroacetic acid in 64 ml water. Use 5 ml aliquot.

Soln (2).—Dissolve 36 g trichloroacetic acid in 44 ml water. Use 4 ml aliquot.

(d) *Kjeldahl solns.*—Including 0.0714 *N* sodium hydroxide.

(e) *Pumice or fine sand.*

METHOD

Preparation of enzyme solutions.—For slightly active materials such as flour, weigh as much as 10 g directly into digestion flasks. For active enzyme preparations prepare a water extract or suspension immediately preceding digestion. (The amount of extract or dilutions thereof used in the digestion mixture may vary up to 1 ml. Appropriate activation technics may be applied to the enzyme extract.)

Digestion procedure.—Weigh 0.625 g (moisture-free basis) of Bacto-hemoglobin into each 125 ml Erlenmeyer flask and add ca 3 g of finely divided pumice. Add 5 g sample of flour to each of two flasks and agitate the mixture by rotation until flour and substrate are intimately mixed. Then add 25 ml of reagent (a) previously warmed to 40° (in thermostat-controlled bath within ±0.1°C.) to each flask and agitate its contents to insure uniform suspension. Place the tightly stoppered flasks in a constant temp. (40°) bath and agitate either continuously or at hourly intervals.

Add a 5 ml portion of trichloroacetic acid soln **c(1)** to one flask of each pair at the end of 15 min. digestion and to the second flask of the pair after 5 hours of digestion. Mix contents thoroly and allow flasks to remain in the bath at 40°C. for exactly 30 min. Centrifuge the suspension for 5 min. at 1800 r.p.m. and filter.* Pipet duplicate 5 ml aliquots directly into Kjeldahl flasks and determine soluble nitrogen.

Follow essentially the same procedure in determining the enzyme activity of an extract. In place of the solid material, use a total of 1 ml of extract or extract plus water. After zero time and 5 hour digestion periods, add to each flask a 4 ml aliquot of trichloroacetic acid soln **c(2)**. Thoroly mix the contents, allow to remain in the water bath for exactly 30 min. and filter without centrifuging; analyze 5 ml aliquots for soluble nitrogen.

Soluble nitrogen.—Proceed as under **2.24, 2.25,** or **2.26.** Use a definite volume of water (350 ml) to dilute the cooled digest and add in such a way as to wash down all the trichloroacetic acid which has condensed in the neck of the flask during the digestion process. Also add the concentrated alkali (one and one-half times the usual quantity) in such a manner as to lave the neck of the flask. After distillation, back-titrate the unneutralized standard acid with 0.0714 *N* sodium hydroxide.

Expression of proteolytic activity.—Proteolytic activity is measured by the difference in back-titration volumes for the 15-min. or zero time digestions and the corresponding 5 hour digestion, expressed in ml of 0.0714 *N* sodium hydroxide. This difference may be translated into mg of soluble nitrogen released from a given weight of the enzyme source.

(8) The tentative method for moisture of soybean flour, **20.77** was changed to read "Proceed as under **20.4,** using a 5 g sample and drying at 130° for 2 hours," and adopted as official, first action.

(9) The tentative methods for ash **20.78,** nitrogen **27.79,** and oil or petroleum benzine extract **20.82** of soybean flour, as revised, *This Journal,* **31,** 81 (1948), was adopted as official, first action.

(10) The following method was adopted as official, first action.

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* Some materials such as flour may remain turbid after the final filtration. Such turbidity may be removed by boiling the centrifuged digestion mixture for a few seconds prior to final filtration. The liquid lost through evaporation should be replaced by the addition of water.

ADDED INORGANIC MATERIAL IN PHOSPHATED FLOUR

QUALITATIVE TEST

Transfer 20 g of flour to dry 250 ml separator, add ca 200 ml carbon tetrachloride, shake well, let stand until solution at bottom is nearly clear, usually ca 15 min. Draw off sediment with a minimum of solution, by turning the stop-cock quickly from side to side, into dry 125 ml separator containing about 100 ml carbon tetrachloride. Again shake 250 ml separator, let stand, with occasional gentle swirling, if necessary, to dislodge sediment from separator sides, until lower portion of soln has cleared. Draw off sediment from 125 ml separator into prepared and weighed Gooch crucible, using suction. Draw off sediment from 250 ml separator into 125 ml separator as before, and let stand with occasional gentle swirling to dislodge sediment from the sides of the separator. . . . When lower portion of liquid has cleared, draw off into Gooch crucible as before. (Care must be taken that no sediment remains on ledge in separator.) Wash the crucible and contents with 25 ml fresh carbon tetrachloride, continue aspirating 2 or 3 min. and weigh at once. Ignite crucible containing the sediment obtained above at 700°C., cool,and weigh as calcium metaphosphate. Weight of calcium metaphosphate, ca $(PO_3)2 \times 1.27 \times 5$ = Percentage of monocalcium phosphate monohydrate, ca $(H_2PO_4)2 \cdot H_2O$ in the flour.

(11) The tentative methods for separation of proteins, **20.32-20.38**, and the tentative method for detection of rye flour in wheat flour, **20.60**, were dropped.

21. COLORING MATTERS

(1) The tentative rapid method for tartrazine, **20.125** (p. 268) as revised and adopted as official, first action, *This Journal*, **31**. 82 (1948), was adopted as official, final action.

(2) The following method was adopted as official, first action.

LEAD IN COAL-TAR COLORS

(Not containing calcium, barium, or strontium.)

REAGENTS

(a) *Standard lead soln.—Methods of Analysis, A.O.A.C.*, Sixth Edition, **29.36(a)**.

(b) *Nitric acid soln, 1%.—Ibid.*, **29.36(b)**.

(c) *Citric acid soln, 50%.—(Special grade—low in lead) Ibid.*, **29.36(d)**.

(d) *Diphenylthiocarbazone (dithizone) soln).*—Stock soln of purified dithizone containing (100 mg. per ml.) chloroform. A working soln containing 20 mg. per liter. *Ibid.*, **29.36(e)**.

(e) *"Stripping" reagent.—Ibid.*, **29.36(f)**.

(f) *Potassium iodide soln, 2%.—Ibid.*, **29.36(g)**.

(g) *Starch soln, 0.5%.*—Weigh 1 gm of soluble starch. Make into a thin paste with several ml of cold water, pour into 200 ml of hot water, and while still hot add 2–3 small crystals of HgI_2 as preservative.

(h) *Sodium thiosulfate soln.*—Make a stock 0.1 *N* $Na_2S_2O_3$ soln. Use 5 ml isoamyl alcohol per liter as preservative. Prepare daily a fresh 0.001 *N* dilution and standardize against standard lead solution.

(i) *Potassium cyanide soln, 10%.*—Dissolve 50 gm. phosphate-free KCN in distilled water and dilute to 500 ml.

(j) *Hydroxylamine hydrochloride soln, 10%.*—Dissolve 10 gm $NH_2OH \cdot HCl$ in 20 ml of water and make slightly alkaline with ammonia. Extract lead with dithizone. Remove excess dithizone with chloroform and boil off any $CHCl_3$ remaining in the aqueous phase. Acidify with HCl and dilute to 100 ml.

(k) *Thymol blue indicator soln, 0.1%.*—Dissolve 0.1 gm thymol blue in distilled water, add sufficient 0.1 *N* NaOH to change the dye to blue and dilute to 100 ml.

DETERMINATION

Transfer a 5.00 gm sample to a 500 ml Kjeldahl flask, add 10 ml concentrated H_2SO_4 and 10 ml concentrated HNO_3, and heat. When evolution of SO_3 fumes begins add 5 ml of concentrated HNO_3 and heat until SO_3 is again evolved. Repeat the additions of concentrated HNO_3 each time SO_3 fumes appear until the dye is completely in solution and the digest is yellow. Then add 10 ml of 1:1 mixture of concentrated HNO_3 and 60–70% $HClO_4$; continue heating until the wet ash is colorless or pale yellow and the bulk of the H_2SO_4 is evaporated.

Cool the flask under running water and neutralize the soln by additions of small portions of concentrated NH_4OH. Add 20 ml of the citric acid soln and then adjust to *p*H 8.5–9 with NH_4OH using four drops of thymol blue indicator. Add 5 ml of 10% KCN soln.

Transfer the alkaline soln to a 250 ml separatory funnel. Extract the lead with a 20 ml portion of dithizone solution containing 20 mg per liter. (*Note:* If there is sufficient iron present to cause excessive oxidation of the dithizone as indicated by a yellow color in the $CHCl_3$ layer, 10 ml of 10% $NH_2OH \cdot HCl$ should be added to reduce the iron.) Allow the chloroform layer to settle and draw off into another separatory funnel. Wash down the floating globule of chloroform with two successive 5 ml portions of weak dithizone (4 mg per liter) and add to the receiving funnel. Repeat the extractions with the stronger dithizone until no more of the red lead dithizonate is observed. Do two more extractions with 10 ml portions of the weaker dithizone soln.

Wash the chloroform extract with 25–30 ml of distilled water containing one drop of concentrated NH_4OH. Draw off cleanly the washed chloroform layer into a third separatory funnel. Add 110 ml. of 1% HNO_3 and shake for one minute. Draw off and discard the chloroform and about 1 ml of the acid layer. Insert a cotton plug into the stem of the funnel to filter the acid layer as it is withdrawn. Discard the first 3 ml of the filtrate. Electrolyze a 100 ml aliquot of the filtrate as directed in 29.41, *Methods of Analysis, A.O.A.C.*

(4) The following methods were adopted as official, first action.

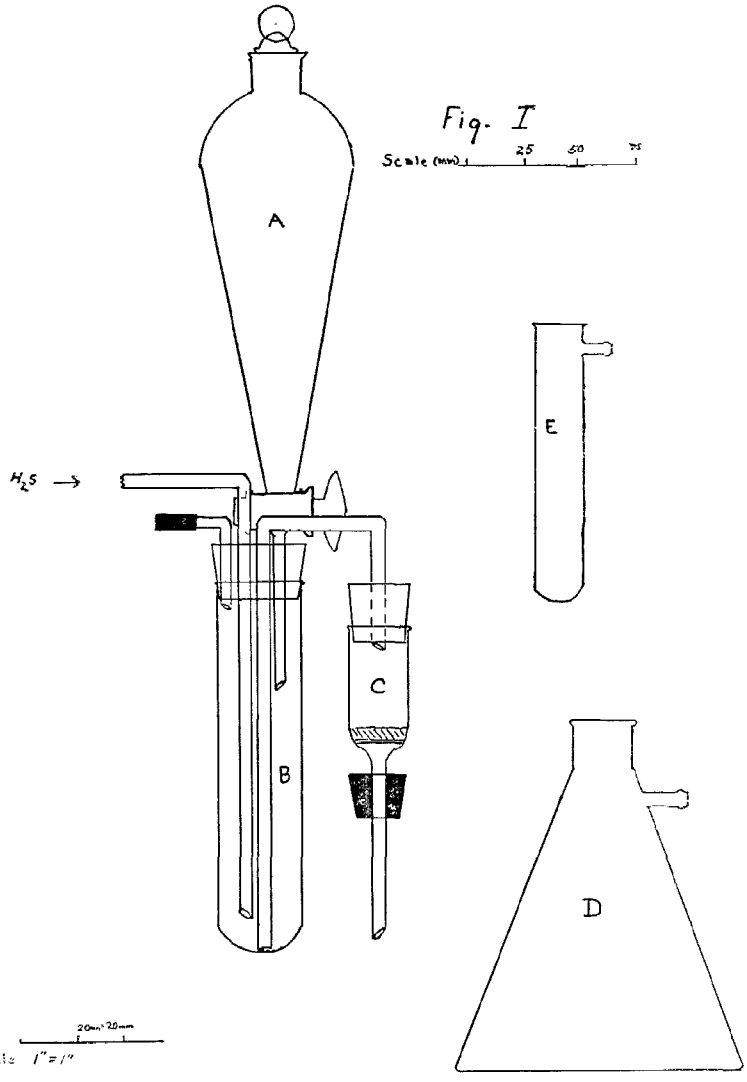
LEAD IN LAKES (ALUMINUM) OF COAL-TAR COLORS

REAGENTS

All reagents should be lead free (**29.36**). Any convenient source of H_2S may be used. The gas should be scrubbed first with (1:1) H_2SO_4 (v/v), then with distilled water, before being passed into the solution.

METHOD

Weigh 2 g of the sample into a 500 ml Kjeldahl digestion flask, add 10 ml of conc. H_2SO_4 and 10 ml of conc. HNO_3, and digest on a low flame until SO_3 fumes appear. Add successive 5 ml portions of conc. HNO_3 (waiting until SO_3 fumes appear before adding each succeeding portion) until all organic matter is in solution. Slowly introduce 5–10 ml of a (1:1) mixture of conc. HNO_3 and 60–70% $HClO_4$, and continue the digestion until the white ppt. formed showed the first signs of spattering. Allow the flask to cool and cautiously add 5 ml of H_2O and then a few drops of conc. NH_4OH. Swirl the flask vigorously and cool under running water. Add 20 ml of 50% (w/v) citric acid soln and adjust the *p*H to 3.0–3.4 (bromophenol blue) with conc. NH_4OH. Add 1 ml of $CuSO_4$ soln containing 1 mg Cu per ml and transfer the soln to the pptn tube (B) of the sulfiding apparatus. (See Fig. I) Bubble H_2S thru the soln at a



rate of ca 2 bubbles per sec. for 3–5 min. and filter the resulting suspension thru (C) at a rate of ca 1 drop per sec. When filtration is complete remove the receiver containing the filtrate and attach a suction test tube as shown in (E). Add 3 ml of hot conc. HNO_3 thru the separatory funnel (A) and draw thru the filter, followed with 2 ml of hot water. Detach the filter and pass an additional 3 ml of hot conc. HNO_3 thru the filter, wetting all sides. Again follow with 2 ml of hot water. If the filter is still colored with PbS, wash again with hot conc. HNO_3 and water. Wash the dissolved sulfides into the pptn tube (B), wetting all sides to take up any residual lead sulfide and then into a 50–100 ml glass-stoppered conical flask. Stopper and shake for a few sec., then remove the stopper and boil until the soln clears, to remove the last traces of H_2S and to coagulate any free sulfur present.

Transfer the soln to a 250 ml separatory funnel. Wash the flask with two 5 ml portions of distilled water and add the washings to the main soln. Add 10 ml of 50% (w/v) citric acid soln, 5 ml of 10% sodium cyanide soln, a few drops of hydroxylamine hydrochloride soln to prevent oxidation of the dithizone, adjust the *p*H to 8.5–9.5 (thymol blue) with conc. NH_4OH and proceed with the dithizone extraction and electrolysis as directed, **29.39, 29.40, 29.41.**

LEAD IN LAKES (CALCIUM, BARIUM, AND STRONTIUM) OF COAL-TAR COLORS

REAGENTS

Sodium carbonate.—Lead free, analytical grade.
Potassium carbonate.—Lead free, analytical grade.
Sodium nitrate.—Lead free, analytical grade.
Sodium carbonate soln.—5% (w/v).
Hydrochloric acid.—(2:5) (v/v).

METHOD

Place 2 g of the lake, 4 g of Na_2CO_3, 6 g of K_2CO_3, and 0.5 g of $NaNO_2$ in a platinum crucible of suitable size. Mix thoroly. Heat carefully until the color is carbonized, then heat to about 850°C. and hold at that temp. for 15 min. If a controlled muffle furnace is available, it is only necessary to place the fusion mixture in the cold furnace and raise the temp. gradually to 850°C. over a two-hour period. Usually 15–30 min. heating at 850°C. is sufficient to complete the fusion.

When fusion is complete, allow the crucible and contents to cool below 100°C., then add 2 or 3 ml of water and heat over a low flame, using care to prevent spattering, until the contents can be separated from the crucible. Transfer the fused mixture to a 150 ml beaker with the aid of about 25 ml of hot water. Boil until the caked material is completely disintegrated, then filter thru a retentive filter paper. Wash the residue on the filter with two 15 ml portions of hot 5% Na_2CO_3 soln. Lead will be in both filtrate and residue. Transfer the filtrate to a separatory funnel and proceed to extract the lead from the filtrate as directed under aluminum lakes. Dissolve the residue on the filter in 10–20 ml of the hydrochloric acid soln, wash the filter with water and add washings to the soln. Boil the soln to expel carbon dioxide, then transfer to a separatory funnel and extract the lead as directed above. Combine with the chloroform extracts from the soluble portion of the fusion products and determine the total lead by the electrolytic method (**29.39, 29.40, 29.41**).

(5) The tentative method for pure coal-tar dye, **24.41** (p. 291), was adopted as official, first action, for pure dye in lakes of D&C Reds Nos. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 31, and 34, and Ext. D&C Red No. 2.

22. DAIRY PRODUCTS

(1) The statement in the method for fat in cheese, **22.130** (p. 337), beginning "Add ca 0.5 g sand" lines 4 and 5, was changed to read "Add a few glass beads or other inert material."

(2) The official sour serum method **22.29** (p. 312) was deleted, first action.

(3) The following method was adopted as official, first action.

WATER-INSOLUBLE ACIDS IN BUTTER AND CREAM

Sampling of butter.—Proceed as directed in *Methods of Analysis, A.O.A.C.*, 6th Ed., **22.107.**

Preparation of butter sample.—Proceed as directed in *Methods of Analysis*, 6th Ed., **22.108** or **22.109**. Weigh 50 g of the prepared sample into a 250 ml centrifuge bottle, add 10 ml of water, if necessary remelt in warm water bath (not steam bath), and add 50 ml of ether. Shake until fat is dissolved.

Preparation of cream sample.—Proceed as directed in *Methods of Analysis, A.O.A.C.*, 6th Ed., **22.60**. Weigh 50 g of the prepared sample into a 250 ml centrifuge bottle, add 20 ml of alcohol, shake, and add 50 ml of ether.

DETERMINATION

Add *N* NaOH in increments of about 0.2 ml to the material in the centrifuge bottle, neutralizing to a decided pink color, using 10 drops of a 1 per cent solution of phenolphthalein as indicator, shaking between additions of the alkali until neutralization is complete. Then add 0.5 ml in excess and shake again for at least 2 minutes. During this and all subsequent shakings release the pressure carefully several times to avoid blowing the stopper out and losing some of the contents. (It is difficult to shake more than one bottle at a time because of greasy stoppers and the pressure which develops.) After shaking remove the stopper and add 50 ml of petroleum ether, shake a few times and centrifuge for 5 minutes at about 1200 r.p.m. (longer if separation not sharp). Set the bottle on a horizontal surface and siphon[1] off the ether fat layer.[2] Wash the aqueous layer remaining in the centrifuge bottle by adding 25 ml of ether, mix thoroly by shaking for several seconds, add 25 ml of petroleum ether and again mix by shaking. Centrifuge, siphon off the ether layer as before, and repeat the washing as above. After each washing the basic red color of phenolphthalein should be permanent; if not, add additional phenolphthalein and alkali to give a decided red (not pink). Add 1 ml of sulfuric acid (1+1) to the residue in the centrifuge bottle and shake vigorously for a few seconds. With butter, add 5 ml and with cream, 10 ml of a 10 per cent sodium tungstate solution and again shake vigorously a few seconds. After the addition of the sodium tungstate, the material should be distinctly acid to congo red paper; if not, add more of the sulfuric acid. Now add 75 ml of ether, shake violently for at least 2 min. and centrifuge. (When working with cream, emulsions may form which do not break completely on centrifuging. These can be broken by adding 10 to 20 ml of alcohol, mixing gently and again centrifuging.) Siphon off the ether layer into a 500 ml separatory funnel. Wash the siphon inside and out with 75 ml of ether in such a manner that the washings drain into the centrifuge bottle, shake violently for at least 2 min., centrifuge and siphon off the ether layer into the separatory funnel. Slight opalescence of the ether layer may be disregarded. Add 100 ml of dilute alcohol (1+1) to the combined extracts in the separatory funnel, neutralize in same manner as before with *N* NaOH to a decided pink color, add 0.5 ml excess and shake violently for an additional 2 min. Immediately add 25 ml of water, mix by single inversion of the funnel, and allow to separate until the water layer is clear. This usually occurs in a few min. Slow separation may sometimes be hastened by playing a fine stream of water on the ether surface. If the volume of the emulsion at the interface is only about 10 ml it may be included in the subsequent extraction. Draw off the aqueous layer into a 600 ml beaker. Add 50 ml of the 1+1 alcohol and about 10 drops of phenolphthalein to the

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[1] The siphon is similar to the delivery tube of an ordinary wash bottle except the intake end is bent, in opposite direction to the outlet end, into a U shape, the opening being ¼ to ½ inch higher than the bottom of the U and cut off horizontally. Excessive constriction should be avoided in the bending. The delivery tube is set loosely enough in the stopper so that it can be raised and lowered. In operation it is so adjusted that the opening of the U bend is about ½ inch above the surface of the aqueous layer. The ether layer can then be blown off gently by means of the customary mouthpiece tube inserted in an adjacent hole in the stopper.

