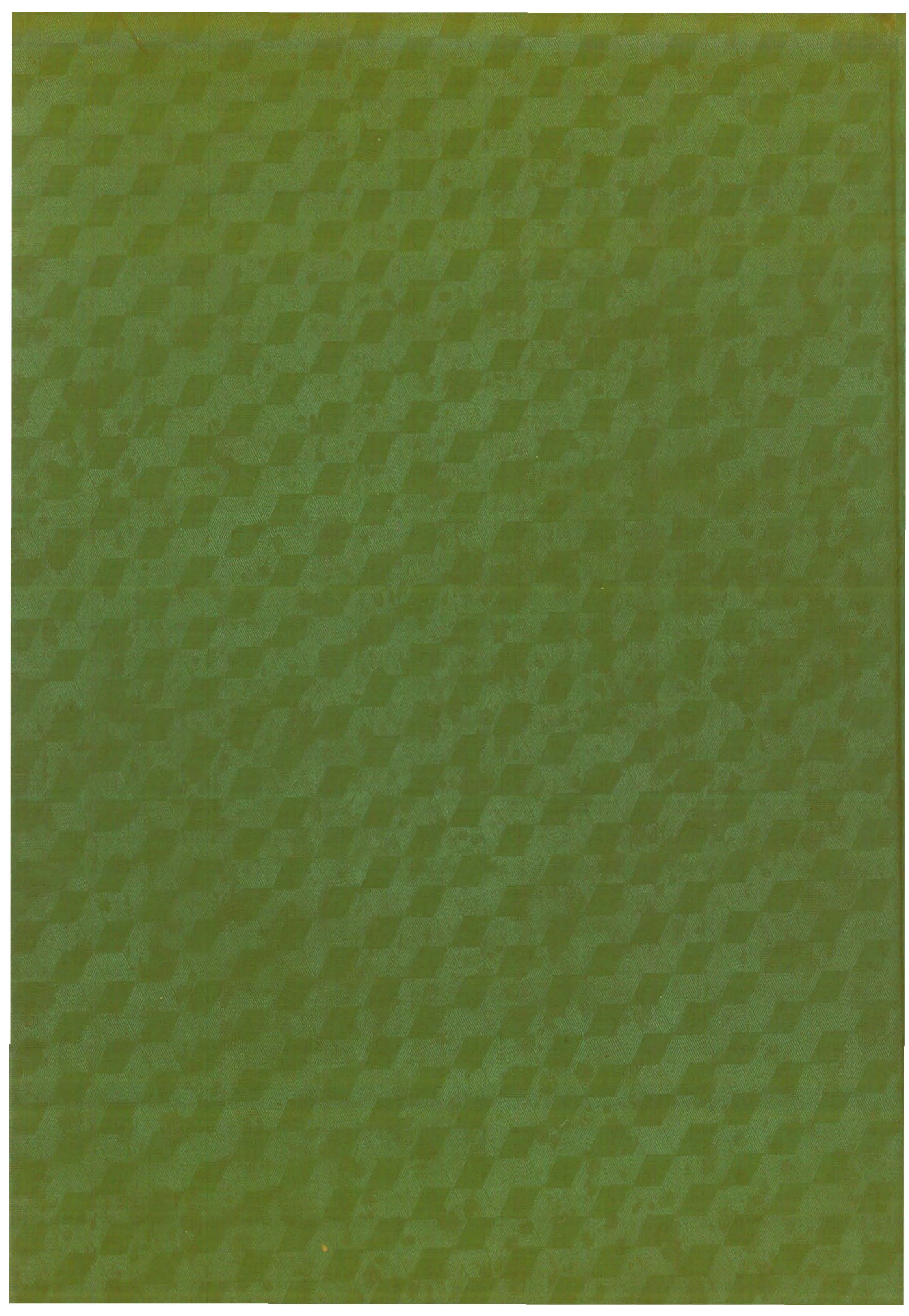

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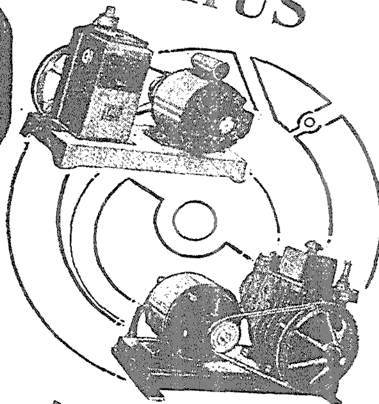

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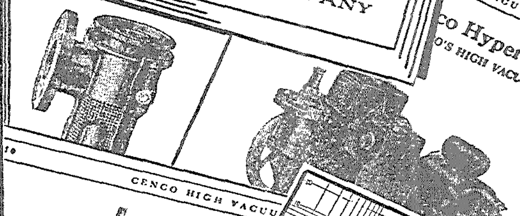

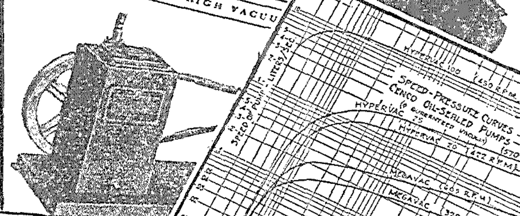

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The 62nd Annual Convention of the Association of Official Agricultural Chemists will be held October 11 to 13, inclusive, at the Shoreham Hotel, 2500 Calvert Street, N.W., Washington 8, D.C.

Hotel accommodations may not be readily obtainable even by next fall; and reservations should be arranged for directly with the hotel and well in advance of the meeting.

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METHODS OF ANALYSIS, 6th edition, 1945

The list price of the new edition will be $6.25, domestic postpaid.

As this number of the *Journal* goes to press, we learn with sorrow of the sudden death of Dr. E. M. Bailey, which occurred on April 13, 1948, at New Haven, Conn.

Dr. Bailey served for many years as Chairman of the Editorial Committee of Methods of Analysis, and of other permanent and special committees; and as President of the Association. His loss will be particularly felt by its members. An obituary will be presented in the next number of the *Journal.*

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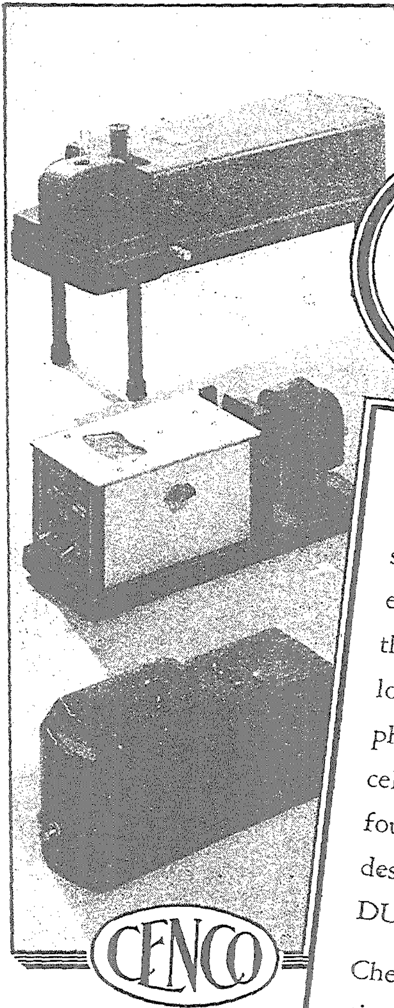

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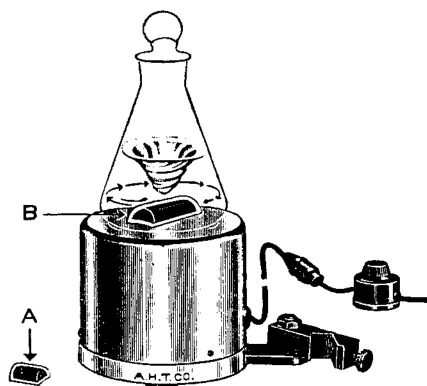

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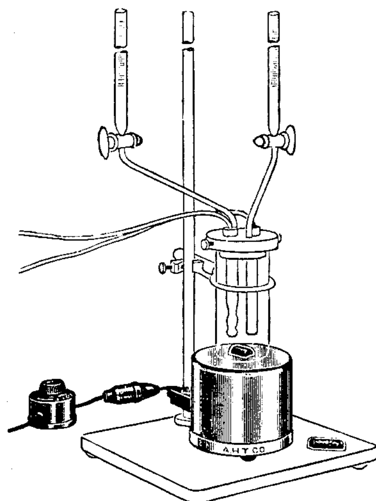

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PROCEEDINGS OF THE SIXTY-FIRST ANNUAL MEETING OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1947

The sixty-first annual meeting of the Association of Official Agricultural Chemists was held at the Shoreham Hotel, Washington, D. C., October 20, 21, and 22, 1947.

The meeting was called to order by the President, J. O. Clarke, on the morning of October 20, at 10:00 o'clock.

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- Lactose in mixed feeds
- Fat in fish meal
- Adulteration of condensed milk products
- Crude fat or ether extract
- Activity of yeast
- Microscopic examination
- Fluorine
- Mineral constituents of mixed feeds
- Crude fiber
- Protein evaluation in fish and animal products
- Hydrocyanic acid glucosides
- Sampling and analysis of condensed buttermilk
- Tankage (hide, hoof, horn, and hair content)

Fertilizers
- Sampling
- Phosphoric acid
- Moisture
- Nitrogen
- Magnesium and manganese
- Acid- and base-forming quality
- Potash and platinum recovery methods
- Sulfur
- Copper and zinc
- Boron

Economic poisons
- Tetra ethyl pyrophosphate
- Herbicides
- Rodenticides
- Benzene hexachloride
- DDT
- Insecticides containing derris or cubé
- Oil emulsions

Disinfectants

Leathers and tanning materials

Plants
- Sampling
- Iodine and boron
- Sugar
- Zinc
- Copper and cobalt
- Carotene
- Sodium
- Cellulose and lignin
- Pectin
- Starch

Spectrographic methods

Soils and liming materials
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- Boron and fluorine
- Zinc and copper
- Exchangeable calcium and magnesium
- Exchangeable hydrogen
- Exchangeable potassium
- Phosphorus

Standard solutions
- Potassium dichromate solutions
- Buffer solutions
- Titanium trichloride solutions

Vitamins
- Vitamin A
- Vitamin B_1
- Vitamin C
- Vitamin D—poultry
- Riboflavin (fluorometric)
- Nicotinic acid
- Carotene
- Pantothenic acid
- Folic acid

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Naval stores
Radioactivity
  Quantum counter
Vegetable drugs and their derivatives
  Chemical methods for ergot alkaloids
  Physostigmine in ointments
  Theobromine and phenobarbital
  Aminopyrine, ephedrine, and phenobarbital
  Quinine
  Chemical methods for penicillin
  Rutin in tablets
  Ethylmorphine in syrups
Synthetic drugs
  Methylene blue
  Sulfanilamide derivatives
  Propadrine hydrochloride
  Carbromal
  Butacaine sulfate
  Spectrophotometric methods
  Trichloroethylene
  Thiouracil
  Phenolphthalein in chocolate preparations
  Pyribenzamine and benadryl
  Synthetic estrogens
Miscellaneous drugs
  Microscopic tests for alkaloids and synthetics
  Mercury compounds
  Organic iodides and separation of halogens
  Alkali metals
  Glycols and related compounds
  Preservatives and bacteriostatic agents in ampul solutions
  Phosphorus, calcium, and iron in vitamin preparations
  Iodine
  Estrone and estradiol
  Chromatographic separation of drugs
  Methyl alcohol
Cosmetics
  Alkalies in cuticle removers
  Hair straighteners
  Cosmetic creams
  Cosmetic powders
  Cosmetic skin lotions
  Deodorants and anti-perspirants
  Depilatories
  Hair dyes and rinses
  Mascara, eyebrow pencils, and eye shadow
  Mercury salts in cosmetics
  Moisture in cosmetics
  Pyrogallol in hair dyes
Coal-tar colors
  Acetates, carbonates, halides, and sulfates in certified coal-tar colors
  Buffers and solvents in titanium trichloride titrations
  Ether extract in coal-tar colors
  Halogens in halogenated fluoresceins
  Identification of certified coal-tar colors
  Volatile amine intermediates in coal-tar colors
  Non-volatile unsulfonated amine intermediates in coal-tar colors
  Sulfonated amine intermediates
  Unsulfonated phenolic intermediates
  Sulfonated phenolic intermediates
  Intermediates derived from phthalic acid
  Mixtures of coal-tar colors for drug and cosmetic use
  Lakes and pigments
  Spectrophotometric testing of coal-tar colors
  Subsidiary dyes in D&C colors
  Lead in coal-tar colors
  Lead in lakes of coal-tar colors
  Subsidiary dyes in FD&C colors
  Hygroscopic properties of coal-tar colors

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Processed vegetable products
- Quality factors
- Moisture in dried vegetables
- Catalase in frozen vegetables
- Peroxidase in frozen vegetables

Fill of container methods (foods, drugs, and cosmetics)

Coffee and tea

Coloring matters in foods

Dairy products
- Phosphatase test in dairy products
- Ash in milk and evaporated milk
- Sampling, fat, and moisture in cheese
- Frozen desserts
- Chlorine in milk
- Acidity of milk
- Preparation of butter samples
- Tests for reconstituted milk
- Sour serum test
- Fat in dairy products

Eggs and egg products
- Added glycerol
- Acidity of fat

Extraneous materials in foods and drugs
- Drugs, spices, and miscellaneous materials
- Dairy products
- Nut products and confectionery
- Baked products, cereals, and egg products
- Fruit products and beverage materials
- Vegetable products
- Sediment test

Decomposition in foods
- Fish products
- Dairy products
- Shellfish
- Apple products

Gelatine, dessert preparations, and mixes
- Jelly strength

Fish and other marine products
- Total solids and ether extract

Gums in foods
- Cheese
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- Creatin in meat products

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- Copper
- Zinc
- Mercury
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- DDT in canned foods

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- Canned fishery products
- Canned meats
- Canned acid foods
- Canned vegetables
- Eggs and egg products
- Nuts and nut products
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Microchemical methods
- Elemental analysis

Nuts and nut products

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- Quarternary ammonium compounds
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- Dichloroacetic acid
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- Dulcin
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Spices and other condiments
- Vinegar
- Volatile oil in spices
- Sugar, ash, and pungent principles in mustards
- Preparation of sample, and fat in mayonnaise and salad dressing

SUBCOMMITTEE D: C. S. FERGUSON (1948) (State Department Public Health, Boston, Mass.), *Chairman;* KENNETH L. MILSTEAD (1950), and J. Walter Sale (1952).