[2] If the ether layer, after centrifuging, is reddish in color, add 10 ml of water, shake and again centrifuge as before. If the reddish color still persists in the ether layer, add 25 ml of ethyl ether, shake and again centrifuge.

contents of the separatory funnel and neutralize with the alkali, shaking vigorously for about 2 min. Add 50 ml of water, mix by single inversion of the funnel and allow to separate until the water layer is clear. Draw off the aqueous layer into the beaker. Add 10 ml of water to the contents of the separatory funnel, mix by single inversion, allow to separate until the water layer is clear, and draw off into the beaker. Place the beaker containing the combined extract and washings on a steam bath (or carefully heat on a hot plate), in order to expel any ether. Evaporate to about 25 ml (a small fan is useful if foaming is serious). (The decided red color should persist thru all these operations and up to the point where the soaps are acidified.) Transfer to a 250 ml beaker with about 25 ml of water. (As an alternate procedure the material may be evaporated to dryness on a steam bath and the residue dissolved in about 50 ml of water.) Dissolve 5 g of anhydrous sodium sulfate in the warm solution, using heat if necessary. Cool to 20° or lower, stirring at frequent intervals in order to keep the soaps from forming a hard crust on the surface. Make acid by adding sulfuric acid (1+1) dropwise, using congo red paper as indicator. Stir vigorously to affect thoro liberation of the fatty acids, mashing all pink soap curds. Add about 500 mg of a filter aid and mix. Filter with suction into a suitable filter.[3] Rinse the beaker with 3 approximately 15 ml portions of water at 20° and transfer the rinsings to the crucible. Maintain suction *for several minutes after visible dripping has ceased,* in order to dry the precipitate. Heavy precipitates can be sucked drier if the cracks are plastered up with some of the precipitate. Filtrate should be clear. Substitute a tared beaker[4] or flask, containing a few glass beads or grains of sand, for the receiving flask of the filtering apparatus. Extract the acids with 4 portions (ca 15 ml each) of ether, breaking up the precipitate with a stirring rod between extractions and thoroly mixing with the ether. The asbestos pad must not be disturped. Evaporate the ether extract (which should be no more than faintly opalescent) on a steam bath and dry the acids in a 100°C oven for one hour. Cool and weigh. Report results as mg of water-insoluble acids (WIA) per 100 g of butterfat.

Dissolve the weighed acids in 10 ml of neutral benzene, and titrate with 0.1 *N* sodium ethylate, using 10 drops of phenolphthalein as indicator, until the end point holds at least 1 minute. (If desired, neutral alcohol and 0.1 *N* NaOH may be used.) Compute the mean molecular weight of the fatty acids by dividing the mg of acids found by the ml of 0.1 *N* alkali used for the titration, and multiplying by 10. The mean molecular weight should not exceed 290. When the amount of acids is below 150 mg per 100 g of butterfat, the mean molecular weight is without significance.

23. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

24. FISH AND OTHER MARINE PRODUCTS

(1) The following method was adopted as official, first action.

CRUDE FAT-ACID HYDROLYSIS

PREPARATION OF SAMPLE

Prepare the sample, according to the type of pack, as directed under par. 24.2 (*Methods of Analysis, A.O.A.C.*, 1945, 359) and keep ground material in a sealed jar. If the jar has been chilled, allow the sample to come to room temperature and shake

[3] The following set-up is convenient: a bell jar; a Gooch crucible, with removable bottom charged with a thin layer of asbestos overlaid with a small quantity of filter aid. The asbestos was a long fiber, amphibole variety, acid and alkali-washed for Gooch crucibles, and washed twice by decantation. The filter aid was Dicalcite Company's "Speedex" added from a suspension in water. Coarse fitted glass crucibles overlaid with a small quantity of filter aid are satisfactory.

[4] Weighed with similar vessel as counterpoise.

jar so that any separated liquid will be absorbed by the fish. Open jar and stir contents with spatula, thoroly contacting the sides and lid so as to incorporate any separated liquid or fat.

METHOD

Weigh into a 50 ml beaker 8 grams of the well mixed sample. Add 2 ml of HCl. Break up coagulated lumps with a stirring rod having an extra large flattened end and continue until a homogeneous mixture is obtained. Add an additional 6 ml of HCl, mix, cover with watch-glass and heat on the steam bath for 90 min. with an occasional stirring. Cool solution and transfer to Mojonnier fat extraction tube. Rinse beaker with 7 ml of ethanol, add to extraction tube and mix. Rinse beaker with 25 ml of ethyl ether in three portions and add to extraction tube, stopper with cork or stopper of synthetic rubber unaffected by usual fat solvents, and shake tube vigorously for one min. Add 25 ml of petroleum benzine (b.p. below 60°) to extraction tube and repeat vigorous shaking. Centrifuge Mojonnier flask 20 min. at ca 600 r.p.m and proceed as directed under par. **20.16** (*Methods of Analysis, A.O.A.C.*, 1945, 240) beginning "Draw off as much as possible of ether-fat soln."

Drying to constant weight takes ca 40 min. for fish. Long heating periods tend to increase the weight of the fat. If a centrifuge is not available, the extraction can generally be made by letting Mojonnier flasks stand until the upper liquid is practically clear, then swirling flask and again letting stand until clear. If a troublesome emulsion occurs, draw off as much of the ether-fat soln as possible after letting flask stand, add a ml or two of ethanol, swirl, and again allow mixture to separate.

25. FLAVORING EXTRACTS

No additions, deletions, or other changes.

26. FRUITS AND FRUIT PRODUCTS

(1) In the official gravimetric cobaltinitrite method for potassium the following was inserted in **26.18(a)** (p. 386) between "10 ml" and "Filter" line 2, "from a previously tested lot of the reagent giving recovery of not less than 98 and not more than 102% with 20 mg amounts of K_2O."

(2) In note (3) of **26.19** (p. 387) the last sentence beginning "If recoveries" was deleted.

(3) The following method was adopted as official, first action.

WATER-INSOLUBLE SOLIDS (RAPID METHOD)

APPARATUS

Waring Blendor (or other suitable comminuting device).

Balance (sensitive to ±1 milligram) and weights.

1-*liter suction flask* (provision for vacuum).

Büchner funnel (Coors #4 5¼″ diam.).

Filter paper.—15.0 cm fast; open texture (Whatman #4, or equivalent).

Weighing dishes.—Aluminum or tinned iron 5¼″ diam. ×¾″ high, with close-fitting cover (16 mm film holders obtainable from camera stores). (All dishes weigh approximately 40 g, tinned iron ca 85–90 g.)

Rapid drying device:

(*a*) Moisture Teller Model 271 T, manufactured by Harry W. Dietert Co., 9330 Roselawn Ave., Detroit 4, Mich., or

(*b*) Forced Draft Drying Oven operating at 100°C.

DETERMINATION

Fit a 15 cm circle of filter paper into a 12½ cm Büchner funnel, add ½ of a 7 cm circle of filter paper (to be used to wipe any insoluble solids from Büchner after filtration and washing the sample), wash with boiling water, apply suction, and dry, using moisture teller and pan. Transfer to weighing dish, cool and weigh in balance using a tare consisting of weighing dish and paper. (Approximate time of drying, 5 min. at 215°F. ±5°F.

Weigh 25 or 50 g of well mixed sample (Waring Blendor) to nearest .01 g, transfer the sample with hot water to a 400 ml beaker, adjust to approximately 200 ml with hot water, stir, and boil gently for a few minutes. Place prepared filter in Büchner, attach to suction flask but do not attach flask to suction line. Pour 50 to 100 ml of boiling water on filter and when a steady flow of water passes thru filter, transfer the sample to the filter, portionwise if necessary. Wash insoluble solids with boiling water and collect approximately 850 to 900 ml of filtrate. (In the washing operation keep the solids from forming a tight mat on the surface by portion-wise additions of the boiling water.) Apply suction after concluding the washing operation and aspirate thoroly. Transfer paper and water-insoluble solids to moisture teller pan, using extra piece of weighed filter paper to complete the transfer, and dry at 215°F. ±5°F. (approximately 15 min., depending on amount of water-insoluble solids). After drying, transfer sample to weighing dish, cool in desiccator, and weigh. Weight of water-insoluble solids + wt. of sample × 100 = % water-insoluble solids.

(4) The following method was adopted as official, first action.

SEEDS IN BERRY FRUITS

Prepare the sample by thoro mixing, using a Waring Blendor. Take 50 g ± .01 g of the sample, transfer with ca 500 ml of hot water to the mixing chamber of Waring Blendor and mix for 1–2 min. Transfer mixture to a 20-mesh screen and use additional hot water to transfer and wash the bare seeds. (Hot water from the tap is suitable for use in this procedure.) Transfer the seeds on the screen to a 70 mm aluminum dish, previously weighed, with close-fitting cover. (This is readily accomplished by transfer to a 7 cm Whatman #4 circle of filter paper in a Coors 2A Büchner funnel with suction. The paper is previously dried and weighed with the aluminum dish.) Dry at 100°C. in a forced draft oven for 30 min. and weigh. To determine average weight of one seed, count out and weigh separately several 100-unit lots. Report average weight of one seed in milligrams; number of seeds per 100 g of sample and after determination of the water-insoluble solids content of the sample, calculate and report the per cent of the total that is due to bare seeds and the per cent that is due to non-seed water-insoluble solids.

27. GRAIN AND STOCK FEEDS

(1) The tentative methods for calcium and phosphorus, *This Journal*, 31, 98 (1948), was adopted as official, first action.

(2) The tentative method for fat in fish meal, *This Journal*, 31, 98 (1948), was adopted as official, first action.

28. MEATS AND MEAT PRODUCTS

No additions, deletions, or other changes.

29. METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

No additions, deletions, or other changes.

30. NUTS AND NUT PRODUCTS

(1) The following methods were adopted as tentative.

Preservation of samples.—Preserve samples in glass top fruit jar or similar air-tight container at 5 to 10°C.

Preparation of sample:

(a) *Nuts in shell.*—Remove meats from shells, being careful to remove all particles of shell from meats. Prepare separated meats as in following.

(b) *Nut meats, shredded coconut, or small pieces.*—Grind not less than 250 g twice thru Enterprise No. 5 food chopper, equipped with revolving knife blade, and plate with holes about $\frac{1}{8}''$ diam. Other types of food choppers, graters, or comminuting devices that give a smooth homogeneous paste without loss of oil may be used. Mix sample well and store in air-tight glass container.

(c) *Nut butters and pastes.*—Transfer sample to container of convenient size and shape, warming semisolid products, and mix carefully with stiff bladed spatula or knife. Electric powered mixers or stirrers may be used instead of spatula or knife if the material is of the right consistency to give uniform mixture. Store sample in air-tight glass container.

Moisture:

Drying with heat.—Dry to constant weight at 95–100° under pressure not to exceed 100 mm of Hg (ca 5 hours), a quantity of the substance representing ca 2 g of dry material. Report loss of weight as moisture.

Crude Fat.

(a) *Direct method.*

Determination.—Large quantities of soluble carbohydrates may interfere with complete extraction of the fat. In such cases extract with H_2O before proceeding with the determination. Extract ca 2 g of sample, dried as directed under anhydrous ether (**27.24**) for 16 hours. Dry extract at temp. of boiling H_2O for 30 min., cool in desiccator, and weigh; continue at 30 min. intervals this alternate drying and weighing until weight is constant. One to 1.5 hours is usually required.

(b) *Indirect method.*—Determine moisture as directed under "Moisture"; then extract dried substance for 16 hours as directed under (a) above, and dry again. Report loss in weight as ether extract.

Crude protein.—Determine *N* as directed under **2.24, 2.25,** or **2.26** (if over 30% protein use **2.26** only), and multiply result by 6.25.

Crude fiber.—See **27.28, 27.29,** and **27.30.**

Ash.—See **34.9** or **34.10** if chlorides present.

Reducing sugars.—See **27.31.**

Sucrose.—See **27.32.**

Sodium chloride:

(a) *Open Carius method.*—Put 2 g of the prepared sample into 250 Erlenmeyer flask or beaker, and proceed as in **24.6,** line 3, beginning "Add."

(b) *With calcium acetate as fixative.*—To 2 g of prepared sample add and thoroly incorporate 10 ml of 10% acetate soln. Then begin with 3rd sentence of **24.7.**

Water-insoluble inorganic residue. See **42.24.**

Added coloring matters. See Chapter 21.

Metals, other elements, and residues. See Chapter 29.

Preservatives and artificial sweeteners. See Chapter 32.

(3) The following tentative methods were deleted: **30.1–30.10,** inclusive, and **30.13–30.17,** inclusive.

31. OILS, FATS, AND WAXES

(1) The modified tentative Bellier Test, **31.47–31.48,** (p. 508), was modified as follows and adopted as official, first action.

31.48 Line 1, after "0.92 g" add "or measure 1 ml."

Line 2, end the first sentence with word "joint"; introduce between "joint" and "add" the new sentence, "If the oil is measured, use a short Mohr pipet with fairly large opening at top, drain to lower mark, hold until meniscus stops rising in pipet, and drain to mark again."

32. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) The official, first action, method for quarternary ammonium compounds in commercial preservatives, *This Journal,* **31,** 105 (1948), table sirup, *ibid,* **31.** 108, bottled beverages containing fruit juices, *ibid.,* **31,** 106, and in beer, *ibid.,* **31,** 108, were adopted as official, final action.

(2) To the official, first action, method for monochloroacetic acid, *This Journal,* **31,** 104 (1948), the following was added and the method adopted as official, final action.

The following equally efficient means of extraction may be used: To 100 ml of sample add 3 ml of H_2SO_4 and shake in a separatory funnel with three equal volumes of ether. Unite the ether extracts and wash by shaking with two 30 ml portions of 1 *N* NaOH. Unite the two NaOH solutions and digest as above.

(3) The official method for monochloroacetic acid (above, No. 2) was adopted as official, first action, for carbonated beverages, and for beer and wine, with the addition "(Use the length of time found necessary for recovery of at least 95% when known quantities of monochloroacetic are extracted in the apparatus)" after "2–3 hours" line 4 of "determination."

(4) The following method was adopted as official, first action.

DETERMINATION OF MONOCHLOROACETIC ACID IN COMMERCIAL PRESERVATIVES

Prepare a dilution of the sample that will permit the measurement of a convenient aliquot containing 50–100 mg of monochloroacetic acid and determine in such aliquot as directed under the method for carbonated beverages.

(5) The following qualitative test for monochloroacetic acid in commercial preservatives was adopted as official, first action.

(A) BARIUM TEST

Dilute 4–5 ml of sample to 100 ml, add 6 ml of H_2SO_4 (1+1), and extract with an equal volume of ether in separatory funnel. In cases where emulsions form, extract in a continuous extractor for 1 hour. Transfer the ether extract to a separatory funnel, add a few drops of phenolphthalein indicator, 5 ml of ± 0.1 *N* $Ba(OH)_2$, and shake for 30 seconds. If the water layer takes on the pink color of phenolphthalein, transfer thru a filter paper to a small beaker. Add ± 0.05 *N* acetic acid until colorless and evaporate to 1–2 ml on the steam bath. Allow the remaining liquid to evaporate

spontaneously in the air and finally in a desiccator. If 5 ml of $Ba(OH)_2$ does not give a pink water layer, add 5 ml more before separating. Repeat the extraction with $Ba(OH)_2$ several times or until a pink soln is obtained, evaporating each barium soln in a separate beaker. Examine the crystals under the polarizing microscope.

Optical-Crystallographic Properties of Barium Monochloracetate

Barium monochloracetate monohydrate crystallizes from water in plates, many of which are hexagonal in habit and frequently forming in over-lapping layers. Even in material that has been finely powdered for microscopic examination, the pointed terminations of the plates, often in pairs, can be observed. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative on the more elongated plates. The plates invariably extinguish sharply with crossed nicols and therefore interference figures were not observed in convergent polarized light (crossed nicols). In view of the fact that the plates persistently lie in one orientation the significant refractive indices were determined by the statistical method, measuring the lowest and highest indices respectively on plates showing the maximum amount of double refraction. These two indices are therefore arbitrarily designated as n_α (the minimum value) and n_γ (the maximum value). The two significant refractive indices are: $n_\alpha = 1.582$ and $n_\gamma = 1.611$, both ± 0.002, and frequently shown on the platy fragments.

(B) BARIUM-INDIGO TEST

REAGENTS

Anthranilic acid reagent.—Dissolve 1 g anthranilic acid in 40 ml of H_2O, add 0.3 g NaOH, and make up to 50 ml.

Caustic soda soln.—Dissolve 10 g NaOH in 10 ml H_2O and filter if necessary.

PROCEDURE

Dissolve 0.17 g of the barium salt in 5 ml H_2O in a 10 ml graduate, add 1.05 ml of 1.0 *N* H_2SO_4, make up to 10 ml, and mix. Let stand until the precipitate settles or filter if preferred. Pipet 3 ml of the clear liquid into small beaker, add 2 ml anthranilic acid reagent and 30 mg Na_2CO_3 (weighed). Test with litmus paper. If acid, add one additional 30 mg of Na_2CO_3. Pour into test tube and heat in water bath for ½ hr. Place the test tube in an oven at 125° ± 5°C. until only a moist residue remains. Remove from the oven, add 2 drops of caustic soda soln directly upon the residue. (If the residue is entirely dry, add 1–2 drops H_2O and let stand until absorbed by the residue before adding the strong NaOH), return to the oven until completely dry (at least 1 hour). Remove from the oven and heat the test tube at 310°–320°C.* until the contents assume an orange color. (This requires 15 seconds to 2 min., but must be carefully watched to remove from the heat as soon as the reaction is complete.) Cool slightly, add 5–7 ml H_2O from a wash bottle, splashing the water to incorporate air into it. Warm over a flame and blow air thru the soln 1–2 min. using a pipet or glass tube. Heat to boiling over the flame and again blow air thru the soln. (As the oxidation progresses, the soln turns red if monochloracetic acid is present, then green or blue or a combination of the two, and finally solid particles of indigo separate out. They have a tendency to rise to the surface at first.) Let the mixture stand about 10 min., then acidify slightly with HCl (1 + 1). After standing further for ½ hr. filter and wash the precipitated indigo with water to remove acid. Allow the paper to dry in the air and preserve as an exhibit.

* For the fusion at 310–320°C use a brass block having a well to contain the test tube and a second well to contain a thermometer. The block is wrapped with a coil of nicrome wire and the heat controlled by a variable voltage transformer. Analysts have used muffle furnaces, micro-burners, Wood's metal, or solder baths, etc., for the fusion with equal success.

(C) INDIGO TEST

Dilute 2 ml of sample to 100 ml, add 3 ml of H_2SO_4 and shake with 100 ml of ether. Add 3 ml of anthranilic acid reagent to the ether extract, evaporate at a low temp., filter off any insoluble matter and apply the indigo test as under (B) beginning "Test with litmus paper, etc."

(D) PYRIDINE TEST

Extract 2 ml of sample as under (C). Transfer the ether extract to a separatory funnel, add a small piece of a universal indicator paper and a sufficient amount of a satd soln of sodium bicarbonate (5 ml are usually sufficient) to the ether extract to make the aqueous layer alkaline (*p*H 7–8) after vigorous shaking. Add enough water to make the total volume of the aqueous layer ca 10 ml and shake again. Draw off the aqueous layer into a small separatory funnel and wash the ether with two successive 5 ml portions of water, also drawing off these wash solns into the small separatory funnel. Wash the combined extracts once with 5–10 ml of ether. Add ca 1 ml of sulfuric acid (1+1) in excess of the amount required to neutralize the alkaline soln (1.5 ml are usually sufficient) and extract the acidified soln with two 25 ml portions of ether. Wash the combined ether extracts once with 1 or 2 ml of water and allow the ether soln to stand a few minutes after drawing off most of the water and swirling to get as complete separation of water from ether as possible. Pour the ether thru a folded filter paper into a 200 ml flask. Wash the separatory funnel and filter paper with two 10-ml portions of ether.

To the ether filtrate add 0.5 ml of pyridine, a small glass bead, mix, and place on a steam bath to remove the ether. Evaporate to a volume of 2 or 3 ml and transfer immediately by means of an eye dropper pipet to a 15 ml centrifuge tube, washing the flask successively with 2, 1, and 1 ml portions of ether. Using the evaporation apparatus, reduce the volume of liquid in the tube to ca 0.3 ml.

Add enough pyridine to increase the volume to ca 0.5 ml (mark on tube is helpful) and place in a constant temperature bath at 60 ± 2°C.

If crystals appear during the heating period the test is positive, and the test need not be continued. If crystals do not appear remove test tube from bath. Remove the excess pyridine by evaporation under reduced pressure using the evaporating apparatus. Placing the tube in a beaker of hot water facilitates the operation. When all of the liquid has been removed, add 0.5 ml of pyridine, mix well, centrifuge, and decant the supernatant liquid. Add ca 5 ml of ether, shake well, centrifuge, and decant. Add 1–3 ml of absolute alcohol (95% alcohol gives results 1–2% lower), depending upon the amount of precipitate, place the tube in a holder, and heat in a hot water or steam bath until the precipitate is dissolved, being careful to swirl the tube gently to avoid superheating and to boil the alcohol so slowly that no loss occurs. Cool in an ice bath, add ca 10 ml of ether, mix well, and allow to stand in the ice bath for about 5 min. Centrifuge, pour off the supernatant liquid, and wash the precipitate once with ca 5 ml of ether. If the tube now contains crystals of pyridine betaine the test is positive.

(6) The following qualitative tests for monochloroacetic acid were adopted as official, first action.

MONOCHLOROACETIC ACID IN CARBONATED BEVERAGES, ORANGE JUICE, AND WINE

QUALITATIVE TEST

Preparation of sample.—Acidify two 100 portions of sample with 3 ml of H_2SO_4 and extract, using either continuous extractors or separatory funnels (state which is used) and apply the indigo test to one extract and the pyridine test to the other.

INDIGO TEST

Add 3 ml of anthranilic acid reagent to the ether extract and evaporate at a low temp. If any insoluble matter (oily or solid) separates out, filter the remaining liquid thru a small wet filter paper into a 50 ml beaker. If no insolubles come out, transfer the residue to beaker, add 30 mg Na_2O_3 and proceed as directed under Barium-indigo test procedure (B) above, beginning "Test with litmus paper," etc.

PYRIDINE TEST

Proceed as under Pyridine test (D) above beginning "Transfer the ether extract."

(7) The tentative method for thiourea in orange juice, *This Journal*, 31, 100 (1948) was deleted and the following adopted as tentative.

THIOUREA IN ORANGES AND ORANGE JUICE

Rapid Oxidation Method

REAGENTS

(a) *Modified Grote.*—Dissolve 0.5 g of sodium nitroprusside in 10 ml of H_2O in 50 ml Erlenmeyer flask. Weigh out 0.5 g of hydroxylamine hydrochloride ($NH_2OH.HCl$) and 1 g of $NaHCO_3$. Mix the two solids quite uniformly in a small beaker or porcelain dish by gentle grinding with a small pestle or flattened glass rod, crushing any lumps in the material. Brush off the rod or pestle and transfer the mixed solid totally to the nitroprusside soln with the aid of a short-stemmed funnel and brush. Do not agitate the flask and allow it to stand until the evolution of CO_2 subsides to only a small evolution. Then swirl to dissolve any remaining $NaHCO_3$. When the evolution of CO_2 practically ceases, add 0.10 ml (11 small drops) of bromine. A second evolution of gas occurs. When agitation no longer produces effervescence, make to 25 ml volume with distilled water and filter. Test the reagent for its efficacy as follows: Dilute 2 ml as in (b), add 1 ml diluted reagent to 10 ml soln composed of 5 ml reagent (c) (diluted 10×), 5 ml H_2O, and 1 drop acetic acid. A strong blue color should develop in 5 min. (If it does not, new reagent (a) should be prepared and the test repeated). Allow to stand at room temp. for 5–10 hours to age the soln. (The soln should be a mahogany brown color. If it is of a greenish cast, it is not as effective a reagent and soon loses its value.) Preserve this stock soln in the refrigerator and it will keep for several weeks.

(b) *Dilute Grote's reagent.*—Dilute one volume of the above reagent with four volumes of water before use and take 1 ml of the diluted reagent for a determination. The diluted reagent will keep for a day.

(c) *Thiourea stock soln.*—Dissolve 100 mg of the pure chemical in water and dilute to 200 ml.

(d) *Citric acid-potassium citrate soln.*—Dissolve 1 g of citric acid (reagent) and 0.84 g of potassium citrate ($K_3C_6H_5O_7+H_2O$ U.S.P.) in water, and make to 100 ml.

(e) *Iodine soln.*—Standard 0.1 *N* soln in KI.

(j) *Sulfuric acid.*—Ca normal soln (0.98 to 1.02 *N*).

APPARATUS

Siphon.—Insert two bent glass tubes in a two-hole cork or stopper, one terminating just below the stopper and the other long enough to reach the bottom of a centrifuge bottle when the cork with tubes is inserted in the mouth of the bottle. Attach another glass tube to the outside end of the longer bent tube by means of a flexible rubber tube. The assembly is used to siphon the lower layer from a centrifuge bottle, and the rate of flow is controlled by squeezing the rubber connection.

Prepare a cap for the inner tube by boring a hole of the same diam. as the tube part way thru a small cork.

PREPARATION OF SAMPLE

Juice oranges in an ordinary reamer, strain out seeds and pulp, and mix well. Measure 125 ml into a 250 ml centrifuge bottle, add 70 ml of ethyl ether, and shake well for 1–2 min. Centrifuge the bottle and contents about 10 min. at 1800 r.p.m. and remove. Cap the short end of the siphon, insert it into the bottle, and lower it thru the top layer into the lower aqueous layer. Push off the cork cap with a glass rod. Lower the tube to the bottom of the bottle, push cork (which carries the siphon) into the mouth of the bottle, and blow in the short tube to start the flow of liquid. Carefully siphon off into a beaker as much of the lower layer as possible and control the rate of flow by squeezing on the rubber connection. Stop the flow when material from the center emulsion layer begins to enter the tube. Add a teaspoonful of Celite (filtercel) to the siphoned liquid in the beaker, stir well, and filter on a Büchner funnel (7–11 cm) with suction, using a Whatman No. 54 or 41 H filter paper. Warm the flask and contents to ca 36°C. (on steam bath) and again apply suction to remove ether.

Pipet 25 ml of the filtrate (clear or nearly so) into a clean 50 ml volumetric flask. In two similar flasks place 25 ml aliquots of citric acid K citrate soln (reagent (d)). To one flask add 2 ml of standard thiourea soln (reagent (c)); the other is a blank. To each of the three flasks, add 5 ml of normal sulfuric acid. Then add 0.1 *N* iodine soln slowly, with rotation, to each flask until the iodine color does not disappear and add 1 ml in excess. Allow the flasks (samples, standard, and blank) to stand 10 min. at room temp. Now add a soln of $NaHSO_3$ (2.5 g/liter) to the contents of the flasks until the iodine color disappears and add 3 or 4 drops in excess. Add gradually and slowly with swirling, 4 ml of 25% sodium acetate soln to each flask and make to volume with water and mix. Designate the oxidized diluted sample as solution "X."

DETERMINATION

Prepare two standards by placing respectively 5 and 10 ml portions of soln from the standard flask in test tubes. Make the first tube to 10 ml by adding 5 ml of liquid from blank soln (no thiourea). Place 10 ml portions of the blank soln and of the sample soln ("X") in two other test tubes. Pipet 1 ml of diluted Grote reagent (b) into each tube with shaking or stirring. Allow the tubes to stand for one hour at about 25°C., or for 10–15 min. in a bath at 45–50°C., to develop the color (blue). Read the developed color of the solns from each tube (sample blank and standards) in a neutral wedge photometer using a filter centered at 610 mμ and a 1-inch photometer cell. From the readings of the blank and standards construct a curve (linear) plotting photometer readings against p.p.m. of thiourea. The oxidized standard in the flask represents 20 p.p.m. (1 mg in 50 ml). A 10 ml aliquot therefore represents 20 p.p.m. and 5 ml corresponds to 10 p.p.m.

A slight correction on the sample color reading obtained as above is necessary because of the natural color present in "X" before addition of the thiourea (Grote) reagent. Obtain readings no the blank soln and the sample soln ("X") as contained in the volumetric flask, without added reagent, using the same photo cell. Subtract the difference between these readings (X − blank) from the sample reading with the thiourea (Grote) reagent. Obtain from the graph the thiourea (p.p.m.) corresponding to the corrected reading. Multiply this value by 2 to obtain the thiourea concentration in the original orange juice.

33. SPICES AND OTHER CONDIMENTS

(1) The official, first action, method for starch in mayonnaise and

salad dressing, *This Journal*, **31**, 108 (1948), was adopted as official, final action.

(2) The official, first action, method for starch in prepared mustard and mustard flour, *This Journal*, **30**, 75 (1947) as revised, *This Journal*, **31**, 108 (1948), was adopted as official, final action.

(3) The tentative method for permanganate oxidation number of vinegar, **33.91–33.92** (p. 554) was deleted and the following adopted as official, first action.

PERMANGANATE OXIDATION NUMBER

REAGENTS

(a) *Sulfuric acid soln.*—1 + 1.

(b) *Potassium permanganate soln.*—31 g per 1000 ml. Standardization is not necessary. Prepare soln according to **43.17**, p. 807.

(c) *Sodium thiosulfate*—0.5 *N*.—Accurately standardized against $K_2Cr_2O_7$. (**43.28**, p. 809) should be modified to correspond to the stronger $Na_2S_2O_3$. It has been found that the following quantities of reagents are satisfactory: 0.50 g $K_2Cr_2O_7$, 10 g KI, 10 ml conc. HCl, and 90 ml H_2O.

(d) *Potassium iodide soln.*—Dissolve 50 g of KI in 100 ml H_2O and filter. Do not use unless colorless.

DETERMINATION

Adjust vinegar to 4 g/100 ml acidity as acetic acid. Steam distil 50 ml of adjusted vinegar and collect 50 ml of distillate. (Distillation should be regulated so that ca 45 ml remain in distilling flask when 50 ml of distillate have been collected. All-glass apparatus is preferable; if not available, cork or rubber stoppers should be covered with Sn or Al foil. The apparatus used for determination of volatile fatty acids in fish products (**24.9**, p. 361) is very convenient. Keep distillate and reagents at 25°C.)

Transfer the 50 ml of distillate to 500 ml glass-stoppered Erlenmeyer flask. Add 10 ml of the H_2SO_4 soln and 25 ml of the $KMnO_4$ soln. The permanganate should be accurately measured, allowing the pipet to drain for a definite time. Hold at 25°C, preferably in a water bath, for exactly one hour. Then immediately add 20 ml of the KI soln and mix well. Titrate the liberated I with the 0.5 *N* $Na_2S_2O_3$.

Conduct a blank determination at the same time, using 50 ml of H_2O, 10 ml of the H_2SO_4 soln, and 25 ml of the $KMnO_4$ soln.

(4) The tentative method for gums in mayonnaise and French dressing, **33.57** (p. 548), was changed to substitute "50 ml" in line 3 of paragraph 2 for "1.5 oz.") and adopted as official, first action.

34. SUGARS AND SUGAR PRODUCTS

(1) The following method was adopted as official, first action.

LAC IN CANDIES

Place 50 g of candy in a 400 ml beaker. Add 50 ml of a mixture of benzol and absolute alcohol (50% by volume) and cover with a watch-glass. Place on the steam bath, heat to boiling, and simmer for a few minutes, stirring occasionally. Decant the liquid into a tared round glass dish of about 100 ml capacity, having a flat bottom about $2\frac{3}{4}$″ in diam. Repeat, using a similar mixture; and finally rinse twice with

two 25 ml portions of absolute alcohol, simmering and stirring each rinsing liquid. With moist sugar candy, avoid overheating to prevent pieces from sticking together.

Add each liquid to the glass dish previously placed over the steam to evaporate the alcohol-benzol mixture. Allow to remain on bath until alcohol is just removed, rotating the dish as it goes to dryness in order to spread the extract uniformly over the bottom surface. Avoid baking the shellac on the dish. If fat appears to be present wash with 3 15-ml portions of petroleum benzene, stirring, and warming. Decant thru a rapid filter.

Add 50 ml of a mixture of 25 ml isoamyl alcohol (B.P. 129–132) and 25 ml benzol, rinsing any solid matter off and filter back into dish. Heat on steam bath with stirring, cool somewhat, and transfer the soln with suspended matter to a suitable (125 ml) separatory funnel. Rinse the dish with 25 ml hot (about 60°) water, adding it to the funnel; shake well, and filter wash water if necessary. Repeat washing *two times* (or until washings are colorless) with water, rinsing the dish well around sides with the first portions of the liquid. Finally, filter the soln of the shellac into the tared dish, rinsing the separator and filters with a little absolute alcohol. Evaporate to dryness on the steam bath, rotating the dish on going to dryness to give a uniform film.

If much fat was extracted in original benzol extraction, wash the final shellac residue with 25 ml petroleum ether, warming and stirring. Decant, dry on steam bath and 100° oven, and weigh.

(2) The tentative method for unfermented reducing substances in molasses, *This Journal*, **31**, 109 (1948) was revised to change reagent (f) from 5 volumes of water to 3, and add after "time" line 6 under fermentation, "an incubator may be used and the flask left overnight," and adopted as official, first action.

(3) The tentative Ofner method for invert sugar in presence of sucrose, **34.47** and **34.48** (p. 575), was adopted as official, first action.

(4) The official refractometric method **34.8** (p. 558) was revised by addition of the following (first action): "In liquid products containing invert sugars, correct the per cent solids obtained from **44.7** by adding 0.022 for each per cent invert sugar present in the sample."

35. PROCESSED VEGETABLE PRODUCTS

No additions, deletions, or other changes.

36. VITAMINS

(1) The following changes were adopted in the tentative method for vitamin A in fish liver oils (pages 599–601) and the method adopted as official, first action:

36.2 Insert "U.S.P. ethyl" before the word "ether" and "anesthesia grade, free from peroxides" after the word "cans" in line 2.

36.3 Delete the word "ground" from end of line 2.

Delete "2 minutes" from line 7 and substitute "allow the mixture to stand (about 2 minutes) until separation is visibly complete, as determined by the absence of refraction streaming and the presence of distinct layers."

36.4 Change "0.4" line 4 to "0.398."

36.6 The density values in the table were corrected as follows:

T.	D.
0.7	2.16
7.0	1.16
11.0	0.959
26.5	0.577
27.5	0.561
29.5	0.530
32.5	0.488
55.5	0.256
63.0	0.201
71.5	0.146

(2) The tentative method for thiamine hydrochloride (vitamin B_1) **36.16–36.23** (p. 606) was adopted as official, first action.

(3) The tentative fermentation method for thiamine, **36.27–36.31** (p. 611–613), was adopted as official, first action.

(4) The 1st paragraph of the section on Preparation of assay solution, 36.25 (p. 610) of the fluorometric method for thiamine, official, first action, *This Journal*, **31**, 112 (1948), was deleted and the following adopted as official, first action.

PREPARATION OF ASSAY SOLUTION

A. EXTRACTION STEP

(a) *For dry or semidry materials that contain no appreciable amount of basic substances.*

(1) Add a volume of 0.1 *N* HCl or 0.1 *N* H_2SO_4 equal in vol. to 10–15 times the weight of the sample in g. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occur agitate vigorously so that all particles come in contact with the liquid, then wash down sides of flask with acid soln.

(2) Digest at 95°–100°C. in a steam bath or in boiling water with frequent mixing, 30 min.; or alternatively autoclave the mixture at 121°–123° for 30 min. Cool and adjust *p*H to 4–4.5 by addition of the 2 *N* $NaC_2H_3O_2$ soln, using brom-cresol green *p*H indicator on spot plate.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances.*

Add HCl soln or H_2SO_4 soln and adjust the mixture to ca *p*H 6.0. Add such an amount of H_2O that the total volume of liquid is equal in ml to 10–15 times the dry wt of the sample in g. Then add the equivalent of 1 ml to 10 *N* HCl or 10 *N* H_2SO_4 for each 100 ml of liquid and proceed as directed under **(a) (1).**

(c) *For liquid materials.*

Adjust the material to ca *p*H 6.0 with either HCl soln, H_2SO_4 soln, or NaOH soln and proceed as directed under **(b).**

B. ENZYME HYDROLYSIS STEP

To a 75-ml aliquot add 5 ml of the enzyme soln, mix, and incubate at 45–50° for 3 hours. Cool, centrifuge mixture until supernatant liquid is clear or practically so, and quantitatively transfer supernatant liquid to a 100 ml volumetric flask. Wash residue by centrifuging with 10 ml, then with 5 ml of 0.1 *N* H_2SO_4. Add washings to the supernatant liquid and dilute to 100 ml with H_2O.

(5) In 36.26 (p. 610) line 8 "(2 seconds)" was changed to "(in 1–2 seconds)."

(6) The tentative method for riboflavin, *This Journal*, **30**, 79 (1947), was deleted and the following adopted as official, first action.

RIBOFLAVIN (VITAMIN B_2)[1]

Microbiological Method

Throughout all stages of the procedure, except where otherwise directed, protect the solns from light that destroys riboflavin.

REAGENTS

(a) *Standard riboflavin stock soln.* I.—Dissolve 50 mg of U.S.P. Riboflavin Reference Standard* in 0.02 *N* acetic acid soln to make 500 ml.[1] Store under toluene at ca 10°. 1.0 ml = 100 micrograms of riboflavin.

(b) *Standard riboflavin stock soln II.*—To 100 ml of (a), add 0.02 *N* acetic acid soln to make 1000 ml. Store under toluene at ca 10°. 1.0 ml = 10 micrograms of riboflavin.

(c) *Standard riboflavin soln.*—Dilute 5.0 ml of (b) with H_2O to make 1000 ml. 1.0 ml = 0.05 microgram of riboflavin. Prepare fresh standard soln for each assay.

(d) *Photolyzed peptone soln.*—Dissolve 40 g of peptone in 250 ml of H_2O, and 20 g of NaOH in 250 ml of H_2O, and mix the solns in a crystallizing dish having a diam. of ca 25 cm. At a distance of ca 30 cm from the dish, place a 100-watt bulb fitted with a reflector, and expose the soln to light from the bulb for 6–10 hours. Maintain the soln during this treatment at temp. not exceeding 25°. Neutralize the NaOH with acetic acid and add 7 g of anhydrous Na acetate and H_2O to make 800 ml. Store under toluene at ca 10°.

(e) *Cystine soln.*—Dissolve 1.0 g of l-cystine in 20 ml of HCl (1 + 3) and add H_2O to make 1000 ml. Store under toluene at ca 10°.

(f) *Yeast supplement soln.*—Dissolve 25 g of H_2O-soluble yeast extract in H_2O to make 125 ml, add 125 ml of a soln containing 38 g of Pb subacetate, and mix the solns. Filter, and add NH_4OH (1 + 2) to the filtrate to *p*H 10. Filter, and add acetic acid to the filtrate to *p*H 6.5. Precipitate the excess Pb with H_2S, filter, and add H_2O to the filtrate to make 250 ml. Store under toluene at ca 10°.

(g) *Salt soln A.*—Dissolve 25 g of KH_2PO_4 and 25 g of K_2HPO_4 in H_2O to make 500 ml. Add 5 drops of HCl and store under toluene.

(h) *Salt soln B.*—Dissolve 10 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.5 g of $FeSO_4 \cdot 7H_2O$, and 0.5 g of $MnSO_4 \cdot H_2O$ in H_2O to make 500 ml. Add 5 drops of HCl and store under toluene.

(i) *Basal medium stock soln.*—

Photolyzed peptone soln	= 50 ml
Cystine soln	= 50 ml
Yeast supplement soln	= 5.0 ml
Dextrose, anhydrous	= 15 g
Salt soln A	= 5.0 ml
Salt soln B	= 5.0 ml

Dissolve the anhydrous dextrose in the solns previously mixed and adjust to *p*H 6.8 with NaOH soln. Finally add H_2O to make 250 ml.

(j) 10 *N* HCl soln.

(k) 1 *N* HCl soln.

(l) 0.1 *N* HCl soln.

(m) 10 *N* NaOH soln.

(n) 1 *N* NaOH soln.

(o) 0.1 *N* NaOH soln.

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[1] See reference at end of method.

* U.S.P. Riboflavin Reference Standard may be obtained from the U.S.P. Reference Standards, 4738 Kingsessing Ave., Philadelphia 43, Pa.

PREPARATION OF INOCULUM

(a) *Stock culture of Lactobacillus casei.*—Dissolve 2.0 g of H_2O-soluble yeast extract in 100 ml of H_2O, add 0.5 g of anhydrous dextrose, 0.5 g of anhydrous Na acetate, and 1.5 g of agar, and heat the mixture, with stirring, on a steam bath until the agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug the tubes with cotton, sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and allow to cool in an upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of Lactobacillus casei,* incubating for 16 to 24 hours at any selected temp. between 30° and 37° but held constant to within ±0.5°, and finally store at ca 10°. Prepare a fresh stab of the stock culture every week, and do not use for inoculum if the culture is more than 1 week old.

(b) *Culture medium.*—To each of a series of test tubes containing 5 ml of the basal medium stock soln, add 5 ml of H_2O containing 0.5 microgram of riboflavin. Plug the tubes with cotton, sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and cool.

(c) *Inoculum.*—Make a transfer of cells from the stock culture of Lactobacillus casei to a sterile tube containing 10 ml of culture medium. Incubate this culture for 16–24 hours at any selected temp. between 30° and 37° but held constant to within ±0.5°. The cell suspension so obtained is the inoculum.

PREPARATION OF SAMPLE SOLUTION

Throughout all stages of the procedure, keep the soln below *p*H 7.0 in order to protect riboflavin.†

Place a measured amount of sample in a flask of suitable size and proceed by one of the methods given below.

(a) *For dry or semidry materials that contain no appreciable amount of basic substances.*—Add a volume of 0.1 *N* HCl soln equal in ml to not less than 10 times the dry weight of the sample in g, but the resulting soln shall contain not more than 0.1 mg of riboflavin per ml. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occurs, agitate vigorously so that all particles come in contact with the liquid, then wash down sides of flask with 0.1 *N* HCl soln.