Alcoholic beverages
- Hops
- Yeast (total and yeast solids)
- Inorganic elements in beer
- Color and turbidity in beer
- Distilled spirits (obscuration test for proof and test for aging)
- Chromatographic absorption of wines
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- Fusel oil in distilled spirits (rapid method)
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- Proteolytic activity of flour
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- Soybean flour in foods (immunological tests)
- Phosphated flour
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- Titration of acids (electrometric)
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- Unfermented reducing substances in molasses
- Drying methods
- Densimetric and refractometric methods
- Honey (free acid and adulterants)
- Confectionery
- Reducing sugars
- Corn sirup and corn sugar
- Color and turbidity in sugar products

Waters, brine, and salt
- Boron in water
- Fluorine in salt

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Subcommittee A

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CRUDE FAT OR ETHER EXTRACT:
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* Subjects listed are only those for which appointments have been made. See page 1 for complete list of subjects. New appointments as made will be announced in the JOURNAL.

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FOLIC ACID:
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Referee: L. F. Curtiss, National Bureau of Standards, Washington 25, D. C.
QUANTUM COUNTER:
Anna E. Mix, Food and Drug Administration, Washington 25, D. C.

VEGETABLE DRUGS AND THEIR DERIVATIVES:
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CHEMICAL METHODS FOR ERGOT ALKALOIDS:
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THEOBROMINE AND PHENOBARBITAL:
Daniel Banes, Food and Drug Administration, Chicago 7, Ill.
AMINOPYRINE, EPHEDRINE, AND PHENOBARBITAL:
C. D. Wright, Food and Drug Administration, Washington 25, D. C.
QUININE:
D. J. Miller, Food and Drug Administration, Buffalo 3, N. Y.
CHEMICAL METHODS FOR PENICILLIN:
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RUTIN IN TABLETS:
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Referee: F. C. Sinton, Food and Drug Administration, New York 14, N. Y.
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SULFANILAMIDE DERIVATIVES:
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CARBROMAL:
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BUTACAINE SULFATE:
L. H. Welsh, Food and Drug Administration, Washington 25, D. C.
SPECTROPHOTOMETRIC METHODS:
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TRICHLORETHYLENE:
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MISCELLANEOUS DRUGS:
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MICROSCOPIC TESTS FOR ALKALOIDS AND SYNTHETICS:
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MERCURY COMPOUNDS:
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ORGANIC IODIDES AND SEPARATION OF HALOGENS:
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HAIR DYES AND RINSES:
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PYROGALLOL IN HAIR DYES:
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COAL-TAR COLORS:
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ETHER EXTRACT IN COAL-TAR COLORS:
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HALOGENS IN HALOGENATED FLUORESCEINS:
N. Gordon, Food and Drug Administration, Washington 25, D. C.
IDENTIFICATION OF CERTIFIED COAL-TAR COLORS:
K. A. Freeman
VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS:
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NON-VOLATILE UNSULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:
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SULFONATED AMINE INTERMEDIATES IN COAL-TAR COLORS:
N. Ettlestein, Food and Drug Administration, Washington 25, D. C.

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SULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS:
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MIXTURES OF COAL-TAR COLORS FOR DRUG AND COSMETIC USE:
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LAKES AND PIGMENTS:
K. A. Freeman
SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS:
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HYGROSCOPIC PROPERTIES OF COAL-TAR COLORS:
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Subcommittee C

PROCESSED VEGETABLE PRODUCTS:
Referee: V. B. Bonney, Food and Drug Administration, Washington 25, D. C.
QUALITY FACTORS:
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MOISTURE IN DRIED VEGETABLES:
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CATALASE IN FROZEN VEGETABLES:
B. M. Gutterman, Food and Drug Administration, Washington 25, D. C.
PEROXIDASE IN FROZEN VEGETABLES:
M. A. Joslyn, College of Agr. Univ. of Calif., Berkeley 4, Calif.

FILL OF CONTAINER METHODS (FOODS, DRUGS, AND COSMETICS):
Referee: Sumner C. Rowe, Food and Drug Administration, Washington 25, D. C.

COFFEE AND TEA:
Referee: Mary E. Ruffe, Food and Drug Administration, Chicago 7, Ill.

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

DAIRY PRODUCTS:
Referee: Guy G. Frary, State Chemical Laboratory, Vermillion, S. Dak.
PHOSPHATASE TEST IN DAIRY PRODUCTS:
George P. Sanders, Bur. Dairy Industry, U. S. Department of Agriculture, Washington 25, D. C.
ASH IN MILK AND EVAPORATED MILK:
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SAMPLING, FAT, AND MOISTURE IN CHEESE:
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CHLORINE IN MILK:
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ACIDITY OF MILK:
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PREPARATION OF BUTTER SAMPLES:
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TESTS FOR RECONSTITUTED MILK:
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SOUR SERUM TEST:
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FAT IN DAIRY PRODUCTS:
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EGGS AND EGG PRODUCTS:
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ADDED GLYCEROL:
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ACIDITY OF FAT:
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EXTRANEOUS MATERIALS IN FOODS AND DRUGS:
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DRUGS, SPICES, AND MISCELLANEOUS MATERIALS:
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NUT PRODUCTS AND CONFECTIONERY:
W. G. Helsel, Food and Drug Administration, Washington 25, D. C.
BAKED PRODUCTS, CEREALS, AND EGG PRODUCTS:
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FRUIT PRODUCTS AND BEVERAGE MATERIALS:
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VEGETABLE PRODUCTS:
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DECOMPOSITION IN FOODS:
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DAIRY PRODUCTS:
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SHELLFISH:
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F. Leslie Hart
CACAO PRODUCTS:
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MAYONNAISE AND FRENCH DRESSING:
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MEAT AND MEAT PRODUCTS:
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SOYBEAN FLOUR IN MEAT PRODUCTS:
O. L. Bennett, Meat Inspection Division, Bureau of Animal Industry, Washington 25, D. C.

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.
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ZINC:
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MERCURY:
A. K. Klein
DDT AS SPRAY RESIDUE ON FOODS:
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DDT IN CANNED FOODS:
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CANNED ACID FOODS:
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CANNED VEGETABLES:
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EGGS AND EGG PRODUCTS:
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NUTS AND NUT PRODUCTS:
William R. North, Food and Drug Administration, Washington 25, D. C.
FROZEN FRUITS AND VEGETABLES:
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Nuts and Nut Products:
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Oils, Fats, and Waxes:
Referee: J. Fitelson, Food and Drug Administration, New York 14, N. Y.
Unsaponifiable Matter:
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Peanut Oil:
Gardner Kirsten
Antioxidants:
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Preservatives and Artificial Sweeteners:
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Saccharin:
Margarethe Oakley
Quarternary Ammonium Compounds:
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Dichloroacetic Acid:
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Mold-inhibitors, Propionates:
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Thiourea:
W. O. Winkler, Food and Drug Administration, Washington 25, D. C.
Dulcin:
L. C. Andrews, State Dept. of Health, New Orleans 7, La.

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:
Dan Unger, Food and Drug Administration, New York 14, N. Y.
Sugar, Ash, and Pungent Principles in Mustards:
F. M. Garfield, Food and Drug Administration, St. Louis 1, Mo.
Sampling, and Fat in Mayonnaise and Salad Dressing:
Sam D. Fine, Food and Drug Administration, Cincinnati 2, Ohio

Subcommittee D

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.

INORGANIC ELEMENTS IN BEER:
Gordon H. Bendix, 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:
M. A. Amerine

CACAO PRODUCTS:
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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:
Frank V. Kenney, Jr., 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:
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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
CARBON DIOXIDE IN SELF-RISING FLOUR:
R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill.
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):
N. H. Walker
BROMATES IN FLOUR:
W. F. Geddes, University of Minnesota, University Farm, St. Paul, Minn.
PHOSPHORUS:
V. E. Munsey
UNSAPONIFIABLE MATTER AND STEROLS IN CEREAL FOODS CONTAINING EGGS:
L. W. Ferris, Food and Drug Administration, Buffalo, N. Y.

BAKING POWDERS AND BAKING CHEMICALS:
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EMULSION FLAVORS:
John B. Wilson
MAPLE FLAVOR CONCENTRATES AND IMITATIONS:
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FRUIT ACIDS:
Fred Hillig, Food and Drug Administration, Washington 25, D. C.
FRUIT AND SUGAR IN FROZEN FRUIT:
R. A. Osborn
WATER-INSOLUBLE SOLIDS:
R. A. Osborn

SUGARS AND SUGAR PRODUCTS
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Lester D. Hammond, National Bureau of Standards, Washington 25, D. C.
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C. F. Snyder
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George P. Walton, Production and Marketing Administration, Beverage and Miscellaneous Foods Division, Washington 25, D. C.

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COLOR AND TURBIDITY IN SUGAR PRODUCTS:
J. F. Brewster, National Bureau of Standards, Washington 25, D. C.

WATERS, BRINE, AND SALT:
Referee: Anna E. Mix, Food and Drug Administration, Washington 25, D. C.
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PRESIDENT'S ADDRESS*

THE DETECTION AND ESTIMATION OF FILTH AND DECOMPOSITION IN FOODS

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Consumers have always wanted clean food. Congress, recognizing the need for consumer protection, enacted the Food and Drugs Act of June 30, 1906, which defines a food as adulterated "if it consists in whole or in part of a filthy, decomposed, or putrid animal or vegetable substance. . . . " Similar definitions are found in many State laws and city ordinances. While the 1906 Federal law used the words "Filthy, decomposed, or putrid," other laws employed such terms as "tainted, rotten, corrupted, offensive, unclean, etc."

The Federal Food, Drug, and Cosmetic Act of June 25, 1938, perpetuates the definition of adulteration in slightly different and more effective language, ". . .if it consists in whole or in part of any filthy, putrid, or decomposed substance. . . ." The 1938 Act went one step further in declaring as adulterated, food which has been ". . .prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth." Thus, the 1938 law not only banned food which is filthy, putrid, or decomposed, but also attempted to correct conditions which may cause food to become filthy or decomposed through insanitary conditions.

Many of the provisions in the 1938 law resulted from changes as the bill passed through the Congress, and there were many bitter controversies on some proposals. It is interesting to note that the provisions dealing with filth, decomposition, and sanitation remained the same from the time the first version was introduced in 1933 until the final passage of the Act in 1938. This demonstrates a practically unanimous recognition by the Congress and the public of the need for sanitation in the production of our nation's food supply.

Dr. E. O. Jordan, in discussing food sanitation in 1931, had this to say—"In addition to the arguments for cleanliness in food handling based on the avoidance of specific food contamination, the aesthetic reasons for maintaining the cleanliness of food must not be lost sight of. Common decency requires that substances repugnant to the average consumer be kept out of any food. Smyrna figs, about which there has been much hue and cry, are certainly more appetizing if known to have been packed under cleanly conditions. Cow dung from healthy cattle may not actually cause

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disease or any sort of intestinal disturbance but that is no reason for allowing any of it to be mixed with milk. Even if it is practicable to manipulate unsound food in such a way that it is not injurious to health and its original condition is completely disguised, no consumer should be exposed to the unpleasantness of eating food that he would not have chosen had he known its history. The food supplied to the modern civilized human being should, in all fairness, be so safeguarded that the consumer is not beset by lurid imaginings about its possible wanderings through back alleys and pigsties. The clean and appetizing preparation and transportation of food is a worthy end in itself.'' (Food Poisoning and Food-Borne Infection, by Edwin Oakes Jordan, Univ. of Chicago Press, April, 1931).

Some of our laws set numerical limits of bacterical counts and other indices of filth or decomposition to guide enforcement officials in carrying out the mandates of the law. The Federal Import Milk Act, for instance, not only requires certain farm inspections but sets definite limits of bacteria counts. The more general terms used in many laws, such as filthy, putrid, decomposed, etc., require the enforcement officer to establish means of determining when food is filthy, putrid, or decomposed. This presents a problem of no mean proportions to the food chemist and other scientific worker to develop methods for the detection of filth and decomposition in foods and to acquire the extensive data necessary to interpret the methods.

While the terms filthy, putrid, and decomposed have been interpreted by many trial courts, only a few interpretations of these terms are to be found in decisions of the higher courts. Presumably this is true because the terms of the law are so plain that there has been little occasion for the higher courts to speak on the subject. The courts have almost unanimously held that the terms filthy, putrid, and decomposed as used in the statute, should be given their common and usual meaning. It does not follow, that food, in order to be classed as filthy, putrid, or decomposed, must have obnoxious organoleptic characteristics to the ordinary consumer. Several court decisions clearly support the use of microscopic methods for the detection of these forms of adulteration. The Courts have also made it clear that contact with filthy substances is sufficient to classify a food as filthy.