Heat the mixture in an autoclave at 121°–123° (1.1–1.2 kg per sq cm) for 30 min. and cool. If lumping occurs, agitate the mixture until particles are evenly dispersed. Adjust the mixture, with vigorous agitation, to *p*H 6.0 with NaOH soln,[2] then add HCl soln immediately until no further precipitation occurs (usually ca *p*H 4.5, the isoelectric point of many of the proteins). Dilute the mixture to a measured volume that contains more than 0.05 microgram of riboflavin per ml and filter thru paper known not to adsorb riboflavin. In the case of a mixture that is difficult to filter, centrifuging and/or filtering thru sintered glass (using a suitable analytical filter-aid) may often be substituted for, or may precede, filtering thru paper. Take an aliquot of the clear filtrate and check for dissolved protein by adding, dropwise, first HCl soln and, if no precipitate forms, then, with vigorous agitation, NaOH soln, and proceed as follows:

(1) If no further precipitation occurs, add, with vigorous agitation, NaOH soln to *p*H 6.8, dilute the soln to a final measured volume that contains ca 0.05 microgram of riboflavin per ml, and if cloudiness occurs, filter again.

(2) If further precipitation occurs, adjust the soln again to the point ofmaximum

* Pure cultures of Lactobacillus casei may be obtained from the American Type Culture Collections, 0?9 M Street, N.W., Washington 6, D. C., as number 7469.

† The concentrations of the HCl and NaOH solns used are not stated in each instance because these concentrations may be varied depending upon the amount of sample taken for assay, volume of sample soln, and buffering effect of sample.

precipitation, dilute to a volume that contains more than 0.05 microgram of riboflavin per ml, and then filter. Take an aliquot of the clear filtrate and proceed as directed under (1).

If the riboflavin content of the sample is too low so that these requirements cannot be met, then concentrate the clear filtrate obtained at ca *p*H 4.5 to a suitable volume with heat under reduced pressure. Filter if necessary and then proceed as outlined above.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances.*—Add dilute HCl and adjust the mixture to *p*H 6.0. Add such an amount of H_2O that the total volume of liquid shall be equal in ml to not less than 10 times the dry weight of the sample in g. Then add the equivalent of 1 ml of 10 *N* HCl soln for each 100 ml of liquid and proceed as directed under (a).

(c) *For liquid materials.*—Adjust the material to *p*H 6.0 with either HCl soln or, with vigorous agitation NaOH soln, and proceed as directed under (b).

DETERMINATION

Prepare standard riboflavin tubes as follows: To duplicate test tubes, add 0.0 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml, 4.0 ml, 4.5 ml, and 5.0 ml, respectively, of the standard riboflavin soln. To each of these tubes add 5.0 ml of basal medium stock soln and H_2O to make 10 ml.

Prepare tubes containing the sample to be assayed as follows: To duplicate test tubes add, respectively, 1.0 ml, 2.0 ml, 3.0 ml, and 4.0 ml of the sample soln. To each of these tubes add 5.0 ml of basal medium stock soln and H_2O to make 10 ml.

After mixing, close the tubes by plugging with cotton, or, covering with caps, and sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm).* Cool, aseptically inoculate each tube with 1 drop of inoculum, and incubate for 72 hours at any selected temp. between 30° and 37° but held constant to within ±0.5°. Contamination of the assay tubes with any foreign organism invalidates the assay.

Titrate the contents of each tube with 0.1 NaOH soln, using bromothymol blue as the indicator, or to *p*H 6.8 measured electrometrically.

Prepare a standard curve of the riboflavin standard titrations by plotting the average of the titration values expressed in ml of 0.1 *N* NaOH soln for each level of the riboflavin standard soln used, against micrograms of riboflavin contained in the respective tubes. From this standard curve, determine by interpolation the riboflavin content of the sample soln in each tube. Discard any values of more than 0.20 or less than 0.05 microgram of riboflavin in each tube. Calculate the riboflavin content in each ml of sample soln for each of the tubes. The riboflavin content of the sample is calculated from the average of the values obtained from not less than 6 of these tubes that do not vary by more than ±10% from the average. If the titration values of less than 6 of these tubes containing the sample soln are within the range of the titration values of the riboflavin standard tubes containing 0.05 to 0.20 microgram of riboflavin, the data are insufficient to permit calculation of riboflavin content of the sample. Titration values exceeding 1.0 ml for the tubes of the standard riboflavin soln series containing 0.0 ml of the soln indicate the presence of an excessive amount of riboflavin in the basal medium stock soln and invalidate the assay.

REFERENCES

[1] *This Journal*, **23**, 346 (1940); **24**, 413 (1941); **25**, 459 (1942); **26**, 81 (1943); **27**, 540 (1944); **28**, 560 (1945); **29**, 25 (1946); **30**, 79 (1947); **31**, 701 (1948).

* Overheating (oversterilizing) of the assay tubes may cause unsatisfactory results.
[1] See footnotes at end of method.

(7) The following method was adopted as official, first action.

RIBOFLAVIN (VITAMIN B_2)

Fluorometric Method

REAGENTS

(a) *Standard riboflavin stock soln I.*—Dissolve 50 mg of U.S.P. Riboflavin Reference Standard* in sufficient 0.02 *N* acetic acid soln to make 500 ml.[1] Store, protected from light, under toluene at ca 10°. 1 ml = 100 micrograms of riboflavin.

(b) *Standard riboflavin stock soln II.*—To 100 ml of (a), add 0.02 *N* acetic acid soln to make 1000 ml. Store, protected from light, under toluene at ca 10°. 1 ml = 10 micrograms of riboflavin.

(c) *Standard riboflavin soln.*—Dilute 10 ml of (b) with H_2O to make 100 ml. 1 ml = 1 microgram of riboflavin. Prepare fresh standard soln for each assay.

(d) 10 *N* HCl soln.

(e) 1 *N* HCl soln.

(f) 0.1 *N* HCl soln.

(g) 10 *N* NaOH soln.

(h) 1 *N* NaOH soln.

(i) 0.1 *N* NaOH soln.

(j) Acetic acid.

(k) 4% $KMnO_4$ soln.

(l) 3% H_2O_2 soln.

(m) $Na_2S_2O_4$ powder.

APPARATUS

Electronic photofluorometer.—Use a fluorometer that is suitable for accurately measuring fluorescence of solns containing riboflavin in concentrations of ca 0.1 to 0.2 microgram per ml.

PREPARATION OF SAMPLE SOLUTION

Throughout all stages of the procedure, protect the soln from light that destroys riboflavin and keep the *p*H of the soln below 7.0.

Place a measured amount of sample in a flask of suitable size and proceed by one of the methods given below.

(a) *For dry or semidry materials that contain no appreciable amount of basic substances.*—Add a volume of 0.1 *N* HCl soln equal in ml to not less than 10 times the dry weight of the sample in g, but the resulting soln shall contain not more than 0.1 mg of riboflavin per ml. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occurs, agitate vigorously so that all particles come in contact with the liquid, then wash down sides of flask with 0.1 *N* HCl soln.

Heat the mixture in an autoclave at 121°–123° (1.1–1.2 kg per sq cm) for 30 min. and cool. If lumping occurs, agitate the mixture until particles are evenly dispersed. Adjust the mixture, with vigorous agitation, to *p*H 6.0 with NaOH soln[2] and add HCl soln immediately until no further precipitation occurs (usually ca *p*H 4.5, the isoelectric point of many of the proteins). Dilute the mixture to a measured volume that contains more than 0.1 microgram or riboflavin per ml and filter thru paper known not to adsorb riboflavin. In the case of a mixture that is difficult to filter, centrifuging and/or filtering thru sintered glass (using a suitable analytical filter-

* U.S.P. Riboflavin Reference Standard may be obtained from the U.S.P. Reference Standards, 4738 Kingsessing Ave., Philadelphia 43, Pa.

[1] To facilitate soln, 50 mg of U.S.P. Riboflavin Reference Standard may be added to ca 300 ml. of 0.02 *N* acetic acid soln and the mixture warmed on a steam bath with constant stirring until riboflavin is dissolved. Then cool and add sufficient 0.02 *N* acetic acid soln to make 500 ml.

[2] The concentrations of the HCl and the NaOH solns are not stated in each instance because these concentrations may be varied depending upon the amount of sample taken for assay, volume of sample soln, and buffering effect of sample.

aid) may often be substituted for, or may precede, filtering thru paper. Take an aliquot of the clear filtrate and check for dissolved protein by adding, dropwise, first HCl soln and, if no precipitate forms, then, with vigorous agitation, NaOH soln, and proceed as follows:

(1) If no further precipitation occurs, add, with vigorous agitation, NaOH soln to *p*H 6.8, dilute the soln to a final volume that contains ca 0.1 microgram of riboflavin per ml, and if cloudiness occurs, filter again.

(2) If further precipitation occurs, adjust the soln again to the point of maximum precipitation, dilute to a volume that contains more than 0.1 microgram of riboflavin per ml, and then filter. Take an aliquot of the clear filtrate and proceed as directed under (1).

If the riboflavin content of the sample is too low so that these requirements cannot be met, then concentrate the clear filtrate obtained at ca *p*H 4.5 to a suitable volume with heat under reduced pressure. Filter if necessary and then proceed as outlined above.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances.*—Add dilute HCl and adjust the mixture to *p*H 6.0. Add such an amount of H_2O that the total volume of liquid shall be equal in ml to not less than 10 times the dry weight of the sample in g. Then add the equivalent of 1 ml of 10 *N* HCl soln for each 100 ml of liquid and proceed as directed under (a).

(c) *For liquid materials.*—Adjust the material to *p*H 6 with either HCl soln or, with vigorous agitation, NaOH soln and proceed as directed under (b).

DETERMINATION

To each of 4 or more tubes[3] (or reaction vessels) add 10 ml of sample soln. To each of 2 or more of these tubes add 1 ml of the standard riboflavin soln and mix, and to each of 2 or more of the remaining tubes, add 1 ml of H_2O and mix. To each of the tubes add 1 ml of acetic acid, mix, add, with mixing, 0.5 ml of $KMnO_4$ soln,[4] and allow to stand for 2 min.[5] Then to each of the tubes, add, with mixing, 0.5 ml of H_2O_2 soln, whereupon the permanganate color must be destroyed within 10 seconds.[6] Shake tubes vigorously until excess O is expelled. If gas bubbles remain on sides of tubes after foaming has ceased, remove bubbles by rotating tubes slowly from end to end.

In a suitable fluorometer, measure the fluorescence of the sample soln containing 1 ml of added standard riboflavin soln and call this reading "A." Next, measure the fluorescence of the sample soln containing 1 ml of added H_2O and call this reading "B." Then add, with mixing, 20 mg of $Na_2S_2O_4$[7] to reduce the riboflavin present, to 2 or more tubes, measure the fluorescence within 5 sec, and call this reading C.[8] The riboflavin content may then be calculated as follows.[9]

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[3] If the fluorometer is of a type that requires tubular cuvettes, all reactions may be carried out in a matched set of these cuvettes.

[4] The amount of $KMnO_4$ soln may be increased for sample solns that contain an excess of oxidizable material, but do not add more than 0.5 ml in excess of that required to complete the oxidation of foreign material.

[5] A riboflavin recovery step is not included in this procedure as no destruction of riboflavin occurs until an elapse of time of ca 3 minutes.

[6] With the precipitation procedure properly followed for preparation of sample soln, no precipitate forms during the above reactions and, therefore, filtration at this point is necessary.

[7] The $Na_2S_2O_4$ must be of high purity. Do not use if unduly exposed to light or air. An amount appreciably in excess of 20 mg may reduce foreign pigments and/or foreign fluorescing substances thereby causing erroneous results. The suitability of the $Na_2S_2O_4$ is checked by the following manner:

To each of 2 or more tubes, add 10 ml of H_2O and 1 ml of standard riboflavin soln containing 20 micrograms of riboflavin per ml, and proceed as directed above with respect to the addition of acetic acid, $KMnO_4$ soln, and H_2O_2 soln. Then upon the addition, with mixing, of 8 mg of $Na_2S_2O_4$, the riboflavin is completely reduced in not more than 5 sec.

[8] After reduction, tubes of A and B should give the same degree of fluorescence.

[9] Most accurate results are obtained when the sample soln is of such a dilution that $B-C/A-B$ approaches 1/1. Any determination in which there is an appreciable deviation from this ratio should be considered a preliminary assay and should be repeated using a dilution of the sample soln that would give readings more nearly approaching this ratio.

$$\text{Mg of riboflavin per ml of final sample soln} = \frac{B-C}{A-B} \times \frac{1}{10} \times \frac{1}{1000}.$$

Calculate the amount of riboflavin in the sample on the basis of the aliquots taken during the analysis.

(8) The official, first action, method for vitamin C, **36.47–36.48**, (p. 620), was revised as follows and adopted as official, final action.

In parenthetical expression under the title after "ferrous Fe" add "Stannous Sn" and "cuprous Cu."

Delete "freshly pulverized stick H_3PO_3" in **36.47(a)** and substitute "glacial HPO_3 pellets, or freshly pulverized stick."

Delete note at end of method (p. 621) and substitute the following:

Products containing ferrous Fe, stannous Sn, and cuprous Cu give values in excess of their actual ascorbic acid content by this method. Following are simple tests to ascertain whether these reducing ions are present in appreciable quantities to invalidate analysis: Add 2 drops of 0.05% H_2O soln of methylene blue to 10 ml of freshly prepared mixture of juice and the HPO_3-acetic acid reagent, mix. Disappearance of methylene blue color in 5–10 seconds indicates presence of interfering substances. Stannous Sn does not give the test and may be tested for by using another 10 ml sample soln to which 10 ml of 25% HCl is added, mix, then 5 drops of 0.05% H_2O soln of indigo carmine, mix. Disappearance of indigo carmine color in 5–10 seconds also indicates presence of interfering substance.

(9) The tentative method for vitamin D in milk, **36.49–36.60** (p. 621), was adopted as official, first action.

(10) The alternate tentative method for carotene, *This Journal*, **31**, 111 (1947), was adopted as official, first action, for carotene in hays and dried plants.

(11) The tentative method for carotene, *This Journal*, **30**, 84 (1947), was deleted for hays and dried plants but continued as tentative for analysis of other materials.

(12) The official, first action, method for nicotinic acid, *This Journal*, **30**, 82 (1947), was revised as follows:

NICOTINIC ACID (NIACIN) OR NICOTINAMIDE (NIACINAMIDE)[1]

Microbiological Method

REAGENTS

(a) *Standard nicotinic acid stock soln I.*—Dissolve 50 mg of U.S.P. Nicotinic Acid Reference Standard* in alcohol to make 500 ml. Store at ca 10°. 1.0 ml = 100 micrograms of nicotinic acid.

(b) *Standard nicotinic acid stock soln II.*—To 100 ml of (a), add H_2O to make 1000 ml. Store under toluene at ca 10°. 1.0 ml = 10 micrograms of nicotinic acid.

(c) *Standard nicotinic acid soln.*—Dilute 10 ml of (b) with H_2O to make 1000 ml. 1.0 ml = 0.1 microgram of nicotinic acid. Prepare fresh standard soln for each assay.

(d) *Acid-hydrolyzed casein soln.*—Mix 100 g of vitamin-free casein with 500 ml of constant-boiling HCl soln (ca 20% HCl) and reflux the mixture for 24 hours.

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[1] *This Journal*, **27**, 105 (1944); **30**, 82 (1947).

* U.S.P. Nicotinic Acid Reference Standard may be obtained from the U.S.P. Reference Standards, 4738 Kingsessing Ave., Philadelphia 43, Pa.

Remove the HCl from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in H_2O, adjust the soln to *p*H 3.5 (±0.1) with 1 *N* NaOH soln and add H_2O to make 1000 ml. Add to the soln 20 g of activated charcoal, stir for 1 hour, and filter. Repeat the treatment with activated charcoal if the filtrate does not appear straw-colored to colorless. Store under toluene at ca 10°. Filter the soln if a precipitate forms upon storage.

(e) *Cystine-tryptophane soln.*—Suspend 4.0 g of l-cystine and 1.0 g of l-tryptophane (or 2.0 g of d, l-tryptophane) in 700–800 ml of H_2O, heat to 70–80°, and add 20% HCl soln, dropwise, with stirring, until the solids are dissolved. Cool and add H_2O to make 1000 ml. Store under toluene at ca 10°.

(f) *Adenine-guanine-uracil soln.*—Dissolve 0.1 g each of adenine sulfate, guanine hydrochloride, and uracil in 5 ml of warm 20% HCl soln, cool, and add H_2O to make 100 ml. Store under toluene at ca 10°.

(g) *Riboflavin-thiamine hydrochloride-biotin soln.*—Prepare a soln containing, in each ml, 20 micrograms of riboflavin, 10 micrograms of thiamine hydrochloride, and 0.04 microgram of biotin by dissolving crystalline riboflavin, crystalline thiamine hydrochloride, and crystalline biotin (free acid) in 0.02 *N* acetic acid soln. Store, protected from light, under toluene at ca 10°.

(h) *p-Aminobenzoic acid-calcium pantothenate-pyridoxine hydrochloride soln.*—Prepare a soln in neutral 25% alcohol to contain 10 micrograms of p-aminobenzoic acid, 20 micrograms of calcium pantothenate, and 40 micrograms of pyridoxine hydrochloride in each ml. Store at ca 10°.

(i) *Salt soln A.*—Dissolve 25 g of KH_2PO_4 and 25 g of K_2HPO_4 in H_2O to make 500 ml. Add 5 drops of HCl and store under toluene.

(j) *Salt soln B.*—Dissolve 10 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of NaCl, 0.5 g of $FeSO_4 \cdot 7H_2O$, and 0.5 g of $MnSO_4 \cdot H_2O$ in H_2O to make 500 ml. Add 5 drops of HCl and store under toluene.

(k) *Basal medium stock soln.*—

Acid-hydrolyzed casein soln	=25 ml
Cystine-tryptophane soln	=25 ml
Dextrose, anhydrous	=10 g
Na acetate, anhydrous	=5.0 g
Adenine-guanine-uracil soln	=5.0 ml
Riboflavin-thiamine-biotin soln	=5.0 ml
p-Aminobenzoic acid-calcium pantothenate-pyridoxine soln	=5.0 ml
Salt soln A	=5.0 ml
Salt soln B	=5.0 ml

Dissolve the anhydrous dextrose and Na acetate in the solns previously mixed and adjust to *p*H 6.8 with NaOH soln. Finally add H_2O to make 250 ml.

PREPARATION OF INOCULUM

(a) *Stock culture of Lactobacillus arabinosus 17-5.*—Dissolve 2.0 g of H_2O-soluble yeast extract in 100 ml of H_2O, add 0.5 g of anhydrous dextrose, 0.5 g of anhydrous Na acetate, and 1.5 g of agar, and heat the mixture, with stirring, on a steam bath until the agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug the tubes with cotton, sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and allow to cool in an upright position. Prepare stab cultures in 3 or more of the tubes, using a pure culture of Lactobacillus arabinosus 17-5,[2] incubating for 16–24 hours at any selected temp. between 30° and 37°, but held constant to within ±0.5°, and fin-

[2] Pure cultures of Lactobacillus arabinosus 17-5 may be obtained from the American Type Culture Collection, 2029 M Street, N.W., Washington 6, D. C., as Number 8014.

ally store at ca 10°. Prepare a fresh stab of the stock culture every week, and do not use for inoculum if the culture is more than 1 week old.

(b) *Culture medium.*—To each of a series of test tubes containing 5 ml of the basal medium stock soln, add 5 ml of H_2O containing 1 microgram of nicotinic acid. Plug the tubes with cotton, sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm), and cool.

(c) *Inoculum.*—Make a transfer of cells from the stock culture of Lactobacillus arabinosus 17-5 to a sterile tube containing 10 ml of culture medium. Incubate this culture for 16–24 hours at any selected temp. between 30° and 37°, but held constant to within ±0.5°. The cell suspension so obtained is the inoculum.

PREPARATION OF SAMPLE SOLUTION

Place a measured amount of sample in a flask of suitable size and proceed by one of the methods given below.[3]

(a) *For dry or semidry materials that contain no appreciable amount of basic substances.*—Add a volume of 1.0 *N* H_2SO_4 soln equal in ml to not less than 10 times the dry weight of the sample in g, but the resulting soln shall contain not more than 5.0 mg of nicotinic acid per ml. Comminute and evenly disperse the material in the liquid if it is not readily soluble. If lumping occurs, agitate vigorously so that all particles come in contact with the liquid, then wash down sides of flask with 1 *N* H_2SO_4 soln.

Heat the mixture in an autoclave at 121°–123° (1.1–1.2 kg per sq cm) for 30 min. and cool. If lumping occurs, agitate the mixture until particles are evenly dispersed. Adjust the mixture to *p*H 6.8 with NaOH soln, dilute with H_2O to make a measured volume that contains ca 0.1 microgram of nicotinic acid per ml, and filter if soln is not clear.

(b) *For dry or semidry materials that contain appreciable amounts of basic substances.*—Add dilute H_2SO_4 and adjust the mixture to *p*H 6.0. Add such an amount of H_2O that the total volume of liquid shall be equal in ml to not less than 10 times the dry weight of the sample in g. Then add the equivalent of 10 ml of 10 *N* H_2SO_4 soln for each 100 ml of liquid and proceed as directed under (a).

(c) *For liquid materials.*—Adjust the material to *p*H 6.0 with either H_2SO_4 soln or NaOH soln and proceed as directed under (b).

DETERMINATION

Prepare standard nicotinic acid tubes as follows: To duplicate test tubes add 0.0 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml, 4.0 ml, 4.5 ml, and 5.0 ml, respectively, of the standard nicotinic acid soln. To each of these tubes add 5.0 ml of basal medium stock soln and H_2O to make 10 ml.

Prepare tubes containing the sample to be assayed as follows: To duplicate test tubes add, respectively, 1.0 ml, 2.0 ml, 3.0 ml, and 4.0 ml of the sample soln. To each of these tubes add 5.0 ml of basal medium stock soln and H_2O to make 10 ml.

After mixing, close the tubes by plugging with cotton, or covering with caps, and sterilize in an autoclave at 121°–123° (1.1–1.2 kg per sq cm).[4] Cook, aseptically inoculate each tube with 1 drop of inoculum, and incubate for 72 hours at any selected temp. between 30° and 37°, but held constant to within ±0.5°. Contamination of the assay tube with any foreign organism invalidates the assay.

Titrate the contents of each tube with 0.1 *N* NaOH soln, using bromothymol blue as the indicator, or to *pH* 6.8 measured electrometrically.

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[3] The concentrations of the H_2SO_4 and NaOH solns are not stated in each instance because these concentrations may be varied dependent upon the amount of sample taken for assay, volume of sample soln, and buffering effect of sample.

[4] Overheating (oversterilizing) of the assay tubes may cause unsatisfactory results.

Prepare a standard curve of the nicotinic acid standard titrations by plotting the average of the titration values expressed in ml of 0.1 *N* NaOH soln for each level of nicotinic acid standard soln used, against micrograms of nicotinic acid contained in the respective tubes. From this standard curve, determine by interpolation the nicotinic acid content of the sample soln in each tube. Discard any values of more than 0.4 or less than 0.05 microgram of nicotinic acid in each tube. Calculate the nicotinic acid content in each ml of sample soln for each of the tubes. The nicotinic acid content of the sample is calculated from the average of the values obtained from not less than 6 of these tubes that do not vary by more than $\pm 10\%$ from the average. If the titration values of less than 6 of these tubes containing the sample soln are within the range of the titration values of the nicotinic acid standard tubes containing 0.05 to 0.4 microgram of nicotinic acid, the data are insufficient to permit calculation of nicotinic acid content of the sample. Titration values exceeding 1.0 ml for the tubes of the standard nicotinic acid soln series containing 0.0 ml of the soln indicate the presence of an excessive amount of nicotinic acid in the basal medium stock soln and invalidate the assay.

37. WATERS, BRINE, AND SALT

No additions, deletions, or other changes.

38. RADIOACTIVITY

No additions, deletions, or other changes.

39. DRUGS

(1) The following method was adopted as offiical, first action.

PHYSOSTIGMINE

Weigh accurately 5 g of the well mixed ointment directly into a 125 ml Erlenmeyer flask and insert a small glass rod for mixing and transfer purposes. Add 10 ml of 2% H_2SO_4, melt the ointment completely on a steam bath and mix the contents thoroly by swirling the flask. Cool in a pan of cracked ice, swirling the contents occasionally until solidification takes place. With the aid of a glass rod, filter the acid solution thru a cotton pledget inserted in the stem of a filter funnel into a 250 ml. separatory funnel. Repeat the extraction procedure 4 more times and wash the filter funnel and cotton pledget with a few ml of distilled water. Continue as in 39.99, line 3, beginning with "Make alkaline to litmus with solid $NaHCO_3$. . . ."

(2) The tentative method for arecoline hydrobromide, 39.73 (p. 689), was adopted as official, first action.

(3) The following method was adopted as official, first action.

TRICHLOROETHYLENE

APPARATUS

Pressure Tubes

Start with a clean dry piece of soft glass tubing 16½ cm long, 6–8 mm int. diam., with wall 1–1½ mm thick (1 mm is easier to work). Heat in center and draw out enough to make int. diam. 3–4 mm at narrow point. Seal both ends securely by heating. When cool, cut at narrow point to make 2 tubes, which should be 8–10 mm long. Ends may instead be sealed after dividing. Narrow ends are left open.

Covered Oil Bath

Any oil bath is satisfactory which permits heating of pressure tubes in approx.

upright position and which protects analyst from burn or injury in case a tube should burst. The chief risk to guard against is that of hot oil being thrown out of bath. The following apparatus is suggested.

Wrap a large Pyrex test tube, 38×300 mm, with sheet asbestos and wire, preferably in such manner that tube may be slid in and out of wrapping. Leave round bottom exposed. Put heavy mineral oil in tube to depth of about 10 cm., or sufficient to cover closed pressure tubes. Support bath in vertical position in hood, with round bottom set into circular hole in a piece of asbestos board. A suitable thermometer will be suspended from a clamp above, with bulb immersed to vicinity of tubes, when bath is being heated.

A small cylindrical wire basket, fitting into bath tube, may be used to place tubes in bath; basket is lowered and raised by an attached wire. Basket and tubes may then be suspended in upper part of bath tube for cooling after reaction. Without basket, tubes may simply be slipped into oil bath loose, and retrieved when cooled by looped wire or other device.

In such bath several tubes may be heated at once. A smaller test tube may be used for bath if only one pressure tube is to be heated at a time.

Monoethanolamine

Colorless and free of chlorine. Commercial monoethanolamine purified by one distillation is usually satisfactory.

DETERMINATION

Tare a pressure tube with suitable support (such as a small beaker, or wire holder). Using fine-tipped pipet, place in tube 0.15–0.17 g of sample. Gently wipe away any of sample on rim or outside of tube, wait until any in upper part of tube has evaporated, and weigh. Immediately add 1.0–1.1 ml of the monoethanolamine and immediately seal open end of tube securely by means of flame, without heating liquids in bottom. When tube has cooled mix liquids completely and place in covered oil bath (see above) at room temp. Tube, or tubes, should rest in approx. upright position and remain in such position until opened later. Suspend thermometer in bath with bulb near tubes, and heat bath to 210–240°C., lowering hood window part way and observing temp. thru window. Maintain in this temp. range 1 hr. Stop heating and remove thermometer with tongs, to avoid placing hands above bath. Remove tubes from oil, but keep safely covered until cool. Use of wire basket as described above is convenient. Tubes may instead be allowed to cool in the oil (a slower procedure) and then removed. Precautions are not needed after tubes have cooled.

Remove oil from outside of each tube. Open tube by filing above liquid and breaking cleanly. With aid of H_2O wash bottle, transfer contents without loss to a 250–400 ml beaker, and dilute with H_2O to 100–120 ml. If necessary, to remove glass particles, filter thru a small cotton pledget into a second beaker, washing entire soln thru. Neutralize with HNO_3 (10–15 drops) and add 1–1.5 ml excess. Heat to 65–70°C. and add an excess of $AgNO_3$ soln, 5% or less (50 ml of 0.1 *N*, T.S., is satisfactory). Coagulate on steam bath with occasional stirring, filter thru a Gooch or fritted glass crucible, wash with hot H_2O, and dry at 130–140°C (½ hr. usually suffices) 1 g AgCl=0.30557 g. C_2HCl_3.

(4) The following method was adopted as official, first action.

CALCIUM, PHOSPHORUS, AND IRON IN VITAMIN PREPARATIONS

METHOD

Transfer a representative portion of the well-mixed sample containing at least

10 mg P, 50 mg Ca, and 1 mg Fe to a 100 ml Pt or porcelain dish. Ash at a temp. not to exceed 525°C, until apparently free of carbon (gray to brown). Cool, moisten with 20 ml H_2O, break up the ash with a stirring rod, and add 10 ml HCl, cautiously under a watch-glass. Rinse off the watch-glass into the dish and evaporate to dryness on the steam bath. Add 50 ml HCl (1+9), heat on the steam bath 15 min., and filter thru quantitative paper into a 200 ml volumetric flask. Wash the filter and dish thoroly with hot water, cool the filtrate, dilute to the mark, and mix.

PHOSPHORUS

Using an aliquot containing 2–5 mg P, proceed as directed in **26.46–7.**

CALCIUM

Transfer an aliquot containing 20–40 mg Ca to a suitable beaker, dilute to 100 ml., and proceed as directed in **12.12.** Correct for $KMnO_4$ consumed in a blank determination.

IRON

Transfer an aliquot containing 0.2–0.5 mg Fe to a 100 ml volumetric flask, add sufficient HCl (1+9) to yield 2 ml of the concentrated acid, and dilute to volume. Proceed as directed in **20.12,** beginning " . . . Pipet 10 ml aliquot into 25 ml volumetric flask. . . . " Determine Fe in sample by comparison with standards prepared as directed.

(5) The official method for iodine **39.202** (p. 728) was reworded as follows and adopted as official, first action:

IODINE

Transfer a quantity of sample that contains not more than 0.1 g of the iodide (0.05 g is ample) to a crucible, preferably Ni. If the sample contains only a slight amount of organic material, add one g of starch. Add 2 or 3 g of solid KOH. If sample is a solid, add 10–15 ml of alcohol before adding the KOH. (It is essential that the alkali be thoroly mixed with the sample to prevent loss of iodine in the muffle. This may be accomplished by stirring, leaving the stirring rod in the crucible, or by heating and swirling on the steam bath until the KOH is in solution.) Dry, and char thoroly. (Use as low a temp. as possible in order to prevent loss of I, in no event more than dull redness.) Extract charred mass with hot H_2O, filter into Erlenmeyer flask, and wash well with hot H_2O.

Neutralize filtrate with H_2SO_4 (1+1), make alkaline again with 4% NaOH soln, and add 1 ml in excess. Heat to boiling and add saturated $KMnO_4$ soln slowly until $KMnO_4$ color remains after several min. boiling. Then add ca 0.5 ml in excess, continue boiling ca 5 min. and allow to cool. (It is essential that the permanganate coloration, not the brown manganese dioxide color, be present at the end of the boiling period. Otherwise insufficient $KMnO_4$ will have been added to completely oxidize all the iodide to iodate.) Add a few ml of alcohol and place on steam bath. ($KMnO_4$ color should be bleached; if it is not, add a little more alcohol.) When precipitate has settled, filter and wash with hot 1% NH_4Cl soln. If the filtrate is not clear, digest on the steam bath until the MnO_2 will be retained on the filter. After cooling, add 1–2 g of KI (crystals), acidify with HCl, and titrate with 0.1 *N* $Na_2S_2O_3$ soln. 1 ml of 0.1 *N* $Na_2S_2O_3$ = 0.00277 g of KI, 0.00250 g of NaI, or 0.00212 g of I.

(6) In the last line, **39.39(b)** (p. 678), the reference **39.17(b)** was changed to **39.170.**

40. MICROBIOLOGICAL METHODS

No additions, deletions, or other changes.

41. MICROCHEMICAL METHODS

No additions, deletions, or other changes.

42. EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) The method for rodent excreta in corn meal, **42.32** (p. 781) official, first action, *This Journal*, **31**, 118 (1948) was adopted as official, final action.

(2) The tentative method for filth in starch **42.38** (p. 782) was deleted and the following method was adopted as tentative.

Weigh out 225 g of starch into a 1500 ml beaker. Add with stirring 1200 ml of cold water (15–20°). Stir out lumps and pour thru a 5–8 in. #140 sieve. Wash with cold running water. Rinse particles from the sieve to a filter paper, using first water and then 80% alcohol. Examine paper microscopically.

(3) The following methods were adopted as tentative.

FILTH IN POPPED POPCORN

Procedure.—Weigh 50 g corn into a 2-liter Wildman trap flask. Add 500 ml hot water, boil for 15 min., cool to room temperature. Add 35 ml gasoline, mix. Allow to stand 5 min. Fill with H_2O, trap off, filter, and examine.

EXTERNAL CONTAMINATION OF UNPOPPED POPCORN, CEREAL GRAINS, PEAS, AND BEANS, ETC.

(*1*) *Macroscopic.*—Examine 225 g[1] in white tray for rodent and insect-damaged and moldy kernels, rodent excreta, other filth, and extraneous materials. Report by weight except rodent pellets. Report them by number and kind.

(*2*) *Microscopic.*—Transfer a separate 225[2] g portion to a 2-liter trap flask. Add 600 ml 40% alcohol. Boil gently, with frequent stirring, for 5 min. Cool. Trap off using gasoline and 40% alcohol, filter, and examine.

(4) The following method was adopted as tentative.

MOLD IN CRANBERRY SAUCE

(*a*) *Strained Sauce*

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

Mix thoroly 50 g of the stirred sauce with 50 g of a 3% pectin soln. Make a mold count of this mixture using official mold count method as directed in **42.57.**

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

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

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[1] Or consumer-size package.

[2] Or consumer-size package when it is 6–10 oz. Between 10–16 oz. split into two portions and test both. Over 16 oz. test an aliquot of at least 8 oz.

(5) In the method for fly eggs and maggots in tomato products, **42.61(b)** (p. 790) the "5 liter" line 4 was changed to "6 liter."

(6) In the method for filth in dried mushrooms, **42.75** (p. 795) after "mushrooms" line 10 add "allowing them to drop thru the screen."

43. STANDARD SOLUTIONS

(1) The official, first action, method for standardization of hydrochloric acid with sodium hydroxide, **43.7–43.8** (p. 803), was adopted as official, final action.

(2) The tentative method for standardization of sulfuric acid by borax, **43.14–43.15** (p. 807), was adopted as official, first action.

(3) In Method II for standardization of titanium trichloride, **21.37** (p. 290), "$K_2Cr_2O_7$" was substituted for "$KMnO_4$" line 1, and adopted as official, first action.

CORRECTIONS FOR VOL. 31, NO. 4

In the paper on succinic acid in decomposed egg products by Henry A. Lepper and Fred Hillig, lines 4 to 7 of paragraph 2 on page 739, beginning "The grades" and ending "Table 2" should be changed to read "The grades as indicated on the carton or on the inspector's collection report are given in Table 2 in which the other details of the samples are also summarized."

In the paper entitled "Test Paper of Urease and Acid-Base Indicator, for Detection of Urea," by J. W. Cook, on page 798, second paragraph under "Preparation of Test Papers," 4th line, after "water" insert "rendered sufficiently alkaline with dilute NaOH to dissolve the dye."

On page iii, beginning of Contents of Volume 31, change "sixty-second" to "sixty-first" annual meeting, and change the dates to "October 20, 21, and 22, 1947."*

* A corrected page is furnished in this number for purposes of binding in Vol. 31. A reprint will be mailed to those who have not subscribed for Vol. 32 (1949).

CONTRIBUTED PAPERS

KJELDAHL DETERMINATION OF NITROGEN IN REFRACTORY MATERIALS*

By C. O. Willits, M. R. Coe, and C. L. Ogg (Eastern Regional Research Laboratory† Philadelphia 18, Pennsylvania)

Since 1883, when J. Kjeldahl (9) introduced his method for the determination of nitrogen in certain compounds pertaining to the brewing industry, many improvements have been made to extend its usefulness for the analysis of nitrogen compounds in general. Investigators have studied innumerable phases of the problem with the object of increasing the accuracy and shortening the digestion time. Even today it is a subject of much interest to analytical chemists.

In the investigation reported here, a study was made of the application of the Kjeldahl procedure to heterocyclic and other aromatic nitrogen compounds generally considered refractory. Many catalysts have been proposed and used in the Kjeldahl procedure, and it was hoped that through the study of these compounds, a better evaluation of catalysts could be made. Nicotinic acid was chosen for most of these studies, since it is a representative compound of this type, can be obtained pure, and meets most of the requirements of a primary standard. Particular attention was given to the catalysts selenium, mercuric oxide, and potassium sulfate, together with times of digestion required for the determination of nitrogen by the Kjeldahl method. Neither copper nor any of its salts were included, since in previous work at this Laboratory (13), copper had no catalytic effect when used with mercury and was inferior to mercury when the two were used separately.

The use of selenium as a catalyst was first recommended by Lauro (10, 11), but later questioned by Osborn and Wilkie (14). Patel and Sreenivasan (15) studied the relationship of selenium to the recovery of nitrogen and to the loss of nitrogen. Sandstedt (18) reported low results for total nitrogen after prolonged digestion with selenium. Davis and Wise (6), Snider and Coleman (20), Dalrymple and King (5), and others reported similar losses of nitrogen, and concluded it does not appear practicable to employ selenium catalysts because digestion time would have to be determined and also controlled in order to obtain the maximum yield of nitrogen. Bradstreet (3, 4) also found progressively lower nitrogen values with increasing quantities of selenium. On the other hand, Illarionov and Soloveva (8) stated that the catalytic action of selenium was

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

† One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

proportional to the amount used. Furthermore, Davis and Wise (6) observed that selenium interfered when the amount of potassium sulfate exceeded 13 g. Shirley and Becker (19) reported that a mixture of selenium oxychloride and mercury is a satisfactory catalyst for the Kjeldahl nitrogen determination of ring-type nitrogen compounds. This was likewise reported by Belcher and Godbert (2), who found that none of the catalysts they tried proved equal in efficiency to the selenium-mercury catalyst.

In addition to having an efficient catalyst for the digestion of nitrogen compounds in the Kjeldahl method, it is important to have the proper amount of potassium sulfate. The first to recognize this was Gunning (7). Later Phelps and Daudt (16) stated that the amount of potassium sulfate and sulfuric acid in the presence of mercury determines the completeness of the hydrolysis.

Alcock (1) reported experiments which showed that a definite relationship exists between sodium sulfate, sulfuric acid, and digestion time in their effect on nitrogen recovery.

PROCEDURE

REAGENTS

1. *Catalysts.*—Metallic selenium powder and mercuric oxide.
2. *Sodium hydroxide-sodium thiosulfate soln.*—Mix 100 ml of 50% sodium hydroxide and 25 ml of 8% sodium thiosulfate soln.
3. *Indicator.*—Mix 400 ml of 0.1% methyl red dissolved in 95% ethanol with 100 ml of 0.1% methylene blue also dissolved in 95% ethanol.
4. *Boric acid.*—Dissolve 4 g of boric acid in 100 ml of distilled water.
5. *Standard acid.*—0.1 *N* hydrochloric acid.

The Kjeldahl method followed differed little from the one in common use. In addition to the sample, the digestion mixture consisted of sulfuric acid, potassium sulfate and 2 catalysts. A sample containing at least 30 mg. of nitrogen was used so that 20 ml. or more of standard 0.1 *N* acid would be required in the titration. In all cases, the digestion was made in a 650-ml. Kjeldahl flask with 25 ml. of sulfuric acid, to which was added different amounts of potassium sulfate, mercuric oxide, and selenium. The mixture was digested for 1 to 8 hours with full boiling; chips were used to maintain uniform ebullition. The time of boiling had no relation to the time required to clear the solution.

The digest was diluted with 250 ml. of distilled water, and made alkaline with sodium hydroxide-sodium thiosulfate solution; 5 g. of 20-mesh zinc granules were then added. The distilling apparatus used a modified connecting bulb (21) and a 500-ml. wide-mouth Erlenmeyer receiving flask sealed with a trap (17). The condenser tube extended below the surface of the 100 ml. of 4% boric acid solution used as the trapping liquid.

RESULTS

Figure 1 shows the effect on the recovery of nitrogen in nicotinic acid of varying amounts of selenium with three different weights of potassium sulfate, with and without 0.4 g. of mercuric oxide. A digestion time of 6 hours was used.

These results indicate that to obtain theoretical recoveries of nitrogen with 5.5 g. of potassium sulfate, the optimum amount of selenium was between 0.25 g. and 0.40 g., whereas with 11.0 or 16.5 g. of potassium sulfate, less than 0.015 g. of selenium could be used. In the absence of

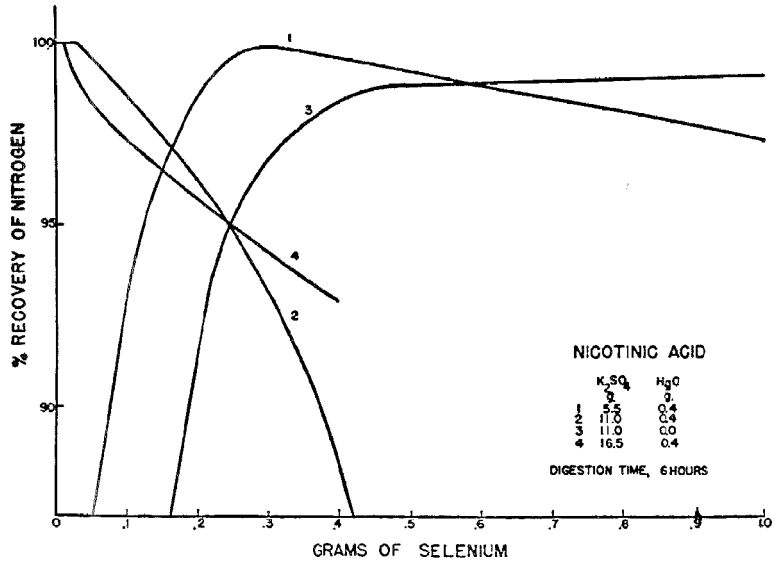

FIG. 1.—Recovery of nitrogen as affected by varying amounts of selenium with different amounts of potassium sulfate.

mercury, as much as 1.50 g. of selenium to 11 g. of potassium sulfate gave incomplete recovery.