The A.O.A.C. has recognized its obligation to develop and study methods to implement the enforcement of sanitary requirements of food laws. This is evidenced by reports of the Association which have for many years included papers on chemical, microscopic, and biological methods designed to detect and estimate filth and decomposition in food.

In their grosser forms, filth and decomposition are easily detected by taste, smell, and vision. Organoleptic methods will frequently reveal decomposition in food before it is processed, mixed, spiced, etc. but such

tests, in many types of food, usually fail after the product has been manipulated, making it necessary to apply tests of a more scientific nature. For example, sour milk is easily detected by taste and odor, but after it has been neutralized and dried it requires a careful chemical examination to determine the true condition of the product. Bread may be made from flour contaminated by weevils and rodents. While the condition of the flour is often obvious before it is sifted and baked into bread, rodent hairs, insect and larva parts and excreta in the bread can only be detected by a careful laboratory examination. Thus the scientific worker is faced with the problem of finding in finished foods evidence of their true condition not obvious by the ordinary senses of sight, taste, or smell.

A number of methods of approach have been employed successfully in the laboratory to determine decomposition. These methods may be classified broadly as follows:

(1) The estimation and identification of various types of microorganisms that develop in food as decomposition progresses (bacteria counts in milk, mold counts on tomato products, and butter, etc.).

(2) The determination and identification of characteristic chemical products of decomposition (indole and volatile fatty acids in seafood, lactic acid in sour milk, etc.).

(3) The disappearances, or marked decrease, of a normal ingredient of the food (sugar in frozen eggs).

The general method of establishing the presence of filth in food has been the separation of insoluble particles and a microscopic study to determine the identity and estimate the amount. In a few non-processed foods the presence of certain bacteria is indicative of contamination of the food with filth. For example, the finding of certain strains of *E. coli* in crabmeat shows that the crabmeat has been contaminated with fecal material.

While I have not attempted a comprehensive study of all the literature on filth and decomposition, I would like to trace the development of a few lines of work which are milestones of progress in dealing with this important subject.

When the Food and Drugs Act of 1906 was passed, tomato puree, catsup, and related foods were not unfrequently made of rotten tomatoes, artifically colored and preserved with chemicals to look like wholesome food. These comminuted products lent themselves readily to the use of decomposed and otherwise unfit raw materials. B. J. Howard, head of the Microanalytical Division of the Bureau of Chemistry, undertook to develop a scientific method for the detection of decomposition in this class of products. Howard developed what has since become known as the Howard Mold Count. The Howard method was one of the first scientific procedures generally used to detect decomposition in food. Since its introduction it has received world-wide recognition not only by food law

enforcement officials but also by the food producing industries.

The Howard Mold Count Method was originally published in 1911 in Bureau of Chemistry Circular 68. It first appeared as an A.O.A.C. method in the 1920 edition of the Methods of Analysis. In the beginning it was applied only to comminuted tomato products. In the latest edition of the Methods of Analysis, we find the Howard Mold Count in modified form, applied not only to comminuted tomato products but also to six other classes of foods, including such dissimilar products as butter and infant foods. While mold is a normal ingredient in a few foods, such as Roquefort and Blue cheese, it is not found in most foods unless they have undergone decomposition. Since the mold count technique measures the amount of dead mold, it can be applied to foods which have been processed by heat, and not only shows that decomposition is present but tells us something about the amount.

J. D. Wildman studied the application of the Howard method to butter and other dairy products. Working with R. L. Vandaveer, Wildman published a very comprehensive study on mold in commercial butter which showed a direct relationship between decomposition in the cream and the mold count of the butter churned therefrom.

Let us now consider methods for the detection and estimation of filth and extraneous matter in food. Since all extraneous matter is not filthy, the usual purpose in examining food for extraneous matter is to determine the amount and kind of filth it may contain. The earliest recorded work in this field was on fluid milk fifty or more years ago, where sediment testing was studied in Europe and this country. The sediment test is intended primarily to reveal contamination of milk with cow manure and other filthy substances. Early workers concerned themselves only with the insoluble portion of the cow manure and other visible elements of filth, and very little progress has been made over the years in the development of methods for estimating the soluble portion of cow manure in milk.

Although detection of the insoluble contaminants in milk is now carried out by a comparatively simple procedure, it is amazing to learn that some of the early workers attempted to make the problem most complex. The simple and obvious method requires only the filtering of the milk through a suitable pad or paper and an estimate of the amount of sediment visually by comparison with pads containing known amounts of sediment, as is standard practice today. The earlier workers, however, first tried to estimate the amount of visible sediment which settled to the bottom of a container on permitting the milk to stand for a definite period of time. This method was refined by applying centrifugal force. Other workers allowed the milk to stand, decanting part off the top and refilling with water, and repeating these operations until the sediment was free of milk. The sediment was then filtered and weighed. Some of the methods

in use in the early days took as long as three days to complete a determination!

Even the earlier workers recognized the desirability of identifying the components of the sediment from milk in order to determine its character, as well as the amount. At the present time many regulatory agencies make microscopic examination of sediment pads.

In the early thirties B. J. Howard and his co-workers undertook the development of a comprehensive set of methods for the detection and estimation of filth in many different types of food. The work was later continued by Henry Welch after Howard's retirement. In his report to the Association as referee on filth methods Welch stated: "The methods which utilize a variety of procedures are, however based upon one or more of three principles:

(1) Solubility of the food or drug and insolubility of the extraneous material.
(2) The preferential wetting of insects, insect parts, rodent hairs, or other extraneous matter by oily liquids whereby they acquire a lighter specific gravity, which allows their separation by flotation methods; and
(3) Selective sedimentation in water or heavier-than-water liquids."

While these fundamental principles are simple, their application can become very complex in many products.

A very significant publication with respect to filth in food appeared in 1944 under the title "Microanalysis of Food and Drug Products." This work, which was prepared under Welch's direction by members of the then Microanalytical Division of the Food and Drug Administration, consists largely of background material on the general subject of filth in foods and drugs and information of value to assist workers in isolating and identifying elements of filth. Included are some of the basic filth methods.

For the first time, the sixth edition of the *Methods of Analysis* contains a chapter concerned entirely with the detection and estimation of extraneous materials in foods and drugs. This chapter of 36 pages presents methods for the determination of extraneous materials in many foods, including canned citrus juices, tea, cocoa, dairy products, nuts, cereals, and others.

The application of the methods to a wide variety of foods tests the skill of the careful worker. We have learned through experience that most of the A.O.A.C. methods yield the best results when followed in the minutest detail. However, since the filth methods do not deal with all types of food, and frequently individual samples present unusual difficulties, the chemist is constantly called upon to introduce variations of the methods of separation with samples having unusual characteristics. The chemist's aim should be to separate the filth as completely as possible so that it can be readily identified and estimated.

Quite probably the work done to date on microscopic methods has been of more value to food officials and to industry than the work on purely chemical methods for detecting filth and decomposition. However, con-

siderable progress is being made in this field. Of particular value is the work of Fred Hillig on the determination of lactic acid and volatile fatty acids as indices of decomposition in dairy products, eggs, fish, etc. In connection with the work on volatile acids in eggs, particularly as applied to dried eggs, we should mention the paper of Lepper, Bartram, and Hillig which brought to bear bacteriological and organoleptic tests as well as chemical ones.

Patterson dealt very comprehensively with the broad aspects of decomposition in his 1944 report as general referee on decomposition. Indole has been studied as an index of decomposition in shell fish and reports have been published on canned oysters by King, Flynn, and Gowanlock, on canned clams and oysters by Beecham, and on shrimp by Duggan and Strasburger. Associate Referee Harris will report at this meeting on galacturonic acid as an index of decomposition (rot) in apple products. It is of interest to note that indole was studied a number of years ago as an index of decomposition in shrimp and at that time it was concluded that indole was not of value. Subsequent to the original investigation, more precise methods of analysis were developed. Using the more adequate methods, it was found that the formation of indole was characteristic of certain types of decomposition in shrimp.

Recently Cook and Steers studied the possible use of the enzymes trypsin and alkaline phosphatase as indicative of rodent and insect excreta in certain types of uncooked foods. Results appear to be promising and the study is being continued.

R. E. Duggan has studied uric acid in frozen eggs and showed that it is valuable in revealing the use of eggs contaminated with manure from dirty shells.

At the time of my own introduction to a food laboratory nearly 35 years ago there were presented many purely chemical and physical problems, such as the determination of protein, fat, fibre, ash, phosphoric acid, benzoic acid, and the host of other procedures required to detect the comparatively crude types of sophistication then employed. Many of you had similar experiences and many of you, as I did, had a wonderful, although sometimes disheartening, time with glycerol in vinegar. Our modern day food chemists are frequently first introduced to the microscope to learn to identify rodent hairs, insect parts, and the other significant elements of filth. They learn how to separate these tiny particles from the food material itself to identify them, and to evaluate their significance. They learn about mold counts and rot counts, and what they mean in terms of filthy and decomposed food. They learn about indole and other chemical indices of decomposition. They learn essentially to establish by laboratory examination the condition of sanitation under which a given lot of food was produced. The modern chemist must also, of course, learn the things that the "old timers" did. All in all, the regu-

latory food chemist of today is in a field embracing a wide variety of interests, a field to challenge his ingenuity and give him an opportunity for outstanding public service.

In the development of evidence of filth or decomposition in food products, the valuable information which can be obtained by means of factory inspection should not be overlooked. Frequently, a thorough factory inspection is the only means available at the present time for determining the true condition of many foods. This is especially true in those industries where filtration, sifting, and other manufacturing procedures successfully remove the evidence of filth or decomposition without removing the existence of filth or decomposition.

While the A.O.A.C. has never attempted to go beyond the collection of a sample in its formal methods, I wonder if we should not consider a study of factory inspection methods as a proper function of the Association. Certainly every food official has a responsibility to establish evidence of violation of the law. Chemists and other scientific workers have provided enforcement officials with most of the evidence in the past frequently using methods of the A.O.A.C. Some of the A.O.A.C. methods, for example, sediment testing of milk, are routinely used by inspectors in the field. Why should we not go one step further and provide the inspector, who is usually a scientifically trained man, with methods of inspection which would have the sanction of the A.O.A.C. and the respect and authority ascribed to the A.O.A.C. by the courts.

While I do not have in mind, at least in the beginning, the general subject of factory investigations, I do have in mind the establishment of a factory inspection technique for a few classes of factories which operate under the same general procedure and principles. For example, a factory inspection technique suitable for primary cheese factories, another to creameries, and another to packers of frozen strawberries. It should be borne in mind that any method which receives the full sanction of the A.O.A.C. must be subjected to collaborative study. I believe this fundamental is so precious that no method should ever be adopted as official until it has been studied collaboratively and found to be satisfactory when used by any competent worker. It is difficult to carry out collaborative work on many of the laboratory methods currently under study by the Association, since perishable foods are involved which are difficult to preserve and distribute for study to a number of laboratories. The problem of collaboration has been successfully overcome in many difficult fields and it need not present an insurmountable difficulty in dealing with factory inspection methods. One solution might be the independent inspection of the same factory at the same time using the same method by different workers and a comparison of their reports for consistency.

That the Association recognizes the importance of further work on filth and decomposition is evidenced by the current activity of two Refer-

ees; one for filth and the other for decomposition, and of six Associate Referees on filth and four on decomposition. Many problems in this field, as in all other fields of science, are pressing for solution. I would like to mention just a few broad fields where methods are badly needed.

Probably the most obnoxious contaminant of food comes from the unclean habits of human beings in handling food. The estimation of *E. coli* in some types of unprocessed food, for example in crabmeat, furnishes evidence of contamination with fecal material. In certain types of unprocessed dairy products, shelled nuts, etc., the presence of *E. coli* furnishes presumptive evidence of contamination with excreta of human or animal origin. Badly needed are methods for the detection of traces of human excreta in processed food. The amount likely to be present, originating as it does from filthy personal habits, will be minute, and this problem is thus one to challenge the imagination of chemists, microbiologists, and other workers in this field.

Methods for the detection of soluble filth should be developed without delay. Occasionally food manufacturers, instead of attempting to bring about fundamental corrections in sanitary practices, will attempt to meet the problem by filtering or sifting out insoluble filth in the hope that the filthy nature of the product will be obscured. (As expressed by Judge Hutcheson of the Fifth United States Circuit Court ". . . it was certainly not intended by Congress to leave packing stock butter manufacturers completely free to use in making their completed product any kind of filthy and putrid material they chose to use, in the faith, the substance of things hoped for, the evidence of things not seen, that, in the homely phrase, it will all come out in the wash . . . .") Factory inspection evidence as to the presence of soluble filth is difficult and expensive to obtain. The A.O.A.C. must provide methods whereby such hidden filth can be detected.

As a food decays, many chemical substances are formed whose determination should give the chemist a measure of decomposition. Possibly some substance or class of substances may be characteristic of protein decomposition, another of fat, and still another of carbohydrates. Unfortunately we are forced to deal with many types of decomposition producing a variety of different classes of chemical substances in food. If a limited number of chemical substances could be found to be characteristic of decomposition in the various types of food, the problem would be simplified. For example, a method for predominantly protein foods and another for predominantly fatty foods would be much more desirable than a method for fish and another for shrimp.