These results indicated that some optimum ratio of selenium to potassium sulfate might be found. To determine this, studies were made with different amounts of potassium sulfate and 0.3, 0.03 and 0.015 g. of selenium. Figure 2 shows that the optimum amount of selenium decreases as the amount of potassium sulfate increases. However, with a prolonged digestion time, in this case 6 hours, it was easily possible to exceed the optimum amount of potassium sulfate to be used with 0.3 g. or 0.03 g. of selenium, with a resultant loss of nitrogen. The long digestion period of 6 hours was chosen so that any loss of nitrogen would be recognized as such and not confused with incomplete digestion. The use of 0.015 g.

of selenium had little influence, since the recovery of nitrogen was essentially the same as when no selenium was used. Three-hundredths gram of selenium gave maximum recoveries when used with 11 g. of potassium sulfate but caused a loss of nitrogen with larger amounts. This increasing loss of nitrogen with increasing amounts of potassium sulfate, as would be expected, was more pronounced when larger amounts (0.3 g.) of selenium were used.

From the preceding experiments, it appeared desirable to establish if, when large amounts of selenium (0.30 g.) and varying amounts of potas-

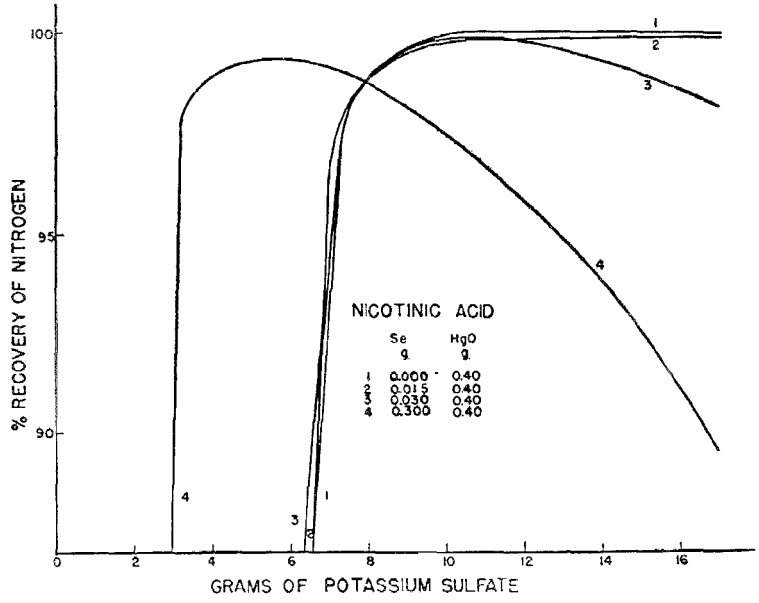

FIG. 2.—Recovery of nitrogen as affected by varying amounts of potassium sulfate with different amounts of selenium.

sium sulfate are used, there is some specific digestion time in which theoretical or maximum recoveries of nitrogen can be obtained. Figure 3 shows that for mixtures containing 0.3 g. of selenium and 5.5 g. of potassium sulfate, 99.8% of the nitrogen of nicotinic acid is recovered after 6 hours digestion, and that there is no apparent loss of nitrogen after 7 and 8 hours. With larger amounts of potassium sulfate, 11 to 15 g., the maximum but less than theoretical recovery of nitrogen occurs with a digestion time of between 2 and 4 hours, and a loss of nitrogen occurs with longer periods. Since the maximum recovery with 11 or more g. of potassium sulfate and 0.30 g. of selenium was less than theoretical, and since the recovery diminished with longer digestion periods, it was decided that

for all future experiments a smaller amount (0.03 g.) of selenium should be used.

It was indicated in Figure 2 that theoretical recoveries of nitrogen could be had without the use of selenium, but there was no indication as to the effect of digestion time on the recovery. A series of determinations was therefore made with varying amounts of potassium sulfate, and 0.4 g. of mercuric oxide with digestion times of 1 to 8 hours. A parallel experiment was made in which 0.03 g. of selenium was added to the digestion

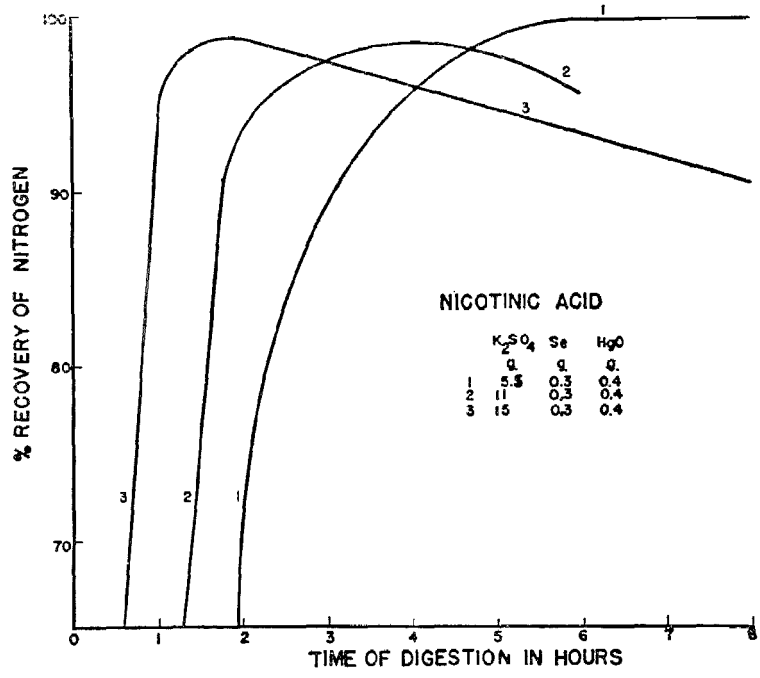

FIG. 3.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of selenium.

mixture. The results of these two experiments are shown in Figures 4 and 5, respectively.

A comparison of Figures 4 and 5 shows that the best digestion mixture contained 15 g. of potassium sulfate, 0.4 g. of mercuric oxide, and no selenium, since it required the shortest digestion period (2 hours) for complete recovery of nitrogen and prolonged digestion caused no loss of nitrogen. With low concentration of sulfate salts, selenium caused a somewhat more rapid rate of nitrogen recovery, whereas with higher concentrations the reverse was true.

In all the preceding experiments, the amount of mercuric oxide, 0.4

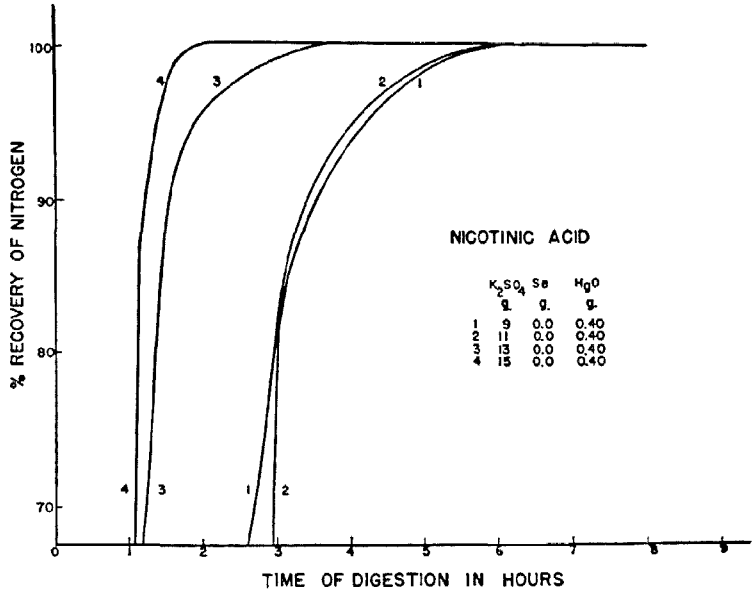

FIG. 4.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of potassium sulfate, without selenium.

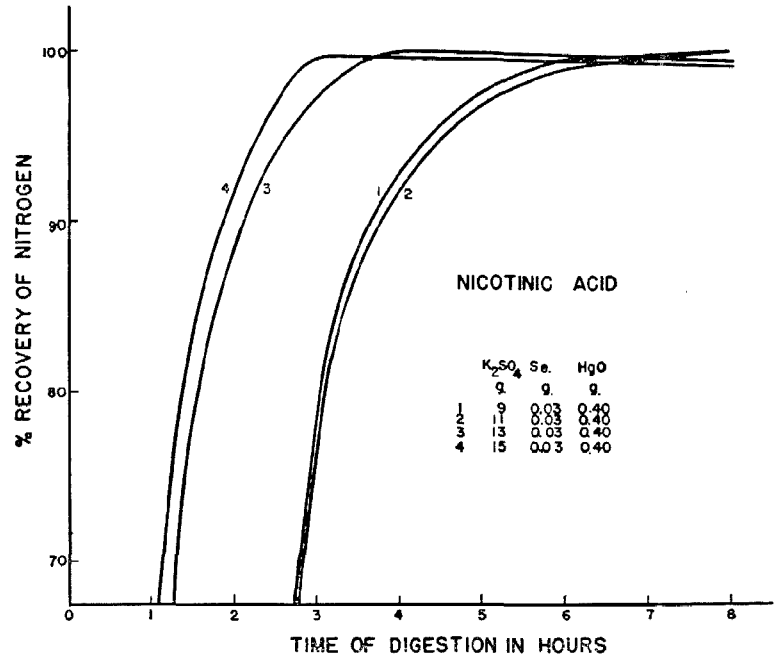

FIG. 5.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of potassium sulfate with selenium in the mixture.

g., was chosen arbitrarily. Experiments were therefore made, as shown in Figure 6, to determine the effect of varying the amount of mercuric oxide. For digestion mixtures containing 15 g. of potassium sulfate and *no mercuric oxide*, less than 55% of the nitrogen was recovered with a 3-hour digestion. The amount of mercuric oxide required with this quantity of potassium sulfate to give complete recovery of nitrogen was not critical, since as little as 0.1 g. or as much as 1 g. gave equally satisfactory results.

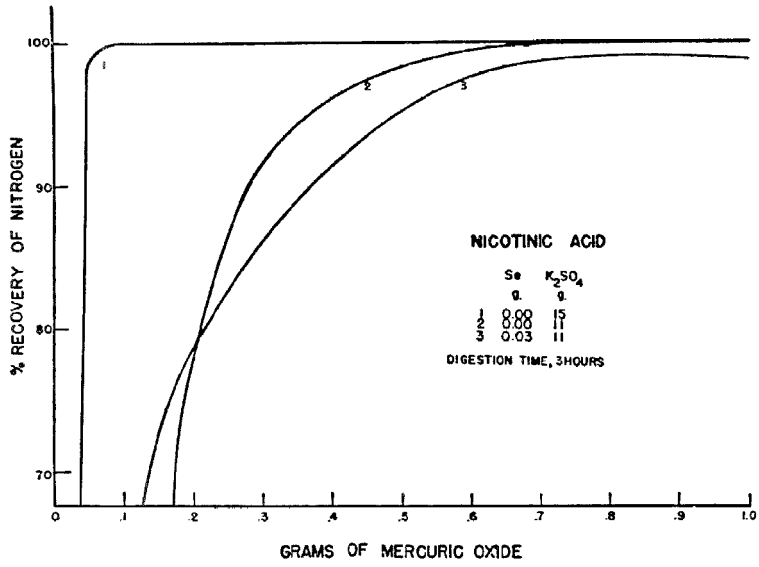

FIG. 6.—Recovery of nitrogen as affected by varying the amounts of mercuric oxide with different amounts of selenium and potassium sulfate.

For the smaller amount of potassium sulfate, theoretical recoveries were obtained only when more than 0.6 g. of mercuric oxide was used. The presence of selenium prevented the complete recovery of nitrogen with all amounts of mercuric oxide used.

Table 1 gives the results of the analyses of other refractory nitrogen compounds. With the digestion mixture consisting of 15 g. of potassium sulfate, 0.4 g. of mercuric oxide, and 25 ml. of sulfuric acid, and a digestion time of 3 hours, the amounts of nitrogen recovered were in close agreement with the theoretical values.

The preceding experiments showed that a simple digestion mixture of potassium sulfate, mercuric oxide, and sulfuric acid was sufficient for refractory compounds, but it was not certain what would happen to a less refractory material when digested for 3 hours or longer. Table 2 shows the recovery of nitrogen from S-benzyl thiuronium chloride with $\frac{1}{2}$- to 6-hour digestion periods. Complete recovery of nitrogen was ob-

TABLE 1.—*Per cent nitrogen found in several typical refractory nitrogenous compounds*

	NITROGEN		
	FOUND BY ANALYSIS		THEORETICAL VALUE
	A	B	
	per cent	*per cent*	*per cent*
Tryptophane	13.60	13.60	13.72
Histidine monohydrochloride	19.94	19.93	20.05
Lysine monohydrochloride	15.24	15.24	15.34
8-hydroxy quinoline	9.61	9.62	9.65
Nicotinic acid	11.38	11.40	11.38

TABLE 2.—*Per cent nitrogen recovered from S-benzyl thiuronium chloride* using the potassium sulfate-mercuric oxide digestion mixture*

DIGESTION TIME, HOURS	NITROGEN RECOVERED
	per cent
0.5	13.81
1	13.82
2	13.85
3	13.83
4	13.84
5	13.82
6	13.83

* Theoretical nitrogen value, 13.82.

tained after half an hour of digestion, and there was no appreciable loss up to 6 hours.

DISCUSSION

These experiments have demonstrated that the time of digestion and the amounts of potassium sulfate and of mercuric oxide used have an interrelated effect on the loss of nitrogen when selenium is present. This may be the reason that many have inaccurately defined the cause of incomplete recovery of nitrogen when selenium was used in the digestion mixture. Since selenium (1) may cause either incomplete recovery of nitrogen or a diminished rate of recovery, and (2) requires a specific time of digestion, its use cannot be recommended.

When selenium is omitted from the digestion mixture, the amount of potassium sulfate, mercuric oxide, and the time of digestion are not critical, insofar as loss of nitrogen is concerned. It was possible, therefore, to increase the amount of potassium sulfate so that complete recovery was obtained in 2 hours with no loss of nitrogen on prolonged digestion.

For the complete recovery of nitrogen from all kinds of materials which may contain refractory nitrogenous compounds, it is recommended as a general procedure that a digestion mixture consisting of 25 ml. of sulfuric acid, 15 g. of potassium sulfate, and 0.6 g. of mercuric oxide be used, with a digestion time of 3 hours. This method, which is a result of the studies reported here, is essentially the same as the A.O.A.C. method (12), and is further proof of its general applicability. If the 15 g. of potassium sulfate is carefully measured, 0.4 g. of mercuric oxide is more than sufficient, as shown in Figure 6. However, if less than 15 g. is added, as is often the case, 0.6 g. of mercuric oxide should be used.

In Figures 4 and 5 the curves for 9 and 11 g. of potassium sulfate, both with and without selenium, almost coincide, whereas the similar paired curves for 13 and 15 g. are markedly different. This peculiarity may explain why different analysts, using presumably the same Kjeldahl method, often obtain different results for refractory compounds. Since potassium sulfate is usually measured by volume instead of by weight, it would be possible for one analyst to add 13 and the other 11 g., and each think that he was following the procedure rigorously. Should both use a 4-hour digestion, one would obtain complete recovery and the other only 92 to 95% recovery.

The desirability of using a mercury compound as a catalyst is beyond question. The omission of mercuric oxide from the digestion mixture always causes incomplete recovery of nitrogen from nicotinic acid. With high concentrations of potassium sulfate, as little as 0.1 g. of mercuric oxide is sufficient.

SUMMARY

Complete recovery of nitrogen can be obtained from heterocyclic nitrogen ring compounds by the Kjeldahl method. The only catalysts required are mercuric oxide and potassium sulfate, used in the ratio of 0.6 to 15 g., with 25 ml. of sulfuric acid, and a digestion time of 3 hours. Because of the danger of loss of nitrogen when selenium is present, the use of selenium as an additional catalyst cannot be recommended.

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A NEW METHOD FOR DETERMINING THE BOILING RANGE OF PSEUDOCUMIDINE IN FD&C RED NO. 1*

By KENNETH A. FREEMAN AND LEE S. HARROW (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

FD&C Red No. 1 is one of the colors certifiable by the U. S. Food and Drug Administration for use in foods, drugs, and cosmetics. In order to be certified, the color must meet the specifications set forth in the Coal-Tar Color Regulations (1). One of the specifications states that the boiling range of crude pseudocumidine used in the production of the color, or of the pseudocumidine obtained by the reduction of the color, must be in the range of 220–245°C.

The Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition (2) gives a tentative method for this determination. This method involves reduction of the FD&C Red No. 1 with stannous chloride and hydrochloric acid, addition of excess sodium hydroxide, and separation of the free amine by means of steam distillation. The amine is finally recovered by ether extraction from the distillate. Experience has shown several objectionable features in this method.

(a) Irritating HCl fumes are produced during the reduction.

(b) Unless the addition of sodium hydroxide to the reduction mixture is very cautiously carried out the reaction mixture spatters.

(c) Low yields of pseudocumidine are produced.

(d) The method is very time-consuming.

In order to overcome these objections, a means was sought for making this determination by another method. Mr. L. Koch (3) of H. Kohnstamm and Company, Inc., suggested the use of sodium hydrosulfite in alkaline solution as the reducing agent. This procedure was found to be superior to the stannous chloride method. Certain modifications were made to provide simultaneous reduction of the color and recovery of the pseudo-

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

cumidine. A modified semi-micro distillation procedure for determining the boiling range of the pseudocumidine has also been developed.

METHOD

APPARATUS

Steam distillation.—A three-liter, two-neck, round bottom flask; one neck is fitted with a dropping funnel; the other with a steam distillation trap which has an inlet for live steam and which is connected to a water-cooled condenser.

Fractionating apparatus.—A 25 ml round-bottom flask fitted with an insulated distilling column, 10–12″ long and ½″ inside diam., packed with 24″ of approximately 24 gauge nichrome wire coiled inside the column in the manner described by Podbielniak (4). An insulated Vigreau column of approximately the same dimensions is also suitable. Provide the column with a 200–260°C Anschutz thermometer.

REAGENTS

Sodium hydrosulfite.—Reagent grade, saturated soln (ca 30%).

Dissolve ca 100 g of color in 2 liters of hot water contained in the round-bottom flask of the steam distillation apparatus. Add 10 g of sodium hydroxide and heat the soln to boiling, then while passing live steam thru the soln at a rate that will produce 5–10 ml of distillate per min., add the sodium hydrosulfite soln dropwise by means of the dropping funnel until the red color disappears (the soln will then be a light yellow-brown). Continue the steam distillation until no more oil distills. Extract the distillate with four 20 ml portions of ether and wash the extracts several times with 10 ml portions of water. Evaporate the major portion of the ether on a steam bath and dry the residue over sodium or potassium hydroxide pellets. Filter the residual soln into the 25 ml round-bottom flask of the fractionating apparatus. Heat cautiously with a heating mantle or a water bath until all the ether has been removed; then continue heating with a Wood's metal bath or an equivalent constant temperature bath. Record the first point of constancy in the temperature rise as the initial boiling point. The final point is taken as the maximum temp. obtainable.

EXPERIMENTAL

A commercial sample of "liquid pseudocumidine" was distilled and the fraction boiling from approximately 218–240°C. was reserved. This material was kept for 24 hours over sodium hydroxide pellets and then divided into two portions. The boiling range of one portion was determined using the apparatus suggested under "Method."

A quantity of FD&C Red No. 1 was prepared from the second portion employing the following procedure:

Dissolve 27 g of crude pseudocumidine (0.2 mol) in 150 ml of 6 *N* HCl contained in a 600 ml beaker. Place the beaker in an ice bath and add 65 g of crushed ice to the soln. Add 100 ml of a cold 21% soln of sodium nitrite, and hold at 5°C. or below. At the end of one hour add small portions of sulfamic acid until a negative test is obtained with starch-iodide paper.

Place in a 2-liter beaker 15 g of sodium carbonate, 15 g of sodium acetate, 6 ml of 30% sodium hydroxide, 67 g of R-salt, and 1 liter of water. When all material is in solution, cool to 5°C. and add the cold diazonium soln from above. Stir for 30 min. holding the temp. at 5°C. or below; then allow the soln to warm to room temp. Heat on steam bath for four hours, cool, and filter. Dry the dye at 135°C. for six hours. By this method a yield of 86 g was obtained (86% of theory).

This color was then reduced according to the proposed method and the boiling range of the resulting pseudocumidine was determined. The results are shown in Table 1.

TABLE 1

BOILING RANGE	% DISTILLED ORIGINAL	RECOVERED MATERIAL
Initial–220°C.	8	10
220–225°C.	55	50
225–227°C.	17	20
227–Upper Limit	19	20

The boiling range of the original material was 218.0°C. to 232.4°C. and that of the recovered material was 218.2°C. to 231.9°C.

DISCUSSION

The proposed method has been employed in the Color Certification laboratories for a period of several months. This experience has shown that the method eliminates the objections of the older method. All of the non-volatile reduction products are soluble in the solution employed. The use of hydrochloric acid is avoided entirely. Neutralization of the reduction mixture is not necessary because the reduction is accomplished in basic solution. The time consumed is greatly reduced, since the reduction of the dye and distillation of the pseudocumidine are occurring simultaneously.

The entire reduction and steam distillation process is carried out in a single piece of apparatus, thus eliminating transfer of material from one apparatus to another (with the consequent loss of material and time consumption).

SUMMARY

A method has been proposed for the evaluation of the boiling range of pseudocumidine in FD&C Red No. 1 (Ponceau 3R). The intermediate is recovered from the color by reduction with alkaline sodium hydrosulfite. The boiling range is determined by a semi-micro fractionating apparatus fitted with an Anschutz thermometer. Experience has shown that the method is more reliable, shorter, and more convenient than the method given in *Methods of Analysis*.

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SPECTROPHOTOMETRIC ANALYSIS OF D&C RED No. 19 (RHODAMINE B)*

By MEYER DOLINSKY (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

One of the specifications (1) for certifiable samples of D&C Red No. 19 is: "Pure dye (as determined by titration with titanium trichloride), not less than 92.0 per cent." The titanium trichloride titration is not very satisfactory since it gives variable, and generally high, results. It was decided, therefore, to study the spectrophotometric analysis of this color, since it appeared that the spectrophotometric determination might be more accurate and precise than the titration method.

As in previous investigations (2) (3), samples sufficiently pure to serve as standards were prepared and spectrophotometric data obtained on solutions of these samples. From these data, the applicability of Beer's law was checked, the location of the absorption peak was determined, and the extinction ratio at arbitrarily selected wave lengths was calculated as an aid in the identification of the color.

EXPERIMENTAL

All optical measurements were made with a General Electric recording spectrophotometer equipped with slit adjustments for an 8 millimicron wave length band. To minimize the effect of the fluorescence of the dye, the cells containing the solutions used in this work were placed at the forward end of the transmission compartment, approximately five inches from the integrating sphere. Calculations indicate that under these conditions less than one per cent of the fluorescent light emitted by the sample should reach the integrating sphere.

Melting points were taken on a Fisher melting point block.

Preparation of Standard Samples.—30 grams of phthalic anhydride (sublimed), m.p. 130.5°C. (literature, 130.8°C.), was fused with 24 grams of m-diethylaminophenol (recrystallized twice from methanol-water and once from chloroform-petroleum ether), m.p. 72–72.5°C. (literature 76°C.), at 165°C. for five hours (4). The Rhodamine B phthalate obtained was treated with ammonium hydroxide and the color base extracted from the ammonium hydroxide solution with several portions of benzene. The D&C Red No. 19 was extracted from the benzene with several portions of hot (1+4) hydrochloric acid and allowed to crystallize.

(a) Part of the D&C Red No. 19 was recrystallized twice from (1+8) HCl; once from (1+20) HCl, and dried at 80°C. to give a product which softened at 185°C. and melted at 193–195°C.

(b) A portion of the crude D&C Red No. 19 was converted to the color

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

base by precipitation from solution with sodium hydroxide. The base was recrystallized three times from ether and dried at 135°C. to give a practically colorless (very light pink) product which melted sharply at 165°C. (literature 165°C.). (5) A separate portion of the color base recrystallized twice from methanol-water had the same melting point and showed identical spectrophotometric characteristics.

(c) Part of the crude Rhodamine B phthalate was recrystallized three times from alcohol and dried at 135°C. to give a product melting at 212°–214°C.

Analytical data on the purified compounds are shown in Table 1.

TABLE 1.—*Analytical data*

	NITROGEN (KJELDAHL)		CHLORINE	
	CALCULATED	FOUND	CALCULATED	FOUND
	per cent	*per cent*	*per cent*	*per cent*
Purified D&C Red No. 19	5.85	5.79	7.4	7.3
Purified D&C Red No. 19 Color Base	6.33	6.30		
Purified Rhodamine B Phthalate	4.60	4.55		

Spectrophotometric Data

(a) A sample of the purified color base, weighed on a semimicro balance, was dissolved in exactly 1000 ml. of (1+999) HCl. Aliquot portions of this solution were diluted with (1+99) NH_4OH to give the solutions for spectrophotometric examination. (All solutions were allowed to stand for approximately one hour at the temperature of the room in which the optical measurements were made, before being made to volume.) Typical results are shown in Table 2 and typical curves in Figure 1.

TABLE 2.—*Extinction values of purified D&C Red No. 19 color base in dilute ammonia*

CURVE NO. (FIG. 1)	CONCENTRATION MG./LITER	$E_{553m\mu}$	$E_{553m\mu}$ / CONCENTRATION
1	1.03	0.260	0.252
2	2.06	0.516	0.251
3	4.11	1.033	0.251
		Average	0.251
Calculated as the hydrochloride		Average	0.233

(Extinction values are corrected for a Signal Lunar White Glass H-6946236 reading of 1.050.)

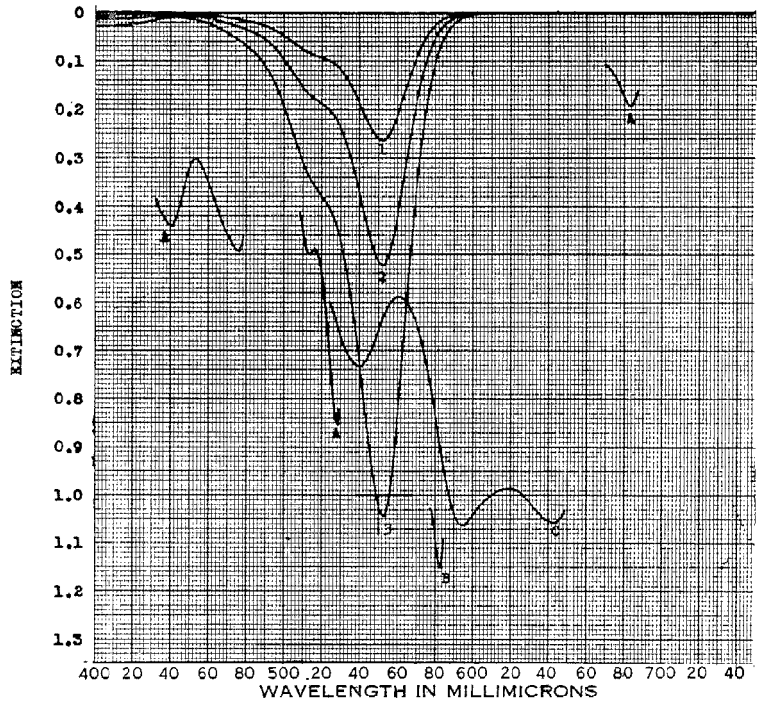

FIG. 1.—Absorption Curves of Purified D&C Red No. 19 Color Base in Dilute Ammonia.

Curve 1—1.03 mg./liter. Curve 3—4.11 mg./liter.
Curve 2—2.06 mg./liter. Cells —1 cm.

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ).
B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 mμ).
C = Signal Lunar White Glass-H-6946236.

(b) A sample of the purified D&C Red No. 19, weighed on a semimicro balance, was dissolved in exactly 1000 ml. of distilled water. Aliquot portions of this solution were then diluted with (1+99) NH_4OH to give the solutions for spectrophotometric examination. Typical results are shown in Table 3.

TABLE 3.—*Extinction values of purified D&C Red No. 19 in dilute ammonia*

CURVE NO.	CONCENTRATION MG./LITER	$E_{553m\mu}$	$E_{553m\mu}$ / CONCENTRATION
1	1.18	0.278	0.236
2	2.37	0.553	0.233
3	4.73	1.093	0.231
		Average.....	0.233

(c) A sample of the purified Rhodamine B phthalate weighed on a semimicro balance, was dissolved in exactly 1000 ml. of (1+99) HCl. Aliquot portions of this solution were then diluted with (1+49) NH_4OH to give the solutions for spectrophotometric examination. Typical results are shown in Table 4.

TABLE 4.—*Extinction values of purified Rhodamine B phthalate in dilute ammonia*

CURVE NO.	CONCENTRATION MG./LITER	$E_{553m\mu}$	$E_{553m\mu}$ / CONCENTRATION
1	1.54	0.282	0.183
2	3.08	0.564	0.183
3	6.15	1.118	0.182
		Average.....	0.183
Calculated as hydrochloride		Average.....	0.232

(d) To equal aliquots of a master solution of the color, acid, alkali or buffer was added and the solution then diluted to a definite volume. The *p*H values of these solutions were determined, using a glass electrode *p*H meter, and the absorption curve run. The results are given in Table 5 and Figure 2.

TABLE 5.—*Absorption curves of D&C Red No. 19 in aqueous solution at various p*H *levels (conc. = 3.66 mg./liter)*

CURVE NO. (FIG. 2)	*p*H	ABSORPTION PEAK	$E_{Absorbtion\ Maximum}$ / CONCENTRATION
1	13.1	553	0.233
2	7.1	553	0.233
3	6.0	553	0.233
4	3.5	554	0.228
5	1.4	556	0.206

DISCUSSION

The absorption curve of D&C Red No. 19 in neutral or basic solution shows a sharp peak at 553 ± 2 mμ with a "shoulder" at 520 mμ. In strongly acid solution the peak shifts to 556 mμ and the extinction per milligram is slightly lower.

Solutions of the color in dilute ammonia follow Beer's law to within $\pm 0.36\%$ if the effect of the fluorescence of the solutions is eliminated. When the effect of the fluorescence is not eliminated, the results at concentrations giving a density of about 1.0 may deviate as much as 4% from Beer's law. The average extinction per milligram per liter for the color in dilute ammonia solution at 553 mμ was found to be 0.233. This figure is

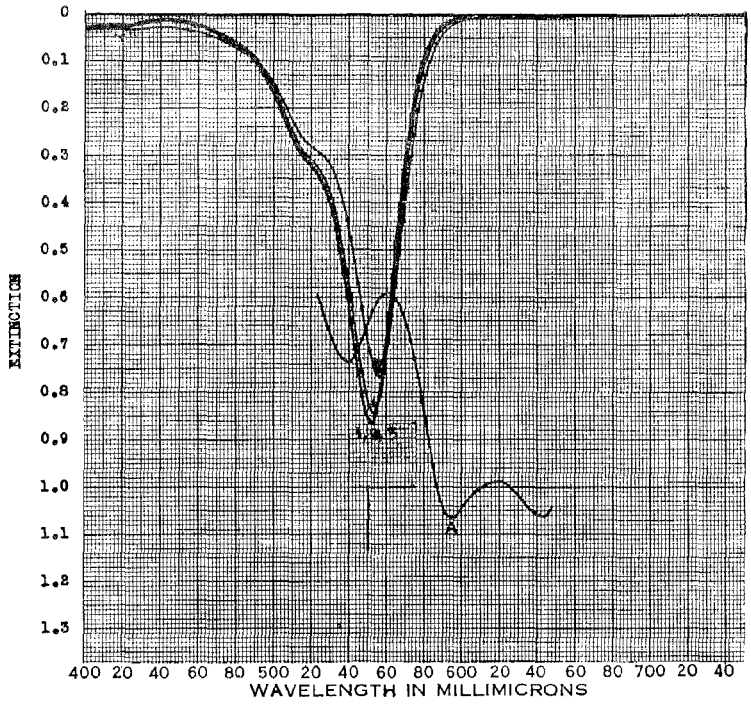

FIG. 2.—Absorption Curves of D&C Red No. 19 in Aqueous Solution at Various *p*H Levels (Conc. = 3.66 mg./liter).

Curve 1—*p*H 13.1
Curve 2—*p*H 7.1
Curve 3—*p*H 6.0
Curve 4—*p*H 3.5
Curve 5—*p*H 1.4
Cells —1 cm.

A = Signal Lunar White Glass-II-6946236.

based on the results of twenty-five determinations. The average deviation from this figure was 0.43% and the maximum deviation 1.07%.

Variation of the amount of concentrated ammonium hydroxide in the solution for spectrophotometric analysis from 1 ml to 10 ml per 100 ml of solution did not change the spectrophotometric characteristics. Solutions stored for twenty-four hours gave curves identical with those of freshly prepared solutions.

APPLICATION TO COMMERCIAL SAMPLES

Three commercial samples of D&C Red No. 19 were analyzed spectrophotometrically, following the procedure described for the standard samples. The data are shown in Table 6.

A commercial sample of D&C Red No. 19, Aluminum Lake, was analyzed spectrophotometrically by dissolving a sample in (1+19) HCl and

TABLE 6.—*Analysis of commercial samples of D&C Red No. 19*

SAMPLE	CONCENTRATION MG./LITER	$E_{553m\mu}$ (IN DILUTE AMMONIA)	DYE SPECTROPHOTOMETRICALLY	DYE BY TITRATION WITH $TiCl_3$	DYE FROM NITROGEN CONTENT
			per cent	*per cent*	*per cent*
D&C Red No. 19	4.0	0.901	96.6	Variable (97–105)	96.8
D&C Red No. 19	4.0	0.886	95.0	98.5	94.4
D&C Red No. 19	4.0	0.904	97.0	95.6	99.6
D&C Red No. 19 Al. Lake	20.0	1.126	24.2	23.0	—

diluting the solution with (1+99) NH_4OH to a suitable concentration of the dye. The result obtained is included in Table 6.

SUMMARY

Spectrophotometric data obtained from dilute aqueous solutions of purified D&C Red No. 19 are presented. Beer's law is shown to be applicable to dilute ammoniacal solutions containing one to five mg. per liter of dye. The average extinction per mg. per liter is 0.233 ± 0.001 at 553 mμ. The extinction ratio $E_{520m\mu}/E_{553m\mu}$ is 0.36 ± 0.01. These data are applied to the determination of "pure color" in commercial samples of the color and a color lake.

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DETERMINATION OF CARBON BLACK IN CHOCOLATE

By ALBERT P. SIDARAS (Walter Baker Chocolate and Cocoa Division of General Foods, Dorchester 24, Massachusetts)

SUMMARY

A method has long been needed for the determination and detection of carbon black in chocolate (when present). Such a method has been developed at the Walter Baker Laboratory.

This procedure for carbon black detection and quantitative estimation

has proven to be very satisfactory. It involves nitration, acetylation, and solvent washing, which leaves the carbon free so that it can be determined by gravimetric analysis. This method is sensitive to one part carbon black in 100,000 parts of chocolate.

INTRODUCTION

For some time some chocolate manufacturers had used carbon black in the formulation of their products, the purpose of which was to darken the chocolate without the use of dutched liquor, which imparted an undesirable flavor. The standard for chocolate products adopted by the Federal Security Administration on Dec. 6, 1944, do not include carbon black as an ingredient. For this reason it becomes an adulterant and a method for its detection and determination is required.

It was originally thought that if the cacao coloring matter could be easily removed from the chocolate, then the carbon black in the chocolate could be detected microscopically. With this in mind the following two approaches to the problem were tried but proved to be unsuccessful.

(A) Digestion with acid and alkali similar to the crude fiber procedure but with increased concentration of acid and alkali and longer digestion periods.

(B) Successive digestion with various solvents, viz, (1) diethyl ether, (2) water Soxhlet extraction, (3) acidified alcohol Soxhlet extraction, (4) refluxing with 5% sodium hydroxide, centrifuging, and decanting liquor; repeating the sodium hydroxide operation with the residue until the residue becomes colorless.

At this point it was realized that an entirely different approach would be necessary and to detect carbon all other matter would have to be removed. To this end the following three techniques were tried. These techniques were based on procedures used to determine carbon black in rubber.*

(A) Nitration with nitric acid (after fat removal) followed by water flotation.

(B) Nitration with nitric acid followed by potassium hydroxide fusion.

(C) Nitration with nitric acid followed by acetylation.

Procedure (A) was satisfactory for a qualitative but not for a quantitative test and, therefore, was discarded.

Procedure (B) was too hazardous and costly so was not given further consideration.

Procedure (C) was the method that was finally adopted as most suitable.

METHOD

Introduce into a 250 ml. flask 100 gm. of the chocolate sample in a molten state,

* *Ind. Eng. Chem. Anal. Ed.*, 9, 278 (1937).

and of temp. ca 40°C. To this add 100 ml of petroleum ether, stopper, and shake the flask until chocolate is completely dispersed in ether. Filter this mixture thru an S&S #589 Black Ribbon paper (15 cm) in a Büchner funnel. (This filter paper should be moistened first with distilled water, the excess of which should be drawn off, as it tends to retard the filtration rate.) Turn off the vacuum after the first portion of ether has passed thru. Soak the residue in the funnel and wash with 3 successive 100 ml portions of petroleum ether, and draw off the ether by vacuum after each soaking and washing. Dry the filter cake by leaving the vacuum on after the final washing. (At this point, the residue should be completely defatted. If there should be a small amount of petroleum ether present in the filter cake it will not interfere with the nitration procedure to follow.) Transfer the filter cake and paper (the paper will not interfere with the nitration) from the funnel quantitatively into a 2-liter beaker and break the filter cake into lumps the size of a pea (Hood Operation). Add 100 ml of 16 normal nitric acid all at once. Stir this mass until it is completely mixed with the acid. In about 4–5 min., the mass will start to froth and give off nitrogen dioxide. At this point place the beaker into an ice bath until the frothing stops. During this period, occasional stirring will be required so as to prevent the froth from climbing over the sides of the beaker. When all activity has ceased, remove the beaker from the ice bath (9–13 min.) and place it in a hot water bath (100°C.). Cover the beaker with a watch-glass and allow the digestion to continue until very little nitrogen dioxide fumes are given off. When this point is reached, fill a 100 ml pipet with 16 normal nitric acid and wash down the side of the beaker and the stirring rod. When the acid wash is completed, cover the beaker again and continue the acid digestion on the water bath until a small volume of fumes is given off. When this occurs, remove the beaker from the hot water bath and dilute the nitration mixture with 100 ml. of cold, distilled water. Filter this mixture completely thru an S&S #589 Green Ribbon filter paper (18.5 cm) in a Bunsen funnel. When the filtration is complete, wash the residue 3 times only, with boiling distilled water. (A fourth washing generally starts to plug the paper.) During these washings, direct the jet of water at the bottom of the filter so as to break up the pulpous skin that forms on the lower part of the filter paper. (Do not try using vacuum for this filtration as it will plug the filter.)

(If the residue at this point is gray or black, then there is definite indication that carbon black is present. For qualitative work, the procedure carried this far would be sufficient to indicate carbon.)

For quantitative results, scrape the residue from the filter paper in the qualitative procedure into a 300 ml glass-stoppered iodine number flask (Cenco #14920), and wash the remaining material on the paper into the flask with a jet of diethyl ether. Evaporate the ether on a hot water bath, using a dry air jet to facilitate the removal of ether fumes. When the evaporation is complete, scrape down the sides of the flask and finish drying any retained moisture in an oven at 120°C.

When the residue is thoroly dry, add a cold mixture of the following reagents which have been previously mixed: 75 ml 95.5% acetic acid; 75 ml acetic anhydride; and 10 ml of sulphuric acid (conc.). Stopper the flask and with occasional shaking let this mixture stand at room temp. or at an elevated temperature of 43°C. until the pulp has made complete solution. At this point, the analyst should be absolutely certain that this pulp is in complete solution for, unless it is, it will make an error in the amount of carbon black finally determined. (Several hours may be required for complete acetylation.)

Dilute this acetylated mixture with 75 ml of 99.5% of acetic acid and filter thru a Gooch crucible which has a heavy asbestos mat. (This crucible should have been previously ignited at 800°C.) After this mixture has completely filtered, wash it with 99.5% acetic acid until the filtrate shows no color; then wash it twice with

chloroform and only twice with boiling, distilled water. Dry at 120°C. for two hours or until constant. Cool in desiccator and weigh.

Ignite the crucible and residue from the acetylation in a muffle furnace at 800°C. for two hours after the furnace has come up to temperature. At the end of the ignition period, remove the crucible to a desiccator and allow it to cool, and weigh for loss. Subtract this weight from the previous weight, and the loss in weight is the carbon if all the pulp has gone into solution previously.

DISCUSSION

To save time and avoid possible error it was first thought that the filter paper might be included in the acetylation procedure. But when this procedure was followed it was found to be objectionable because of the increase in digestion time and the error caused in the final results.

The final procedure and duplicate samples of the chocolate used at Walter Baker Laboratory were submitted to the Central Laboratories of the General Foods Corporation for collaborative work and evaluation. The results checked closely.

The chocolate used in all these experiments contained 25.7 mg of carbon black per 100 g.