After a substance is found to be characteristic of decomposition in a food product, extensive studies are required to interpret the results on examination of specific lots of food. Ideally, the most valuable index is a substance which is completely absent in sound food but plentiful in de-

composed food. Unfortunately, many indices now used are present in minute amounts when no decomposition has occurred, and only when larger amounts are found can we establish decomposition as a fact. We hope and expect that work in this field will reveal many indices of the first type.

Methods for the determination of filth and decomposition should be as simple as possible and, to be of most value, should be applicable to field use.

The Howard Mold Count Method and the sediment test methods on milk have been most valuable because they lend themselves to field use. Although it would be difficult to use many of the present methods in the field it is not too much to expect that some of them may later be modified and made available for field use. A field method for lactic acid (not titratable acidity) in dairy products is badly needed.

The type of research needed in the field of filth and decomposition is very time consuming, extensive, and as a rule, exceedingly complex. I have every confidence that the many able young men and women now engaged in this work are capable of giving us the answers to these numerous and difficult problems. These young workers are doing outstanding research, and bringing to bear intense interest and original thinking. As I study the reports of these real scientists, I feel humble in the face of the progress which they are making.

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 THE EDITORIAL BOARD

By H. A. Lepper, *Chairman*

At our last meeting progress in the sale of the sixth edition of *Methods of Analysis* was reported as being the disposition, during the first year, of over one-half of the number printed. This year we sold all but 650 copies of the remaining 4,000.

Advantage has been taken by over 6,000 purchasers of the Book of the offer of the Association to furnish reprints of the report on changes in methods adopted at meetings subsequent to the publication of the book. This, together with the favorable comment on this service shows it has fulfilled a definite need. Reprints of changes made each year until publication of the seventh edition will be sent to those who have asked to be placed on the mailing list.

As with previous editions, the reviews devoted to the book in the scientific journals of the world have been most favorable. A few complaints have been directed to the decimal system of numbering the sections of the chapters on the assumption that with decimal numbers, for example, 24.41 should follow 24.4. With the use of the book, however, this possible confusion is soon overcome, and with the next printing the book can be avoided by the use of a colon or hyphen in place of the decimal point.

This year there has been no need for a committee on revision of methods, so we are to have only the report by Dr. White for the Committee on the Journal. The report of the Treasurer shows that the Journal has continued to be self-supporting.

Approved.

REPORT OF THE EDITORIAL COMMITTEE OF "THE JOURNAL"

W. B. White, *Editor and Chairman*

Despite annoying delays caused by the paper shortage and production difficulties at the printing plant, the *Journal* has shown gratifying progress

during the year. Total pages will be 706 against 448 pages for Volume 29. There will be 38 excellent contributed papers which is 10 more than the number in Volume 29. More and more, contributors are finding that the *Journal* is an excellent medium from many standpoints: wide circulation, discriminating readers, speed of publication, and the high scientific caliber of our reviewers, to name only a few.

In the matter of our wide circulation, it may be of interest to say that all 48 States, the District of Columbia, Alaska, Puerto Rico, Hawaii, Panama, and the Philippines are represented. Beside Canada, England, Scotland, and Ireland, there are subscribers in 8 other British lands "overseas." Central and South America and the West Indian republics number 11 countries on our list, Europe 14 (including Iceland), Asia 4, and Africa 1 (Egypt). Subscribers now number nearly 1800, compared with about 1500 for Volume 29.

We have instituted the practice of promptly acknowledging receipt of manuscripts, with no commitments as to their acceptability. The "Notice to Contributors," which appeared in Volume 28, page 35 (Feb. 1945), is being enclosed with the acknowledgement. It will be mailed to others on request.

In closing, the Chairman wishes, on behalf of the Committee, to thank our reviewers for their conscientious and able reviews, and our contributors for the patience and unfailing courtesy they have shown toward editorial suggestions.

Approved.

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

Your Chairman is pleased to report that the ninth edition of *Standard Methods for the Examination of Dairy Products*, to be published by the American Public Health Association, is in galley form and should be in print about January 1, 1948.

This edition contains 13 chapters, the first and last of which initiate a new policy. Chapter 1 is devoted essentially to explanatory matter for guidance of the less well informed administrator in the selection of tests to determine sanitary quality and in the interpretation of the results obtained by such tests.

Chapters 2 to 12 outline microbiological methods for milk, cream, butter, cheese, frozen dessert ingredients, and frozen desserts, and for the coliform group and certain pathogenic species of bacteria therein; also methods for sterility tests for dairy equipment, sediment and extraneous matter in dairy products, vitamin assays, phosphatase methods to determine pasteurization, and certain chemical procedures.

Chapter 13 bears the title "Screening Tests." It contains a selected group of procedures which permit determinations routinely on many more samples than could be made in case determinations were made by one or more of the refined technics. Where litigation may be elected, workers are warned not to depend upon the results obtained by screening tests. Where contests are expected, the methods should be such as will leave no doubt as either to the representative character of the sample or the dependability of the method used.

Representatives from Laboratory Committees in the American Public Health Association wish the members of this Association to know that they appreciate the priviledge granted to them to quote in the A.P.H.A. Methods certain sections from your Book of Methods.

Approved.

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

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

By L. S. WALKER, *Chairman*

*Official Final Action**

GUARANTEEING IN TERMS OF ELEMENTS

All fertilizer components with the exception of potash (K_2O) and phosphoric acid (P_2O_5), if guaranteed, shall be stated in terms of the elements.

Fused tricalcium phosphate is a product composed chiefly of the alpha form of the compound represented by the formula $Ca_3(PO_4)_2$. Its fineness and content of available phosphoric acid, (P_2O_5) shall be stipulated. Example: Fused tricalcium phosphate—twenty-five per cent (25%) available phosphoric acid (P_2O_5).

Calcium metaphosphate is a product composed chiefly of the vitreous compound indicated by the formula $Ca(PO_3)_2$. Its fineness and its content of available phosphoric acid (P_2O_5) shall be stipulated. Example: Calcium metaphosphate—sixty per cent (60%) available phosphoric acid (P_2O_5).

Potassium metaphosphate is a product composed chiefly of the crystalline compound represented by the formula KPO_3. Its fineness and content of phosphoric acid (P_2O_5) and of potash (K_2O) shall be stipulated. Example: Potassium metaphosphate—fifty-five per cent (55%) available phosphoric acid (P_2O_5): thirty-seven per cent (37%) potash (K_2O).

Double sulfate of potash and magnesia (Langbeinite). Double sulfate of potash and magnesia (langbeinite) is a commercial product containing not less than twenty-one per cent (21%) of potash (K_2O), nor less than fifty-three per cent (53%) of sulfate of magnesia and not more than two and one-half per cent (2.5%) of chlorine.

* NOTE.—The above report was approved by vote of the Association with respect to the official, final action, definitions of terms and interpretation, and the proposed definitions. The recommendation that the Association of Official Agricultural Chemists discontinue the work on the Definition of Terms and the Interpretation of Results on Fertilizers and Liming Materials, after being duly moved and seconded for adoption, was laid on the table by a vote of 14 to 9.

PROPOSED DEFINITION

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).

Your committee has been asked by the Executive Committee to study the question of relinquishing the activities involved in the study of Definitions of Terms and Interpretations of Results on Fertilizers and Liming Materials. Anticipating this change, your chairman communicated with each member of the committee requesting an expression of views on this subject. The Committee has voted in favor of this change by a vote of four to two. It is therefore recommended that the Association of Official Agricultural Chemists discontinue the work on the Definitions of Terms and Interpretations of Results on Fertilizer and Liming Materials.

Approved.

REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

WM. F. REINDOLLAR, *Chairman*

One of the chief duties of the Chairman of this Committee is to coordinate the reports of the referees and associate referees and to encourage and stimulate them to complete their assignment and to submit reports at a date sufficiently in advance of the meeting for them to receive due consideration by the proper subcommittee. During the past year this was accomplished by sending out three series of letters, one in the early part of the year, one in the spring, and the last in the late summer. While the response to these efforts has been rather gratifying, it was felt that an undue burden was placed on the referees by requiring them to send copies of their reports to several individuals. In order to standardize the procedure for handling reports and at the same time to minimize inconvenience to all concerned, the following plan will be followed in the future.

1. Associate referees are requested to prepare not less than six copies of their respective reports and mail them not later than a definite date which will be announced by direct correspondence.

2. All six copies are to be mailed to the office of the Secretary-Treasurer in Washington, D. C. The ribbon copy is to be included in this group unless the worker plans to be present and give his own paper, in which event it is to be turned in immediately after presentation. The associate referee does not have to mail copies to anyone else.

3. Upon receipt of the reports the Secretary-Treasurer will immediately mail one copy to each member of the appropriate subcommittee, one to the appropriate general referee, and one to the chairman of the Committee on Recommendations of Referees.

4. The Secretary-Treasurer will also keep these individuals posted as to which associate referees will have no reports or delayed reports.

5. General referees will be requested to review the work of their associates and to submit their own reports not later than a definite date which will be announced.

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 G. E. Grattan (Department of Agriculture, 79 Sussex St., Ottawa, Can.), *Chairman*; H. A. Halvorson, and E. L. Griffin

FEEDING STUFFS

It is recommended—

(1) That the following editorial corrections be made on page 419, method **27.59,** line 11. The following to be inserted before the words, "compare. . .$KMnO_4$" "Cool, make to measured volume of 50 or 100 ml, and mix."

(2) 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.
(e) Activity of yeast.
(f) Fluorine.
(g) Protein evaluations in fish and animal products.
(h) Hydrocyanic acid glucosides.
(i) Sampling and analysis of condensed buttermilk.
(j) Microscopic examination of feeds.
(k) Tankage (hide, hoof, horn, and hair content).

(3) That the methods for calcium and phosphorus as outlined by the Associate Referee be made tentative and work continued.

(4) That the acetone method for fat in fish meal be adopted as tentative.

(5) That editorial changes in the method for crude fiber be made as recommended by the Associate Referee and the study continued.

(6) That the work on fat in cooked animal feeds be discontinued.

(7) That the method for nitrogen, **2.26,** be made preferential when applied to feeding stuff materials containing 30% or more of protein and that the work be discontinued.

FERTILIZERS

It is recommended—

(1) That line 6 of section **2.1,** *Methods of Analysis* (1945) be changed to read "If less than 100 bags, sample not less than 20 bags."

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* 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.

That the following be added in section **2.1,** line 7, before the final sentence: "In sampling a bulk lot of fertilizer draw not less than 20 cores from different regions of the lot. In sampling fertilizer in small containers (10 lbs or less) a single package may constitute a sample for the lot;" and the whole section be made official, first action, and the study of sampling equipment and methods be continued.

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

(a) the ammonium citrate method and its applicability to basic slag;
(b) evaluation of sintered, fused, and calcined phosphates as fertilizers;
(c) the aging of molybdate solutions.

(3) that the recommendations of the Associate Referee on P_2O_5 be adopted and the methods be made official, first action.

(4) That attempts to work out improved methods for moisture be continued.

(5) That work on methods for nitrogen be continued with special emphasis on fertilizers which contain ammonium nitrate.

(6) That work on methods for manganese and magnesium be continued.

(7) That the study of problems relating to acid- and base-forming qualities of fertilizers be continued.

(8) That the work on potash be continued.

(9) That 95% ethyl alcohol be adopted instead of 80% alcohol in the determination of potash (official, first action).

(10) That work on analysis of sulfur be continued.

(11) That the study of methods for copper and zinc be continued.

(12) That the work on methods of analysis for boron be continued.

(13) That editorial correction in section **2.10(b)** of "323.81" to "324.03" line 1 and of "32.38" to "32.40" line 2 be made.

ECONOMIC POISONS

It is recommended—

(1) That the method for total chlorine in emulsions containing DDT, solvent, emulsifying agent, and water, as recommended by the Associate Referee, be adopted as tentative.

(2) That the methods for DDT be amended as recommended by the Associate Referee and retained as tentative.

(3) That specific methods be sought for the determination of 1080 and Antu in rodenticides.

(4) That methods for fluorine and nicotine be discontinued.

(5) That studies on tetra ethyl pyrophosphate, benzene hexachloride, insecticides containing derris or cubé, oil emulsions, and herbicides be instituted.

(6) That the title of the section on Insecticides and Fungicides be changed to "Economic Poisons."

DISINFECTANTS

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

LEATHER AND TANNING MATERIALS

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

PLANTS

It is recommended—

(1) That the Associate Referee on copper and cobalt be relieved of the assignment as requested.

(2) That the Associate Referee on carbohydrates be relieved of his assignment as requested and the title changed to "sugar."

(3) That Associate Referees be appointed to study sodium, cellulose and lignin, pectin, and starch.

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

(5) That the studies on boron be continued.

(6) That the studies on zinc 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 naphthalene-3, 6-disulphonic acid, or "chromotrope-B" be studied as a suitable reagent for the determination of boron in soils.

(4) That further studies in *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 "500" be substituted for "900" (p. 13, section **1.32,** line 3).

(7) That a study be made as to the adequacy of $Ca(OH)_2$ as a fixative for fluorine in soil charges of 1 to 1 proportion with calcination at 500 C in 5 to 60 minute periods.

(8) That the direct distillation of unignited soil with H_2SO_4 at 165°C with sequential distillation of an aliquot at 135°C be studied collaboratively.

(9) That the "2 point" titration procedure for the determination of exchangeable H in soils be studied further in relation to liming practice.

(10) That the survey and comparison of methods for the determination of phosphorus (a) that fraction in "available" state and (b) the propor-

tions of organic-inorganic forms therein be continued (*This Journal*, **30**, 43).