TABLE 1

Analyst	*Amt. of carbon in mg/100 g of choc.*
No. 1.	28.8, 30.4, 26.1, 25.1, 30.1, 26.0, 30.1, 26.0, 30.5, 26.7, 26.0, 30.5, 26.0, 30.5, 26.0, 26.7, 25.9, 26.2, 27.0, 26.5, 25.9, 26.2, 25.5, 26.3
No. 2.	25.0, 27.0, 30.5

TABLE 2

The average carbon for Analysts No. 1 and No. 2 in Laboratory 1 was 28.2 mg.

Range of Weight—5.5 mg.
Std. Deviation—1.88 mg.

CONCLUSION

This method is satisfactory for the detection and estimation of carbon black in chocolate. The accuracy of this method depends largely upon the complete digestion of the pulp during the acetylation and may be said to be ± 5 mg. if all conditions have been fulfilled.

ACKNOWLEDGMENTS

The writer acknowledges the cooperation of Mr. N. Ishler and Mr. T. P. Finucane of General Foods Central Laboratories, also Miss C. McDermott and Mr. P. J. Downey of the Walter Baker Chocolate and Cocoa Division of General Foods.

A VOLUMETRIC METHOD FOR THE DETERMINATION OF MAGNESIUM*

By L. J. HARDIN and W. H. MACINTIRE (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

It is to be expected that the trend toward guarantee of magnesium contained in fertilizers will result in the necessity for determination of that element in fertilizer control. This development will call for many additional magnesium analyses in the laboratories of manufacturers and state control officials. The factors of time and convenience then will concern all fertilizer chemists.

The conventional gravimetric method (1) for the determination of magnesium is slow. Even when several samples are analyzed simultaneously, the unit time required is large and the final result cannot be obtained conveniently in less than 30 hours of lapsed time. In case a separation from calcium is necessary, a still longer period is required. As the ignition step of the gravimetric procedure, several hours are required to bring the electric furnace to the requisite 1000°C., at which it must be held for 1 hour, and to cool and weight the ignited precipitate. Therefore, any time-saving modification in the last stage of the determination would be helpful.

The precipitation of magnesium as magnesium ammonium phosphate has been adopted universally as the analytical standard, and no modification of that step was attempted. There is a volumetric procedure, (2) however, whereby the precipitate of magnesium ammonium phosphate is filtered by gravity, washed with ammonium hydroxide, air-dried to remove ammonia and the filter then is returned to the beaker where it is titrated with 0.1 *N* acid. As an alternative, the filtration is made onto a Gooch crucible where it is washed with ammonium hydroxide and with alcohol. The precipitate is then dissolved from the crucible by means of 0.1 *N* acid and the resultant solution and washings are back titrated. Using either alternative, this procedure is somewhat shorter than the one in which the precipitate is ignited in the furnace, although the drying requires considerable time and the dried precipitate does not undergo dissolution readily in the 0.1 *N* acid.

In the attempts to shorten the time for the magnesium determinations, the final steps of the established gravimetric and volumetric procedures were subjected to four variations. These were (a) filtration by suction on a weighed fritted glass crucible of "G" porosity and drying in an atmosphere of 50 per cent relative humidity (induced by means of 43.5 per cent H_2SO_4 in a desiccator) after which the dried $MgNH_4PO_4 \cdot 6H_2O$ was weighed; (b) procedure as in (a), but dried at 40°C. and the $MgNH_4PO_4$

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

·$6H_2O$ weighed; (c) filtration by suction on a weighed fritted crucible of "G" porosity and ignition at 500°C; (d) filtration by suction on a pad of filter paper pulp in a Shimer tube, the precipitate washed five times with 1+19 NH_4OH and then four times with neutral 95 per cent alcohol. The pad that contained the alcohol-washed precipitate then was transferred from the filter tube to the flask or beaker in which the precipitation was made, and disintegrated by means of a jet of water. After the addition of 0.5 ml. of Bromcresol green indicator (0.2 per cent) and a 5 ml. excess of 0.1 *N* acid (HCl or H_2SO_4) the thoroughly mixed solution-suspension was allowed to stand 15 minutes in an atmosphere free of fumes, and then back titrated with 0.1 *N* NaOH (0.05 *N* NaOH may be preferred). Appearance of the initial medium green (*p*H 4.6) has been found to be the correct end-point. A reference color standard, prepared with the same amount of indicator and filter paper suspension, is helpful in recognizing the end-point. The net titer of 0.1 *N* acid times 2.015 gives *mgm* of MgO.

ANALYTICAL RESULTS

The results presented in Table 1 were obtained by means of the several modifications in the analysis of aliquots of a 5-gram per liter solution of magnesium nitrate prepared by use of the hexahydrate. The com-

TABLE 1.—*Comparison of results obtained by means of four modified procedures for the determination of magnesium with those obtained by means of the standard gravimetric and volumetric procedures*

	MgO VALUES					
ADDED (a)	GRAVIMETRIC (b)	VOLUMETRIC (c)	METHOD I (d)	METHOD II (e)	METHOD III (f)	METHOD IV (g)
mgms	*mgms*	*mgms*	*mgms*	*mgms*	*mgms*	*mgms*
19.63	19.99	19.60	19.71	19.14	20.09	19.92
19.63	19.70	19.60	20.58	18.53	20.09	19.80
19.63	20.27	—	19.35(h)	19.17	20.38	19.80
19.63	—	—	—	—	19.80	19.80
19.63	—	—	—	—	19.29	91.90(i)
19.63	—	—	—	—	—	20.00(i)
Average	19.99	19.60	19.88	18.94	19.93	19.87
Time required (j)	7 hrs.	3 hrs.	25 hrs.	25 hrs.	3 hrs.	30 min.

(a) Computed from formula $Mg(NO_3)_2 \cdot 6H_2O$.
(b) Precipitated $MgNH_4PO_4 \cdot 6H_2O$ was filtered by gravity and ignited 1 hr. at 1000°C.
(c) The precipitated $MgNH_4PO_4 \cdot 6H_2O$ was filtered on paper, dried and titrated with 0.1 N acid, using bromcresol green indicator.
(d) Dried 24 hrs. over H_2SO_4 at 50% relative humidity (fritted crucible).
(e) Dried 24 hrs. at 40°C. (fritted crucible).
(f) The precipitated $MgNH_4PO_4 \cdot 6H_2O$ was filtered on fritted crucible and ignited 1 hr. at 500°C.
(g) The precipitated $MgNH_4PO_4 \cdot 6H_2O$ was filtered on Shimer filter with suction, washed with 1+19 NH_4OH, then with alcohol, and titrated with 0.1 *N* acid, using bromcresol green.
(h) The precipitate was exposed 72 hours.
(i) The determination was made after single precipitation.
(j) For completion of determination after the final filtration of magnesium ammonium phosphate.

parisons given in Table 2 were obtained when the proposed procedure was used in the analysis of acid-digested charges of magnesium-fortified fertilizer mixtures that had been analyzed by the gravimetric method. The conventional double-precipitations were used, except as noted.

DISCUSSION

The findings by the conventional gravimetric method were slightly higher than the theoretical values. Similar values were obtained through

TABLE 2.—*Magnesium content of fertilizer mixtures as determined by the proposed volumetric procedure*

	MgO VALUES					
	1		2		3	
	mgms	*per cent*	*mgms*	*per cent*	*mgms*	*per cent*
Added in aliquot (a)	8.68	21.70	9.00	9.00	4.81	4.81
Determined, gravimetrically (b)	8.68	21.70	9.00	9.00	9.62	4.82
Determined, volumetrically (c)	8.60	21.50	8.90	8.90		
			9.20	9.20		
Added in aliquot (a)	21.70	21.70	18.00	9.00	9.62	4.81
Determined, volumetrically (c)	21.60	21.60	17.70	8.85	9.40	4.70
	21.50	21.50	—	—	9.00	4.50
					9.50	4.75
					9.60	4.80
					9.40	4.70
					9.30(d)	4.65(d)
					9.60(d)	4.80(d)
					9.30(d)	4.65(d)

(a) Calculated from gravimetric values.
(b) Average of check determinations.
(c) Final filtration was made on Shimer suction filter; precipitate was washed 5 times with 1+19 NH_4OH, then 4 times with small portions of 95% ethyl alcohol.
(d) Same as (c) with the exception that 1+9 methyl-ethyl alcohol corresponding to "Formula 30" was used instead of the 95% ethyl alcohol.

the use of the fritted crucibles and ignition at 500°C.; care must be used, however, to avoid breakage of the crucibles through sudden cooling.

The results obtained by means of the conventional volumetric procedure were in agreement with the theoretical values, when the filter paper and precipitates were air-dried to eliminate ammonia prior to the titration. When compared to the usual ignition, however, this volumetric procedure did not effect appreciable saving of time and the air-dried precipitate did not dissolve readily in 0.1 *N* acid.

The precipitates that were filtered on a fritted crucible and dried in an atmosphere of 50 per cent relative humidity did not reach constant weight in 24 hours. The period of exposure then was extended to 72 hours, after which the weights were virtually constant, and the findings were in satisfactory agreement with the established values. This procedure, however, did not afford the desired rapidity.

The lowest values were those obtained when the precipitate was dried 24 hours at 40°C. Since constant weight was not obtained in briefer periods of drying, no time advantage was obtained through the use of this procedure.

Only one-half hour was required for the entire operation of filtration of the precipitated $MgNH_4 \cdot 6H_2O$ on a Shimer filter, washing with 1+19 NH_4OH and then with 95 per cent alcohol, return to the precipitation flask or beaker, period of standing and titration. The resultant values were in agreement with the theoretical values and with those obtained by the gravimetric procedure. Repeated determinations gave satisfactory checks. Since the results through single precipitations also were in close agreement with established values, it appears that double precipitation may be omitted, unless unusual accuracy is required.

Filtration upon a Shimer filter and titration of the alcohol-washed, moist precipitate is deemed preferable to either the conventional gravimetric or the volumetric method. When only a few determinations are to be made at a time, the gravimetric method is particularly inconvenient and high in unit time requirement. The step of gravity filtration is slow, an electric furnace is required and from 5 to 6 hours is necessary to attain 1000°C. requisite for a 1-hour ignition, whereas additional time is required for the cooling and the weighing of the crucibles.

In contrast, the proposed rapid procedure of filtration through a pad of pulped filter paper in the Shimer filter and titration of the alcohol-washed precipitate is equally adaptable to a single analysis and to the multiple routine determinations that may become necessary in case magnesium becomes designated as a guaranteed element in fertilizers. Satisfactory results were obtained when the 1+9 methyl-ethyl mixture was used in lieu of 95% ethyl alcohol. This mixture corresponds to the commercial, tax-free, denatured product, "Formula 30."

SUMMARY AND CONCLUSIONS

The conventional steps for the analytical preparation, purification and precipitation of magnesium as magnesium ammonium phosphate as prescribed in the "official" method (1) were used in the present comparison between four modified techniques and the conventional gravimetric and volumetric procedures. The proposed modifications in technique relate solely to the treatment of the precipitate of magnesium ammonium phosphate.

When dried at 40°C., the $NH_4MgPO_4 \cdot 6H_2O$ apparently underwent slight dehydration, whereas complete drying was not effected through a 24-hour exposure in an atmosphere of 50 per cent relative humidity. The time required for desiccation was inadmissible in both cases.

Filtration through a fritted crucible and subsequent ignition of the precipitate at 500°C. was time saving, in comparison with the gravity

filtration and ignition in the electric furnace. However, because of the high breakage of crucibles, this technique is not deemed practical.

The proposed volumetric procedure prescribes filtration of the magnesium ammonium phosphate precipitate upon a pad of filter paper pulp on a Shimer filter, washing the beaker and the pad with dilute NH_4OH and then with either 95 per cent neutral alcohol or reagent "Formula 30." After the washed pad is returned to the beaker and dispersed by means of a fine jet of water, the magnesium content is determined by dissolution of the precipitate in a slight excess of 0.1 *N* acid and back titration with 0.1 *N* NaOH against bromcresol green indicator. When several samples are run concurrently, the 30-minute requirement per determination can be decreased to as little as 10 minutes.

The results obtained through the use of the proposed procedure were in agreement with those obtained by means of the conventional gravimetric determination.

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THE EVALUATION OF ROTENONE IN DERRIS ELLIPTICA ON THE BASIS OF TOTAL CHLOROFORM EXTRACTIVES

By CALEB PAGAN and DAVID G. WHITE[1]

The use of substitutes for rotenone in the past 5 years has not been entirely satisfactory for the control of many insect pests according to the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture (2). It is predicted that rotenone may be in greater demand than heretofore. The selection of high-yielding plants is often a problem because of the rather complicated analysis required to determine rotenone content. A simpler method is to determine the content of rotenone plus rotenoids colorimetrically; this value is closely correlated with the actual rotenone content (1).

The present paper deals with a critical evaluation of data from three experiments with respect to rotenone content, rotenone plus rotenoids, and total chloroform extractives of roots from different varieties of *Derris elliptica* (Wall.) Benth. and of *Lonchocarpus utilis* A. C. Smith. In all cases, the roots were air-dried to constant weight and then ground in a Wiley mill through a 0.5 millimeter screen. Rotenone analyses were

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made according to the official method.[2] Rotenone plus rotenoids were determined colorimetrically as described by Jones (1). The total chloroform extractives were determined by evaporating 10-milliliter aliquots of chloroform extracts, used in rotenone determinations, on a steam bath followed by drying in an oven at 105°C. to constant weight. Determinations of the total extractives required less equipment and time than either of the other tests.

The data were expressed on a percentage basis and the ratios of rotenone to rotenone plus rotenoids, and of rotenone to total chloroform extractives, were calculated. The ratios were analyzed statistically to establish the standard deviations from the means and the data are summarized in Table 1.

TABLE 1.—*The ratios and standard deviations of rotenone to rotenone plus rotenoids and to total extractives in roots of Derris elliptica, from three experiments*

PLANTS REPRESENTED—VARIETIES AND CLONES	AGE AT HARVEST	RANGE IN ROTENONE	RATIO OF ROTENONE TO ROTENONE PLUS ROTENOIDS		RATIO OF ROTENONE TO TOTAL EXTRACTIVES	
			MEAN	STANDARD DEVIATION[4]	MEAN	STANDARD DEVIATION[4]
	Months	*per cent*				
Varietal experiment[1]						
Sarawak Creeping	25–29	3.6–5.8	0.374	0.031	0.340	0.030
St. Croix	25–29	1.2–2.0	.389	.025	.333	.023
Río Piedras Changi III	25–29	3.3–4.2	.552	.023	.479	.021
All 3 varieties	25–29	1.2–5.8	.438	.048	.384	.106
Clonal experiment[2]						
MG Changi III	36	6.2–8.7	.489	.034	.436	.016
Harvesting experiment[3]						
Sarawak Creeping	14	3.5–6.6	.344	.027	.286	.024
Sarawak Creeping	20	3.2–6.3	.389	.024	.341	.021
Sarawak Creeping	26	3.4–7.4	.403	.029	.361	.019
Sarawak Creeping	32	4.0–6.3	.366	.024	.332	.018
All 4 ages at harvest	14–32	3.2–7.4	.375	.036	.330	.044

[1] Reported by Jones, Merriam A., and Pagan, Caleb. A comparison of three varieties of *Derris elliptica*. *Trop. Agr.* 23(4): 76–80, 1946.

[2] Reported by Jones, Merriam A., White, David G., and Pagan, Caleb. Evaluation of some clones of *Derris elliptica*. *Trop. Agr.* 23(5): 89–93, 1946.

[3] Reported by White, David G., Pagan, Caleb, and Jones, Merriam A. Production of *Derris elliptica* in relation to type of cutting and age at harvest. *J. Agr. Research* (In press).

[4] Standard deviations were calculated using the following formula:

$$\text{S.D.} = \sqrt{\text{variance}\left(\frac{y}{x}\right)} = \sqrt{\frac{\bar{y}^2}{\bar{x}^2}\left[\frac{(\text{Var. } \bar{y}}{\bar{y}^2} + \frac{\text{Var. } x}{\bar{x}^2} - \frac{2\ \text{Covar. } xy)}{\bar{x}\bar{y}}\right]}$$

Where y = individual rotenone content and x = individual rotenone + rotenoids content (or total extractives as the case may be); and $\bar{x}$ = mean of x and $\bar{y}$ = mean of y.

[2] *Methods of Analysis, A.O.A.C.*, 5th ed. (1940), p. 64.

The rotenone content of the roots in the three experiments ranged from 1.2 to 8.7 per cent, total extractives from 3.8 to 21.4 per cent, and rotenone plus rotenoids from 3.3 to 17.0 per cent.

It is of interest to note the good agreement between the mean ratios of Sarawak Creeping in the varietal and harvesting experiments and between the high yielding MG Changi No. 3 clones and the low yielding Río Piedras clones. If we consider the case of same clonal material of one variety, the agreement is even closer. For example, cuttings from the MG Changi No. 3 clones were propagated in the field and at the end of 2 years the roots of 32 plants were dug and analyses made for rotenone, total chloroform extractives, and rotenone plus rotenoids. The mean ratio of rotenone to total extractives was found to be 0.437 as compared with 0.436 for the original material.

These results indicate that the ratio of rotenone to rotenone plus rotenoids, or to the total chloroform extractives, is reasonably constant among varieties, although the least deviation occurred within a variety. Within the Sarawak Creeping variety there were small deviations from the mean ratios of roots harvested at 14 to 32 months but for all practical purposes the ratios will hold with reasonable accuracy between these age limits.

Samples of roots of *Lonchocarpus utilis* selected at random from plants grown in Peru were similarly analyzed.[3] The rotenone content of these plants ranged from 2.3 to 6.6 per cent. In 49 sample plants the mean ratio of rotenone to rotenone plus rotenoids was found to be 0.447 ± 0.039, and the mean ratio of rotenone to total extractives was found to be 0.364 ± 0.024.

Under uniform field conditions, determination of rotenone for each of a large number of samples may not be necessary. Duplicate analyses of rotenone in a composite sample should be made, together with total chloroform extractives, and a ratio established. The rotenone content of individual samples can then be based upon total chloroform extractives. Determination of total chloroform extractives in each sample appears to be the easiest and quickest method of evaluating large numbers of samples with reasonable accuracy.

SUMMARY

(1) A comparison has been made of the ratios of rotenone to total chloroform extractives, and of rotenone to red color value, for roots of *Derris elliptica* plants.

(2) A simple and rapid method of estimating the rotenone content of roots of *Derris elliptica* based on total chloroform extractives, is presented.

[3] Plants collected by E. C. Higbee, Office of Foreign Agricultural Relations, United States Department of Agriculture.

(3) Less extensive data are presented for *Lonchocarpus utilis* which show that total chloroform extractives may give a good approximation of the rotenone content of these plants.

LITERATURE CITED

(1) JONES, MERRIAM A., 1945. "The application of a modified red-color test for rotenone and related compounds to *Derris* and *Lonchocarpus*." *This Journal*, 28, 352–359 (1945)

(2) ROARK, R. C. 1946. Abstracts of recent patents relating to rotenone. E-706 (Processed).

BOOK REVIEW

Standard Methods for the Examination of Dairy Products, Ninth Edition. Published by the American Public Health Association, 1790 Broadway, New York City 19, N. Y. (1948). 6×9½ inches, 373 pages, index, 37 figures, cloth. Price $4.00.

The manual contains analytical procedures to guide regulatory agencies charged with determining and maintaining the healthfulness of dairy products. It is of equal value to industrial workers desiring to have their products conform to standards of wholesomeness.

The methods deal chiefly with sanitary (bacteriological) determinations, pages 1–221 and 336–343. To satisfy additional needs in public health laboratories, the Association has been granted permission to include certain chemical procedures and filth methods for dairy products as recognized in *Official and Tentative Methods of Analysis*, Sixth Edition, by the Association of Official Agricultural Chemists; and certain vitamin assay procedures as recognized in the *United States Pharmacopoeia*, Twelfth Revision, including the First Bound Supplement thereto, by the Board of Trustees of the United States Pharmacopoeial Convention.

The Ninth Edition is characterized distinctively by an orderly separation in Chapter 1 of directions for guidance of administrative officials. Succeeding chapters outline aseptic sampling procedures and directions for the microbiological examination of fluid milk and cream, butter, cheese, frozen desserts and the ingredients therein; for sterility tests to be applied to dairy equipment; for residual phosphatase in heat-treated products; for vitamin assays; for sediment and extraneous matter; and for certain chemical determinations. Assembled in the last chapter are certain "Screening Methods," the use of which will provide administrators with more continuous information about the sanitary character and the chemical composition of certain dairy products than could be obtained with identical funds and personnel using the more refined, time-consuming methods. Obviously, the results of tests obtained by use of Screening Methods are not regarded as satisfcatory in case litigation is required.

The new format makes the manual distinctively helpful to laboratory workers also. This has been accomplished by maintaining uniformity of arrangement and style, by using a system of simple cross-references, and by improving the index. While there has been some sacrifice of historical matter, bibliographical references supply abundant data for the critical investigator or administrator.

A. H. ROBERTSON

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