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

(12) That the direct titration against bromcresol green (Method II, *This Journal* **30**, 297 (1947), be adopted as optional for the determination of the neutralizing value of blast furnace slags.

(13) That the tentative procedures in "Liming materials" be annotated by the statement "without correction for sulphide content."

(14) That the correction for sulphide content be studied further.

STANDARD SOLUTIONS

It is recommended—

(1) That the method for the preparation and standardization of sodium thiosulphate solution (**43.28**) be adopted as official, final action.

(2) That the method recommended by the National Bureau of Standards for the standardization of potassium permanganate solution be adopted as official, first action, and that it replace the official method appearing in the sixth edition, *Methods of Analysis*.

(3) That the method for the preparation and standardization of bromide-bromate solutions be adopted as official, first action.

(4) That the methods for the standardizing of titanium trichloride solutions be studied further.

(5) That the study on buffer solutions be continued.

(6) That the study of the preparation and standardization of potassium dichromate solutions be continued.

(7) That the method for the preparation and standardization of thiocyanate solutions be adopted as official, final action.

VITAMINS

It is recommended—

(1) That the collaborative procedure for the determination of carotene be studied further, with different samples of alfalfa meal and other types of food and feed materials.

(2) That the collaborative procedure as outlined for the determination of carotene in hays and dried plants be adopted as an alternate tentative method.

(3) That the General Referee consider the question of recommending the inclusion of both saturated steam pressure and temperature for sterilizing, in view of the disagreement in the *U.S. Pharmacopoeia XII* and the *Handbook of Chemistry and Physics* (19th Ed.).

(4) That the thiochrome method for thiamine (**36.24–36.26** incl.) be made official (first action).

(5) That further work be conducted on the fluorometric method for

vitamin B_2 and that further consideration be given to the present microbiological method.

(6) That the method for folic acid be given further study.

(7) That the other vitamin methods for which there were no reports be given further study.

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 reports were received. It is recommended that studies on various methods of measurement of radioactivity be undertaken as Associate Referees are available.

SPECTROGRAPHIC METHODS

The Referee submitted a report outlining the quantitative technique used in his laboratory for the determination of metals and phosphorus in plant materials and illustrating the precision that had been obtained. He recommends that samples of various types of plant materials be submitted to collaborators for analysis by their own techniques and that these techniques then be compared as to their relative accuracy. At a later date the one technique found to be most promising would be submitted to all collaborators for further study. The committee concurs in these recommendations.

VEGETABLE DRUGS AND THEIR DERIVATIVES

It is recommended—

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

(2) That the study of methods for determining physostigmine be continued, with particular attention being paid to the determination of this alkaloid in ointments.

(3) That the study of the determination of theobromine and phenobarbital in mixtures be continued.

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

(5) That the study of the determination of quinine by the Herd and other methods 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.

(6) That the method proposed by the Associate Referee for the determination of ephedrine be adopted as official (first action) and the subject closed.

(7) That the modification of the N. F. VIII method for the determination of camphor in spirit of camphor proposed by the Associate Referee be adopted as official (first action) and also referred to the Committee on the National Formulary, and that the subject be closed.

(8) That the study of chemical methods for penicillin be continued.

(9) That a study be made of the determination of rutin in tablets.

(10) That a study be made of the determination of ethylmorphine in syrups.

SYNTHETIC DRUGS

It is recommended—

(1) That study of the determination of methylene blue be continued, with particular attention being paid to spectrophotometric methods.

(2) That the study of methods for the determination of sulfanilamide derivatives be reassigned.

(3) That the method proposed by the Associate Referee for the determination of atabrine (quinacrine hydrochloride) be adopted as official (first action) and the topic closed.

(4) That the distillation and extraction methods for demerol previously outlined by the Associate Referee [*This Journal,* **28**, 712 (1945)] be adopted as official (first action) and the subject closed.

(5) That the study of the determination of propadrine hydrochloride be continued.

(6) That the study of the estimation of carbromal by the determination of bromine be continued.

(7) That the method proposed by the Associate Referee for the determination of dihydrocodeinone be adopted as official (first action) and the subject closed.

(8) That the study of butacaine sulfate be continued.

(9) That the study of spectrophotometric methods for drugs be continued.

(10) That the study of the determination of trichloroethylene be continued, with particular attention being paid to the Rauscher method for chlorine in organic compounds.

(11) That the method proposed by the Associate Referee for the determination of thiouracil in tablets be adopted as official (first action) and the propyl derivative of this compound studied next year.

(12) That the study of methods for the determination of phenolphthalein in chocolate preparations be continued.

(13) That a study be made of the detection and determination of pyribenzamine and benadryl.

(14) That a study be made of the determination of synthetic estrogens.

MISCELLANEOUS DRUGS

It is recommended—

(1) That the title of the Associate Refereeship for microchemical tests for alkaloids and synthetics be changed to "Microscopic tests for alkaloids and synthetics," and the subject continued.

(2) That study of the ethanolamine method for mercury be continued.

(3) That study of the separation of chlorides, bromides, and iodides be reassigned.

(4) That the Associate Refereeship on organic iodides be discontinued and work on this subject be combined with that on the separation of the halogens.

(5) That the method proposed by the Associate Referee for the assay of compound ointment of benzoic acid be adopted as official (first action) and the topic closed.

(6) That study of the separation of the alkali metals be continued.

(7) That study of the determination of glycols and related compounds be continued.

(8) That study of methods for preservative and bacteriostatic agents in ampul solutions be continued.

(9) That study of the determination of phosphorus, calcium, and iron in vitamin preparations be continued.

(10) That the recommendation of the Associate Referee that his proposed modification of the method for total iodine be submitted to collaborative study be approved.

(11) That the subject of superheated steam in separation of drugs be dropped.

(12) That the study of estrone and estradiol be continued.

(13) That the study of the chromatographic separation of drugs be continued.

(14) That the tentative method for methyl alcohol, sections **39.161–39.162**, be studied, together with the directions in **16.25**, to bring about uniformity in these procedures.

DRUG BIOASSAYS

No reports were received. Since it appears to be impractical, if not impossible, for the Association to obtain qualified collaborators for work of this sort, it is recommended that the Refereeship on drug bioassays be abolished.

COSMETICS

It is recommended—

(1) That the periodate consumption and formic acid titration methods proposed by the Associate Referee be made official (first action); and study of other cosmetic cream problems be undertaken.

(2) That the following topics on which no reports were received be continued:

Alkalies in cuticle removers
Hair straighteners
Cosmetic powders
Cosmetic skin lotions
Deodorants and antiperspirants
Depilatories
Hair dyes and rinses
Mercury salts in cosmetics

(3) That the Associate Referee's recommendation of further study of methods for mascara, eyebrow pencils, and eye shadow be approved.

(4) That the study of the determination of moisture in cosmetics be continued.

(5) That the study of the determination of pyrogallol in the presence of henna be continued.

COAL-TAR COLORS

(6) That the following topics on which no reports were received be continued:

Acetates, carbonates, halides, and sulfates in certified coal-tar colors
Buffers and solvents in titanium trichloride titrations
Ether extract in coal-tar colors
Identification of certified coal-tar colors
Unsulfonated phenolic intermediates in coal-tar colors
Mixtures of coal-tar colors for drug and cosmetic use

(7) That the following topics on which no reports were received be continued and reassigned:

Halogens in halogenated fluoresceins
Nonvolatile unsulfonated amine intermediates in coal-tar colors
Sulfonated amine intermediates in coal-tar colors
Intermediates derived from phthalic acid
Sulfonated phenolic intermediates in coal-tar colors

(8) That the method proposed by the Associate Referee for the determination of aniline in coal-tar colors be adopted as official (first action), and that study of the applicability of this method to other volatile amines be continued.

(9) That adoption of the method proposed by the Associate Referee for the determination of pure dye in lakes and pigments be deferred until the method has been tested on other dyes.

(10) That study of the following topics be continued as recommended by the Associate Referees:

Spectrophotometric testing of coal-tar colors
Subsidiary dyes in D&C colors

(11) That the following new topics be studied:

Lead in coal-tar colors
Lead in lakes of coal-tar colors

Subsidiary dyes in FD&C colors
Hygroscopic properties of coal-tar colors

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By JOSEPH CALLAWAY, U. S. 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 a moisture in dried vegetables be continued.

(3) That studies of methods for the estimation of the enzymatic activity in 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 the Referee review the chapter on coffee and tea and make recommendations for additional work where this appears desirable.

COLORING MATTERS IN FOOD

It is recommended—

(1) That the method for the detection of small amounts of tartrazine in alimentary paste as modified by the Associate Referee be made official, first action.

(2) That work be continued on the separation and quantitative determination of tartrazine (FD&C No. 5) and sunset yellow FCF (FDC No. 6).

(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 A.B.), FD&C Yellow No. 4 (Yellow O.B.), FD&C Orange No. 2 (Orange S.S.), and FD&C Red No. 32 (Oil Red X.O.).

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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.

DAIRY PRODUCTS

It is recommended—

(1) That the Sanders-Sager method for phosphatase in fluid milk and cream, in cheddar type cheese, and in soft uncured cheeses, be made official, first action. That this test with modification making it applicable to other designated types of cheese, ice cream mix, sherbet mix, chocolate-flavored milks and skim milks, butter, sweet cream and cultured buttermilks, fermented milks, goats' milk, cheese whey, and concentrated milks, be adopted as tentative and subjected to collaborative tests; and that the present tentative methods for phosphatase (**22.43** to **22.57**) be dropped.

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

(3) That studies of methods for the detection of chlorine added to milk be continued.

(4) That the rapid screening method for moisture described by the Associate Referee be adopted as tentative, and that methods for the preparation of samples of various types of cheese, processed cheeses, and related products, and methods for the determination of fat in these foods, be further studied.

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

(6) That the use of mechanical shaking devices and of artificial methods of cooling suitable for use in the present official method for the preparation of butter samples be studied and that the mechanical stirrer method described by the Associate Referee be adopted as tentative.

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

(8) That the official method (**22.146b**) for the preparation of samples for frozen dessert and other methods for the preparation of samples be further studied.

(9) That an associate referee be appointed to study and make recommendations for methods for the determination of various fruits and other characterizing ingredients of ice cream and other frozen desserts.

(10) That studies be initiated to ascertain whether the present Babcock method for fat in milk should be modified when used for determining fat in homogenized milk.

(11) That the Roese-Gottleib method in dairy products be studied.

(12) That the editorial changes in wording recommended by the Referee in section **22.149** (fat in ice cream) be made.

(13) That there be inserted in sections **22.28 (a)**, **22.29**, and **22.30** directions for converting readings made on Bausch and Lomb instruments to corresponding readings on Zeiss instruments, and that a study of these sections be continued.

(14) That the test for sediment in fluid milk **(22.40, 22.41,** and **22.42)** be dropped and that the American Public Health Association method for sediment in fluid milk be adopted as tentative and further studied.

(15) That the editorial changes in the wording of section **22.33** recommended by the Referee be made.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That the method for the determination for added glycerol in liquid and frozen eggs, and egg yolks, be further studied.

(2) That the method for the acidity of the ether extract of dried egg and dried egg yolk proposed by Kline and Johnson be further studied, utilizing if possible samples of dried egg products of known history.

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, first action.

(2) That methods for extraneous matters in fruits be subjected to collaborative studies.

(3) That methods for extraneous matters in drugs be further studied.

(4) That the changes in wording of section **42.57** (mold in tomato products) providing for adjusting the total solids in the sample by the use of the Abbe refractometer, as recommended by the Associate Referee, be made.

(5) That the changes in wording of section **42.11** (manure fragments in dairy products), recommended by the Referee, be made.

DECOMPOSITION IN FOODS

It is recommended—

(1) That the method described by the Associate Referee for the determination of indole in shrimp, oysters, and crabmeat be adopted as tentative and work on other indices of decomposition in these foods be continued.

(2) That the method for the determination of galacturonic acid in apple products described by the Associate Referee be further studied and that substances other than galacturonic acid be investigated as indices of the use of rotten apples in the manufacture of apple products.

(3) That studies be continued on fish and dairy products.

GELATIN, DESSERT PREPARATIONS, AND MIXES

It is recommended—

(1) That changes in the method for the determination of jelly strength in gelatin **(9.6)** recommended by the Associate Referee, be made; and that this method as revised and also the method for determination of jelly strength in dessert powders **(9.12)** be subjected to collaborative study.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the composition of the substances extracted by ether and by mixtures of ether and petroleum ether after the acid hydrolysis of the fish be further studied, with the idea of determining whether the material extracted is largely fat, before final adoption of this method for the determination of fat in fish.

(2) That work be continued on selecting a suitable method for determining total solids in fish, and, if practicable, that such method be subjected to collaborative study.

GUMS IN FOOD

It is recommended—

(1) That the method for detection of gums in soft uncured cheeses be further studied as to its quantitative application and as to whether it detects the presence of soluble alginates.

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

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

(4) That studies be continued on the detection of gum in mayonnaise, French dressing and related salad dressings.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That work be continued on 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 nonfat dry milk solids in meat products.

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

METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

It is recommended—

(1) That methods for the determination of cadmium be further studied.

(2) That the methods for the determination of copper of Greenleaf, and of Bendix and Grabenstetter, be subjected to collaborative study.

(3) That methods for the determinations for DDT in spray residue and canned foods be further studied.

(4) That methods for the determination for mercury in spray residue be further studied.

(5) That methods for the determination of zinc be further studied.

MICROBIOLOGICAL METHODS

It is recommended—

(1) That work be continued on eggs and egg products, sugar, canned

vegetables, canned meat products, canned fish, acid canned foods, nuts and nut products.

(2) That methods for the examination of frozen fruits and vegetables outlined by the Associate Referee be studied and if practicable be subjected to collaborative study.

MICROCHEMICAL METHODS

It is recommended—

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

NUTS AND NUT PRODUCTS

It is recommended—

(1) That methods for the preparation of samples and the determination of moisture and fat in nuts and nut products be further studied.

OILS, FATS, AND WAXES

It is recommended—

(1) That the method for unsaponifiable matter (**31.40**) be made official, final action.

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

(3) That the F.A.C. Method for unsaponifiable matter (**31.37** to **31.39** inclusive) be deleted, final action.

(4) That the method for squalene (**31.41–31.43**) be made official, final action.

(5) That studies of methods for determining the stability of fats be discontinued for the present.

(6) That studies of methods for the estimation of peanut oil be continued.

(7) That studies on methods for the detection and determination of antioxidants in fats be continued.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That work on the esters of benzoic acid be continued and if practicable that methods also be found for the detection and determination of esters of vanillic acid.

(2) That methods for the quantitative determination of saccharin in baked goods be further studied.

(3) That the method for saccharin described by the Associate Referee *This Journal*, **30**, 492 (1947), be further studied.

(4) That the method for estimation for volatile fatty acids in bakery products described by the Associate Referee be adopted as tentative and

work on this subject continued with the idea of utilizing if possible the chromatographic procedure for the separation of propionic acid.

(5) That the tests for the detection of thiourea in orange juice, described by the Associate Referee, be adopted as tentative and work continued. That the quantitative methods for thiourea in orange juice and frozen peaches, described by the Associate Referee, be adopted as tentative and the work continued.

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

(7) That work be initiated on the detection and estimation of the artificial sweetener having the composition 1-propoxy 2-amino 4-nitrobenzene.

(8) That work be initiated on the detection and determination of dichloracetic acid in foods.

(9) That work be continued on the development of improved methods for the detection of formaldehyde in foods and that unnecessary and unused methods now carried as official method be recommended for deletion.

(10) The the method for the determination for monochloracetic acid (applicable to carbonated beverages and fruit juice, *This Journal*, **27,** 199, 1944), with the provision as to the use of alternate method of extraction recommended by the Associate Referee, be made official, first action; that further collaborative work be done on other fruit juices and beverages and that the application of this method to other foods be studied; and that collaborative work on the indigo and pyridine qualitative tests for monochloracetic acid be continued.

(11) That the ferricyanide method for quaternary ammonium compounds in commercial preservatives, *This Journal*, **29,** 312 (1946), be made official, first action; that the method for the determination of quaternary ammonium compound in table syrup, *This Journal*, **29,** 325 (1946), be made official, first action; that the method for the determination of quaternary ammonium compound in beverages containing fruit juices, *This Journal*, **29,** 322 (1946), be made official, first action; that the method for the determination of quaternary ammonium compounds in beer, *This Journal*, **29,** 325 (1946), be made official, first action; and that studies be continued of methods for the determination of quaternary ammonium compounds in fruit juices, soda waters, milk, salad dressings, sandwich spreads, pickles, relishes, and related products.

SPICES AND CONDIMENTS

It is recommended—

(1) That the method for the determination of caramel in vinegar **(33.78)** be dropped and that studies be made of other tests for caramel, such as Mathers test, and the test for caramel in wine **(15.39),** to determine their applicability to the detection of caramel in vinegar.

(2) That the permanganate oxidation method be applied to a number of samples of distilled vinegars of known history in order to ascertain if it has value in differentiating this type of vinegar from a dilute acetic acid.

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

(4) That studies of the determination of tartaric acid and tartrates in vinegar be continued.

(5) That the method, as recommended by the Associate Referee for starch in mayonnaise and salad dressing, be made official, first action, and that work be initiated looking toward the adoption, as official, of methods for the preparation of samples and determination of total fat in mayonnaise and salad dressing.

(6) That the present tentative method for starch in prepared mustard and mustard flour, modified as suggested by the Associate Referee, be adopted as official, first action.

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

(8) That the official method for copper-reducing substances **(33.40)** be dropped, final action.

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

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

(11) That studies be continued for methods for the determination of volatile oils 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 the following official (first action) methods for malt be adopted as official, final action: Sampling **(14.39)**; Preparation of sample **(14.40)**; Bushel weight **(14.41)**; 1,000 Kernel weight **(14.45)**; Moisture in caramel malt and black malt **(14.52)**; Diastatic power **(14.61)**.

(2) That the following official (first action) methods for beer be adopted as official, final action: Extract of original wort **(14.7)**; Real degree of

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

fermentation or real attentuation **(14.8)**; Apparent degree of fermentation or apparent attenuation **(14.9)**; Total acidity—Indicator Titration method **(14.10)**; Total Acidity—potentiometric titration method **(14.11)**; H-ion concentration (*p*H)—Electrometric method **(14.12)**.

(3) That the following official (first action) methods for hops be adopted as official, final action: Sampling **(14.80)**; Physical examination **(14.81)**; Preparation of sample for chemical analysis **(14.82)**; Moisture **(14.83)**; Resins **(14.84–14.89,** inclusive).

(4) That the following official (first action) methods for brewing sugars and syrups be adopted as official, final action: Extract **(14.90)**; Non-extract (apparent water) **(14.92)**; Fermentable extract—**(b)** Rapid Fermentation method **(14.92)**; Protein **(14.93)**; Iodine reaction for unconverted starch **(14.95)**; Acidity **(14.96)**; H-ion concentration (*p*H) **(14.97)**; Ash **(14.98)**; Total reducing sugars—Munson-Walker general method **(14.99)**.

(5) That the following official, first action methods for wort be adopted as official, final action: Specific gravity **(14.103)**; Original extract or original gravity **(14.104)**; Fermentable extract **(14.105)**; Iodine reaction **(14.106)**; Total acidity **(14.107)**; H-ion concentration (*p*H) **(14.108)**; Color **(14.109)**; Protein **(14.110)**; Total reducing sugars **(14.111)**.

(6) That study of methods for determination of essential oil in hops be continued.

(7) That the study of the tentative method for color (*This Journal*, **30,** 68, 1947), and the photoelectric beer color evaluation, as well as work on beer turbidity methods, be continued.

(8) 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 collaboratively.

(9) That study of methods for testing soluble starches used in diastatic power determination of malt be discontinued.

(10) That study of methods for determination of total solids and yeast solids be continued in accordance with this year's report of the Associate Referee.

(11) That the Milos test for caramel, **14.35** be deleted, first action.

(12) That the Mathers test for caramel, described in this year's report of the Associate Referee on Wine, be studied collaboratively with respect to its application to beer.

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

(14) That the official modified Denigés' method for methanol, section **16.25,** be studied together with the directions in **39.161–39.162,** to bring about uniformity in these procedures.

Wines:

It is recommended—

(1) That study of the spectrophotometric examination of wines be discontinued.

(2) That chromatographic studies in wines be continued.

(3) That study of methods for methanol in wines and distilled liquors be continued.

(4) That the official Milos test for caramel (**15.38**) be deleted (first action).

(5) That the Mathers test, as described in the report of the Associate Referee this year, be adopted as official, first action.

(6) That the tentative confirmatory test for caramel (**15.39**) be modified as described in the report of the Associate Referee for this year.

Distilled Liquors:

It is recommended—

(1) That the study of the obscuration method for determining the true proof of blended spirits be continued.

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

(3) That method **16.46 (b)** be changed as recommended by the Associate Referee in this year's report and that study of cordials and liqueurs be continued.

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

(5) That the Fulton test for caramel, described in this year's report of the Associate Referee, be adopted as tentative, for distilled liquors and for cordials and liqueurs.

(6) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official (first action) for Distilled Liquors.

(7) That the Mathers test for caramel, described in this year's report of the Associate Referee, be adopted as official (first action) for cordials and liqueurs, and that it be included by reference in the chapter on cordials and liqueurs in **16.60**, in place of the modified Marsh test, **16.39**, and the Milos test, **15.38**.

(8) That an Associate Referee be appointed to study a rapid colorimetric method for fusel oil.

(9) That the method for fusel oil, **16.22** (p. 196) be further studied.

CACAO BEAN AND ITS PRODUCTS

It is recommended—

(1) That the work on lecithin (*This Journal*, **25**, 717) be continued.

(2) That the work on the determination of maltose and of lactose in the presence of other reducing sugars be continued.

(3) That the method for tannins and pigments in this year's Referee Report, be adopted as tentative.

(4) That the three procedures for fat in refractory beverage bases, described in this year's report of the Associate Referee, be further studied.

(5) That work be continued on the tentative method for pectic acid, **19.16**, with particular reference to milk chocolate.

CEREAL FOODS

It is recommended—

(1) That both procedures proposed by the Associate Referee for the determination of phosphorus in cereals and cereal products be adopted as official (first action) and that the study be continued.

(2) That the dry ashing method for iron **20.9–20.12**, inclusive, be made official, final action, for enriched macaroni products, degerminated, bolted, and whole corn meals, and that the study be discontinued.

(3) That the wet ashing method for iron (*This Journal*, **30**, 71, 1947), be made official, final action, and that study be discontinued.

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

(5) That the tentative method for the determination of fat acidity in grain, flour, corn meal, and whole wheat flour (**20.18–20.21**, inclusive) be further studied and also that the relationship of acidity to unsoundness be studied.

(6) That the Associate Referee continue his work reported this year on the application of the method for reducing and non-reducing sugars in flour **20.28–20.30**, inclusive, to the determination of sugars in bread and other bakery products, with special consideration to the article on this subject published by R. M. Sandstedt and G. C. Fleming (*This Journal*, **30**, 550–2).

(7) That the tentative method for benzoyl peroxide in flour, **20.53**, be continued for rye flour and the title changed to Benzoic Acid in rye flour; and that the method proposed by the Associate Referee replace it for wheat flour.

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

(9) That the method for the determination of lactose in bread (*This Journal*, **24**, 630) be further studied.

(10) That the determination of milk fat in bread, **20.86**, be further studied.

(11) That the methods for the determination of proteolytic activity of flour be continued.

(12) **(a)** That the method for moisture in soy flour, **20.77**, may be changed to read—"Moisture—*see* **20.2** or **20.4**, with the exception that a 5 g sample be dried 130° for 2 hours." **(b)** That the method for ash, **20.78**, be changed to read—"Ash—*see* **27.9**." **(c)** That the method for nitrogen, **20.79**, be changed to read—"Proceed as directed under **2.26**,

using 10 g K_2SO_4, or Na_2SO_4 and 0.7 g of HgO or its equivalent in Hg, with the additional option of using sodium alizarin sulfonate." **(d)** The the method for oil, **20.82,** be changed to read "*See* **31.07** except that ca 2 g full-fat flour or 5 g low or defatted soy flour be extracted for 5 hours" and that the study be continued on these methods.

(13) That studies be made on the detection and determination of soy bean flour in cereal products.

(14) That the method proposed by the Associate Referee for determination of the amount of added inorganic material in phosphated and self-rising flour be further studied.

(15) That the method referred to in *This Journal* **25,** 83–4, for the determination of unsaponifiable matter and sterols in noodles be studied to determine their applicability to other foods containing eggs.

(16) That studies of methods for the determination of albumin in noodles and other farinaceous egg-containing products be continued.

(17) That the method for the determination of total solids, **20.84(b),** for raisin bread and bread containing raisins and fruits (official, first action, *This Journal,* **30,** 72, 1947) be adopted as official, final action, and that study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

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

(19) That the study on the determination of bromates in flour be continued along the lines suggested in this year's report of the Associate Referee.

(20) That the study on the determination of apparent viscosity measurements of flour be discontinued.

BAKING POWDER

It is recommended—

(1) That the present official method for determination of residual carbon dioxide in baking powders be deleted (final action under suspension of the rules), for the following reasons:

(a) The wording and description of the method is susceptible to too many interpretations.

(b) The manner of heating and treating the sample does not yield a true residual because all of the available CO_2 is not driven off.

(c) The method is not applicable to all types of baking powder, particularly the pyrophosphate types.

(2) That the modified Quartermaster Corps method as described in the report of the Associate Referee this year, be adopted as a tentative method, adding the words "or electricity" after the phrase "The bath should be heated with gas."

(3) That the modified Q.M.C. method referred to in recommendation (2) be subjected to further study wherein the single evaporation to dryness is carried out in a moisture oven instead of on a water bath and at temperatures ranging from 70 to 100°C.

(4) That the modified Q.M.C. method referred to in recommendation (2) also be studied using a neutral saturated sodium chloride solution as a medium instead of distilled water.

(5) That the expression "or subtract 17.8 from 17.6" in method **17.9,** available carbon dioxide—official, be deleted, first action, and that the tentative available CO_2 be determined by subtraction of the tentative residual CO_2 from the total CO_2, official **17.6.**

(6) That an investigation be made on modifying the present A.O.A.C. gravimetric method, **17.2** and **17.3,** by changing from H_2SO_4 and KOH absorption bulbs to the use of "Caroxite" or "Ascarite and Anhydrone."

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 spirts 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 vanillin 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 section **25.2** be changed to read as follows:

"**25.2** Alcohol-Official—Proceed as directed in **16.6** or **16.7** but measure the sample used at 15.56°C. in a pycnometer (see pages 192–3) calibrated at that temperature."

(13) That the Referee study collaboratively the modification of **25.23** as given in his report.

(14) That the Referee study collaboratively the modification of **25.54** as given in his report.

(15) That **25.64** be changed to read as follows:

"**25.64** Alcohol-Official—Proceed as directed in **16.6 (b).**

FRUITS AND FRUIT PRODUCTS

It is recommended—

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

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

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

(4) That the study of methods for the determination of water insoluble solids be continued.

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the Java method (*This Journal*, **29**, 242) for unfermented reducing substances in molasses be adopted as tentative, and that work be continued.

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

(3) That study be continued on tables of density of sucrose solution at various temperatures.

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

(5) That the study of the applicability of electro-deposition to the direct quantitative determination of dextrin in honey and honeydew honey be discontinued.

(6) That the study of the characteristic properties of dextrins of honey and honeydew honey be discontinued.

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

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

(9) That collaborative study of the method described in last year's report of the Associate Referee, for the determination of resinous glaze in confectionery be continued, with special reference to large amounts of lac.

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

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

(12) That the procedures in N.B.S. Circular C440, pp. 324–334, for measurement of transmission of solutions of commercial sugar products, be considered with a view to their future adoption as tentative methods.

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

(14) That changes in accordance with the International Sugar Scale be adopted as official, final (special action) in sections **34.18** and **34.19.**

WATERS, BRINE, AND SALT

It is recommended—

(1) That studies of methods for the determination of borate be continued.

(2) That methods for determination of fluoride in salt be studied collaboratively.

REPORT OF A.O.A.C. REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL

The Crop Protection Institute congratulates the A.O.A.C. on its record of 61 years of very worth-while accomplishments, and sends greetings and best wishes for a continuation of the successful and fine record of the past and may the future have still better things in store for your useful organization.

The Board of Governors of the Crop Protection Institute continues to hold at least two meetings a year to discuss the progress of projects and consider new projects. The various project committees are always working and the Chairman of Board is perpetually active.

In the early years of the Institute, the work was largely with inorganic compounds. In recent years the material to be tested for crop protection are largely synthetic organic compounds. The progress which has been made in many fields are marvels of efficiency and in directions which were undreamed of a few years ago. Great progress has been made in developing organic insecticides and fungicides which are harmless to man and animals. Sprays have been developed which will prevent fruit from dropping from the trees. Much has been accomplished in developing weed killers with selective characters. So now lawns can be freed of objectionable weeds without injury to the grass. Much work has been done and much accomplished in protecting man and animals from objectionable and disease carrying insects and plants.

While much progress has been made in developing synthetic organic products for the control of objectionable plants, insects, and animals, though this field is in its infancy it offers a great opportunity for the research worker. This Association should encourage its members to promote

this field of research and cooperate whenever possible with the entomologist, botanist, and pathologist in solving their problems.

The Crop Protection Institute has about the same number of projects under way as in former years. The numbers of people or firms who come to the Institute for help and research are increasing. The Institute continues the policy of cooperating with State experiment stations and university laboratories whenever possible. There is no doubt but that commercial industries need and appreciate having a neutral agency like the Crop Protection Institute to assist them in developing and testing their products.

We recommend that A.O.A.C. continue its active cooperation with the crop Protection Institute and direct its way such commercial work, of a more or less private character, as it is prepared to handle.

H. J. PATTERSON
W. H. MACINTIRE

REPORT OF COMMITTEE ON CLASSIFICATION OF METHODS

Your Committee has made a careful survey of all methods in the 1945 Edition of the Association's "Methods of Analysis."

Those methods clearly designated "Official" need no comment, other than to urge that any methods which have been for some time in the "First Action" status should be promptly adopted as Official, Final Action. If doubt has arisen as to their merit, referees should resolve such doubt at once by directing further collaborative study, looking toward either final adoption or deletion, as the findings dictate.

There are a few chapters in the "Methods of Analysis" which use the footnote device: "All methods are tentative unless otherwise designated." It is urged that this ambiguous device be abandoned. Each individual method should be plainly tagged; and incidentally, such a procedure would use up no additional space.

As to those methods designated "Tentative" (and those which now bear no designation), these seem to fall into the following broad groups:

(1) Those which could, and we feel should, be made official with or without further collaborative study, as the findings justify.

(2) Those which have been thought, we believe erroneously, to be, from their very nature, incapable of collaborative study.

(3) Those which are frankly for the purpose of making rough approximations rather than precise measurements.

(4) Those which are clearly obsolete.

A brief discussion of these four classes follows:

(1) No worthy method should be saddled with the term "Tentative," if by worthy we mean producing reliable results in capable hands. Any

method found unworthy of official adoption should, we believe, be dropped rather than continued as tentative; and referees should reach a decision as promply as possible. Pending such decision on methods not palpably unworthy or obsolete, the methods should be designated "Pending Official" or the equivalent. Such methods may be regarded as interim methods to be made official for inclusion in the revision of "Methods of Analysis" which follows their adoption. If found unworthy they should be dropped. It is not intended that the Book include methods in the "pending official" status.

(2) We believe that any method of analysis that is of proven usefulness is, almost by definition, capable of collaborative study. Take as an illustration the methods for the isolation of filth from foods in Chapter 42. These, we believe, could and should be studied collaboratively, not as methods of measurement which they are not, but rather as to whether they do or do not cleanly isolate the filth from the food itself. In such a study the collaborator would report a method as "satisfactory" if no food remained with the filth to obscure it. Satisfactory methods of isolation should then be adopted as official. We believe that other purely isolational methods could be handled in the same way.

(3) This class of methods serves the sole purpose of indicating to the analyst whether he needs to go through the longer and more accurate procedure, or whether he has no further regulatory or research interest in the sample. We believe that such methods should not be designated as either official or tentative, but rather called just what they are: "screening methods." Whatever the name, it should be uniformly used by all referees.

(4) The obsolete methods need little comment. The sooner they are deleted the better, for their omission will give the "Methods of Analysis" badly needed elbow room. Any doubt on the question should be promptly resolved by collaborative studies instigated by the referee.

By way of final comment, there is need for making a clear-cut distinction among (1) "Sampling Methods," which we feel should be rigidly restricted to the selection, from the "lot," of unit packages (or material, in the case of bulk shipments) for further attention; (2) "Drawing the Sample," which is concerned with exactly how the inspector breaks into the units selected, and withdraws, consolidates, and packs the sample so withdrawn; and (3) "Preparation of Sample," which describes just how the *analyst* treats it in order to make it homogeneous, before he weighs out the analytical charge. We recommend that referees adopt this plan of nomenclature, making any necessary changes in present headings.

A brief discussion of these three classes of sampling and subsampling follows:

(1) There are numerous treatises on the art of sampling in this re-

stricted sense of the word. It is enough to say that each individual task is always unique in some respect, and that therefore only the very broadest principles can be formulated for any given material. It is our view that all "methods" of this character should be deleted as at once too broad and (when "official") too restrictive.

(2) These, on the other hand, do have a place in the "Methods of Analysis," but we believe no such method should be retained unless the procedure has stood the test of collaboration, and then it should promptly be adopted as "official." Such collaboration needs to be wisely and carefully planned in advance, so that the experiment will, to the extent possible, isolate the sampling variations from the analytical ones. If, as seems improbable, a method is so abstract or abstruse as to make a collaboration impossible, it should be deleted.

(3) These methods are pretty well subject to the comment under (2), and little need be added here. It must be remembered that the choice is between a resort to some general language (such as "stir until homogeneous"), in which case the preparation is left entirely to the judgment of the analyst; and a set of directions for stirring, shaking, grinding, sieving, etc., which he must perforce follow. Which course to take in any specific case should be a matter of deep, and somewhat philosophical, reflection on the part of the responsible referee. The first course would call for no formal method of sample preparation at all; the second would call for a rigid "official" procedure.

We might point out certain headings that are somewhat difficult to classify, e.g. "Preliminary Examination," "Physical Examination," "Macroscopic Examination." In the main these deal with broad generalizations, directions, and the like. Perhaps referees should re-examine these as to their value, and then either delete them or give them some general heading, such as "General Suggestions" which would take them entirely outside the "Methods" classification.

In this connection, and also in connection with the above discussion on the entire process of sampling and sub-sampling, it might be wise to include a statement analogous to the first two parenthetical sentences in Chapter 1. These are: "(In view of the variability of soils, it seems impossible to devise an entirely satisfactory method for sampling. It is obvious that details of procedure should be determined by the purpose for which the sample is taken.)"

To sum up, your Committee makes the following recommendations to the Association:

To chart a course (including any necessary changes in the Constitution and By-laws) which, by the time the next edition of *Methods of Analysis* is issued, will have accomplished the following objectives:

(1) The complete deletion of all so-called "tentative" analytical and

sampling procedures which are palpably obsolete or unworthy.

(2) Adoption as "official" of such currently "tentative" procedures as are found worthy.

(3) The designation as "pending official" of those tentative methods not covered by (1), pending further study by the appropriate referee.

(4) Adoption as "official final action" of all those methods long designated as "official first action" which are found worthy of such adoption.

(5) Adoption of useful methods of the "screening" type, under a suitable heading which will not include either of the terms "tentative" or "official."

(6) The possible adoption of a heading, again without the words "official" or "tentative," to cover any useful suggestions as to physical, preliminary, or macroscopic examination, or as to handling and storage of the inspector's or analyst's sample.

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

REPORT OF THE SECRETARY-TREASURER

By HENRY A. LEPPER

The meeting of the executive committee was held on Sunday, October 19, 1947, at 10 a.m., in the Green Room of the Shoreham Hotel. All members were present and ex-president J. W. Sale served as the additional member in the absence of Dr. Ross. The audit of the firm of Snyder, Farr, and Company was presented and accepted.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, INC.

BALANCE SHEET—SEPTEMBER 30, 1947

ASSETS

Current Assets:		
Cash, Lincoln National Bank	$23,091.40	
Office cash fund	61.12	$23,152.52
Accounts receivable	$ 4,038.42	
Less reserve for doubtful accounts	163.90	3,874.52
Accrued interest receivable, Government bonds		250.00
Inventories		5,057.87
Total Current Assets		$32,334.91
Investments		42,319.00
Total Assets		$74,653.91

LIABILITIES AND SURPLUS

Current Liabilities:		
Accounts payable		$ 2,245.16
Snrplus;		
Balance, October 1, 1946	$61,344.53	
Add net profit, year ended September 30, 1947,	11,064.22	
Balance, September 30, 1947		72,408.75
Total Liabilities and Surplus		$74,653.91

A vacancy on the Committee on Recommendations of Referees, due to the change in position of C. S. Ladd, no longer eligible to active membership, was filled by appointment of A. H. Robertson. The present chairman of the committee, W. F. Reindollar, was reappointed. The vacancy occurring on the editorial committee of *The Journal,* was filled by the appointment of J. B. Smith. Because of the practically complete sale of the 9,000 copies of the sixth edition of Methods of Analysis, the Secretary was instructed to negotiate the publication of an additional 1,000 or 2,000 copies, if the rate of sale indicates a sufficient demand therefore between now and the contemplated issue of the seventh edition in 1950. Authority for the purchase of an addressing machine and an adding machine for the business office was granted. The Secretary was instructed to arrange with other associations engaged in establishing methods of analysis involving the same topics as those of our Association a means if possible whereby uniformity in direction could be adopted by both, using the present harmonious relationship with the American Public Health Association as a guide.

Approved.

REPORT OF COMMITTEE ON NECROLOGY

ANDREW LINCOLN WINTON

Dr. Winton was born at Westport, Connecticut, January 26, 1864, and died October 17, 1946. After attending high school at Bridgeport, Connecticut, he graduated in 1884 from Sheffield Scientific School of Yale University with the degree of Ph.B. After graduation he became a member of the staff on the Connecticut Agricultural Experiment Station where he began his work on vegetable histology. Winton's first attendance at the Association Meetings was in 1891 at which time he presented a paper on the Determination of Potash. He was president of this Association in 1898. His interest in our Association continued to the end when only nine days before his death he wrote, "I have been looking ahead to the A.O.A.C. Meeting."

HENRY E. BARNARD

Dr. Barnard was born November 14, 1874, in Dumbarton, New Hampshire. He graduated from the University of New Hampshire in 1899. He received two honorary degrees, Ph.D. from Hanover College in 1913 and Sc.D. from New Hampshire in 1928. In 1901 he organized the New Hampshire State Laboratory of Hygiene and in 1905 performed the same duty for Indiana. He drafted the first food and drug law to be adopted after the passage of the Federal Law. He served as Federal Food Administrator during World War I and organized the Corn Industries Research Foundation and the National Farm Chermurgic Council during World War II, in both of which he was director. Dr. Barnard was principal Employment Specialist in Chemistry for National Personnel of Scientific and Specialized Personnel. He was active in the American Chemical Society, serving as a director from 1917 to 1920. He died December 31, 1946, in Indianapolis, Indiana, after an illness of five months.

ANDREW L. FELKER

Andrew L. Felker, New Hampshire's beloved Commissioner of Agriculture, died suddenly October 17, 1946, at the home of his son, Louis K. Felker at Laconia at the age of 77. He died at the end of a hard days work in the interest of his fellow farmers. He was born in Barrington July 6, 1869, and was appointed the first Commissioner of Agriculture for New Hampshire on January 10, 1914. At that time he had little help while today it requires 28 full time employees to handle the department. He organized and administered control and eradication of all diseases of live stock and poultry, laws controlling sale of seeds, feeds, fertilizers, insecticides and fungicides; dairy plant inspection; insect and plant disease disease control; apiary inspection and seed potato certification, and had the interest of all farmers in the State and was quick to establish laws for their benefit. He was the oldest commissioner of agriculture in the United States in years of continuous service, held the honorary degree of LL.D. and was active in many organizations. His place will be difficult to fill.

CHARLES ALBERT BROWNE

On February 3, 1947, Dr. Browne died in Washington, D.C., at the age of 76 years. His obituary published in the August 15, 1947, number of the *Journal* is an appreciation of his life and work and need not be repeated here. Suffice it to say that he achieved high rank in his chosen field, agricultural chemistry, particularly the chemistry of sugars, and withal was a charming scholar and gentleman. He was a warm supporter of this Association, serving as its president in 1925. He attended the meeting last fall and enjoyed to the full the contact with his many friends. In his passing we have all suffered loss.

WILLIAM HORACE ROSS

Dr. Ross was born on December 27, 1875 at River John, Nova Scotia, Canada, and died on May 16, 1947. His work on agricultural chemistry, started in Arizona in 1907 and continued after 1912 in the U. S. Department of Agriculture until his retirement in 1945, has been extremely fruitful. He has made many contributions to the development of methods for fertilizer analysis and has had a strong influence on the methods adopted by this Association. His work is more fully described in his obituary which appeared in the August 15, 1947, number of this *Journal*. The President's address, given at the October 1946 meeting of this Association, showed his broad understanding of the factors involving soil fertility and productivity. His counsel in the Association will be seriously missed.

BERNARD A. LINDEN

Born on December 17, 1893, in New York City, he died on March 9, 1947. Mr. Linden joined the staff of the Bureau of Chemistry in 1914 as a laboratory helper. He studied bacteriology and other biological sciences at Emerson Institute and George Washington University night schools and was promoted to the professional service in 1925. In his long career as a food bacteriologist he combined a high degree of technical skill and investigational ability in his work, notably on decomposition of fishery products, sanitation in food packing plants, and in the field of food poisoning. At the time of his death he was serving as supervising analyst in the Bacteriological Section, Division of Microbiology.

L. S. WALKER, *Chairman*
W. B. WHITE
E. L. GRIFFIN

REPORT OF THE COMMITTEE ON NOMINATIONS

President: G. H. Marsh, Director Chemistry Division, Montgomery, Ala.

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

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

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

Additional Members of the Executive Committee: W. A. Queen, Washington, D. C.; H. A. Halvorson, State Chemist, St. Paul, Minn.; W. B. White, Washington, D. C.; J. O. Clarke, Food and Drug Administration, Chicago, Ill.

W. H. MACINTIRE, *Chairman*
FRANK H. WILEY
LESLIE E. BOPST

Approved.

REPORT OF COMMITTEE ON RESOLUTIONS

Whereas, the officers of our Association have capably and efficiently performed their duties during the past year and have planned and carried through successfully this the 61st meeting at the close of the 63rd year of our organization, therefore, be it

Resolved, that we express our appreciation and thanks to President J. O. Clarke, Vice-president George H. Marsh, Secretary-Treasurer Henry A. Lepper, and other members of the Executive Committee, for unselfish and able service rendered.

Whereas, the progress of the work of this Association depends so much upon the effectiveness of the services of our Referees and Associate Referees, therefore, be it

Resolved, that the Association extend this expression of genuine appreciation to these workers who have given so generously of their time and efforts toward the accomplishment of the aims of this organization.

Whereas, the registration at the present meeting reveals the presence of representatives of State and Federal agencies and institutions in all parts of the Nation, and from Canada, therefore, be it

Resolved, that we hereby express our thanks to those agencies responsible for making possible the attendance of so many representatives; Be it further

Resolved, that the Secretary express to the management of The Shoreham Hotel the thanks of our Association for making available rooms and other facilities suitable for the successful conduct of the meeting.

GUY G. FRARY, *Chairman*
E. M. NELSON
J. J. TAYLOR

Approved.

CHANGES IN OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE SIXTY-FIRST ANNUAL MEETING, OCTOBER 20, 21, AND 22, 1947*

The changes in the methods of the Association recorded below become effective March 17, 1948, as provided in section 8 of the by-laws, thirty days from the date of publication of this Report, Feb. 15, 1948.

1. SOILS

(1) The tentative method for fluorine, section **1.32** (p. 13), line 3, was changed by substituting "500" for "900."

2. FERTILIZERS

(1) The official method for sampling, section **2.1** (p. 20), was changed

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

by substituting "20" for the first "10" in line 7 and by addition of the following sentences between "bags" and "Thoroly" in line 7:

"In sampling a bulk lot of fertilizer, draw not less than 20 cores from different regions of the lot. In sampling fertilizer in small containers (10 lbs or less) a single package may constitute a sample for the lot."

The whole section, as revised, was adopted as official, first action.

(2) The official volumetric method for phosphoric acid was changed, and the method as revised was adopted as official, first action, by—

(a) substituting "324.03" for "323.81" line 1 and "32.40" for "32.38" line 2, in section **2.10(b)**;

(b) deleting "and dilute to 200 ml with H_2O" from section **2.11** (p. 23) and designating the section as "**(a)**";

(c) by adding to section **2.11** the following:

"**(b)** *Not applicable in preparation of solns by sulfuric acid digestion.*—Proceed as directed under **2.8 (a)**, **(b)**, or **(c)**, preferably **(a)** when these acids are a suitable solvent, to point where acid digestion of sample is completed. Add 25 ml of 10% $BaCl_2$ soln to the hot digestate, boil ca 2 min, and continue as directed under **2.8**."

(d) adding to section **2.12(a)** (p. 23) the following as the first sentence: "Prepare soln of sample as directed under **2.11(a)**."

(e) deleting the phrase, in section **2.12(b)** first line, "Not applicable to superphosphates and other fertilizers that contain sulfates (5)," and adding the following as the first sentence: "Prepare soln of sample as directed under **2.11(b)**."

(f) adding to section **2.12** a new paragraph, as follows:

"**(c)** *Not applicable to superphosphates and other fertilizers that contain sulfate or to solns prepared with the aid of sulfuric acid.* (5).—Prepare soln of sample as directed under **2.11(a)**. Proceed as directed under **(b)**."

(3) The official method for potash was changed and the method as revised was adopted as official, first action, by substituting "95%" for "80%" in section **2.40(d)**, (p. 31), line 1, and section **2.42** (p. 32)' lines 12 and 15.

3. AGRICULTURAL LIMING MATERIALS

(1) The following method was adopted as tentative for neutralizing value of blast furnace slag.

Blast Furnace Slags.—Weigh 0.5 gram charge ground to pass 80-mesh sieve and transfer into a 250 ml Erlenmeyer flask. Wash down with small portions of water and introduce 35 ml of 0.5 N HCl while swirling. Heat to a gentle boil over Bunsen burner, *agitating the suspension continuously* until the bulk of the sample has dissolved. Maintain the boiling for 5 min.; cool to room temperature; then dilute with CO_2-free H_2O to about 150 ml and add 1 ml of 30% H_2O_2 and 3 drops of a 1% soln of Bromcresol green. (Methyl red indicator, 4 drops of a 2% soln, may be substituted for Bromcresol green with some advantage in visibility of end point. This indicator is very unstable, however, and requires renewal of buffer soln every few days.) Back titrate with 0.5 N NaOH, adding the first 15 ml rapidly and continue titration dropwise, contents of stoppered flask being agitated vigorously after each addi-

tion until the indicator tint matches or slightly oversteps that of a *p*H 5.2 phthalate buffer soln of like volume and indicator concentration after agitation for 2–3 seconds.

(2) The tentative procedures, section **3.3**, p. 42, and the one above are to be annotated by the statement "without correction for sulphite content."

4. COSMETICS

The following method was adopted as official, first action:

GLYCEROL IN VANISHING CREAM

REAGENTS

Potassium periodate.—.02 *M*. Dissolve 4.6 grams of C. P. KIO_4 in ca 500 ml of hot water. Dilute to about 900 ml with water, cool to room temperature, and make to 1 liter.

Sodium hydroxide.—0.02 *N*. (See **43.5** or **43.6**)

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

Propylene glycol.—A pure product containing no free acidity or liberating acidic substances on oxidation is desirable. A product B.P. 85–86°C/10 mm is suitable.

DETERMINATION

Isolation of glycerol.—Place 2 to 10 gm of sample in a separatory funnel, add 25–50 ml of water, acidify slightly with dilute H_2SO_4 (10 gm/100 ml), and extract with successive portions of chloroform. Usually 4–5 portions of chloroform, each ca 35 ml, are sufficient to remove all chloroform soluble material. Wash the combined chloroform extract with 10 ml of water. Filter the aqueous solution and wash water through a cotton plug to remove droplets of chloroform, and receive filtrate in a 250 ml volumetric flask. Add 3 drops of bromcresol purple indicator to the filtrate and neutralize with carbonate free alkali (0.1 *N* NaOH is satisfactory) making the final adjustment with the .02 *N* NaOH. Dilute almost to the mark with water, and if necessary, add more alkali so as to maintain a light but definite purple color in the solution, and then complete the dilution to the mark.

Periodate oxidation.—Transfer an aliquot of the neutral soln preferably containing 30–40 mg of glycerol to a 100 ml volumetric flask and add 50 ml of .02 *M* KIO_4. Make to mark with water and allow to stand ca 1 hour. (Test for excess periodate which must be present in the oxidation mixture by adding $NaHCO_3$ and KI to a test portion; if excess is present, iodine will be liberated.)

Formic Acid Titration.—(Applicable in absence of substances yielding acid on periodate oxidation.) Transfer a 50 ml aliquot of the oxidized mixture to a titration flask, add 10 drops of propylene glycol (ca 0.5 ml), mix well, wash down the sides of the flask with water and allow to stand for 10 min. Add 3 drops of bromcresol purple indicator and titrate with 0.02 *N* NaOH to a light purple end point.

1 ml 0.02 *N* NaOH = 1.84 mg glycerol

5. ENZYMES

No additions, deletions, or other changes.

6. INSECTICIDES AND FUNGICIDES

(1) The following method was adopted as tentative.

TOTAL CHLORIDE IN EMULSIONS CONTAINING DDT, SOLVENT, EMULSIFYING AGENT, AND WATER

Weigh a quantity of well-mixed sample containing about 0.75 g of DDT in a

tared weighing bottle. Wash into a 100-ml volumetric flask and make to volume with 99% isopropanol. Transfer a 10-ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. Place on a steam bath, and expel the isopropanol and water in a current of air. Remove last traces of solvent and water from the cooled flask. If drops of water still remain, add 10 ml of isopropyl alcohol and repeat the evaporation. Proceed as directed in method (6) *This Journal*, **30**, 65 (1947), line 2, beginning "Add 25 ml of 99% isopropanol. . . "

(2) The tentative method for DDT by total chlorine, *This Journal*, **30**, 64 (1947), was changed by deleting from the first "and," line 2, and ending with "and," line 6, and substituting therefor "add 10 ml thiophene-free benzene, dissolve the sample, make to volume with 99% isopropanol. Transfer a 25 ml aliquot to a 250–500 ml standard tapered Erlenmeyer flask. Add. . ."

(3) The tentative method for total chlorine in dusting mixtures containing DDT in absence of organic matter, *This Journal*, **30**, 64 (1947), was changed by deleting beginning with "From" line 3, p. 65, through "bath" line 4, and substituting therefor:

"Evaporate on steam bath until most of the benzene is removed. It is not desirable to evaporate to dryness, as DDT may decompose with loss of hydrochloric acid. Add 25 ml of 99% isopropanol and 2.5 g of metallic sodium in the form of ribbon or cut in small pieces and shake flask to mix sample with the alcohol. Proceed as in (1) *This Journal*, **30**, 64 (1947) beginning "Connect the flask."

(4) The tentative method for total chlorine in dusting mixtures containing DDT in presence of organic matter such as coloring matter, plant resins, etc., *This Journal*, **30**, 65 (1947), was changed by deleting beginning with "Proceed" line 7, through "water. . ." line 8, and substituting therefor:

"Evaporate on steam bath until most of the benzene is removed. It is not desirable to evaporate to dryness, as DDT may decompose with loss of hydrochloric acid. Add 25 ml of 99% isopropanol and add 2.5 g of metallic sodium in form of ribbon or cut in small pieces, and shake flask to mix the sample with the alcohol. Connect to a reflux condenser and boil gently for at least ½ hour. Shake flask occasionally. Eliminate excess metallic sodium by cautiously adding 10 ml of 50% isopropanol through the condenser at the rate of 1–2 drops per second. Boil for an additional 10 min., then add 60 ml of H_2O."

(5) The tentative method for total chlorine in mineral oil sprays containing DDT in the absence of organic matter (plant extractives material, organic thiocyanate) was changed by deletion beginning with "From," line 2, through "isopropanol," line 3, and substituting "Add 25 ml of 99% isopropanol and 2.5 g of metallic sodium and proceed as in (1) *This Journal*, **30**, p. 64 (1947), beginning "in the form of ribbon."

(6) The tentative method for p, p′ DDT, section **6.152** (p. 86), was changed by deleting beginning "Reflux" line 3, through the second "Add," line 4, and substituting therefor "Place in a water bath at 25° for 35–60 min, add 10 ml of H_2O and add . . ."

(7) The title of chapter 6 was changed to "Economic Poisons."

7. CAUSTIC POISONS

No additions, deletions, or other changes.

8. NAVAL STORES

No additions, deletions, or other changes.

9. GELATIN, DESSERT PREPARATIONS, AND MIXES

(1) The tentative method for jelly strength section **9.6** (p. 104), was deleted.

(2) The following method was adopted as tentative.

JELLY STRENGTH

"To 7.5 g of sample add from pipet, with stirring, 105 ml of water at 10° to 15°C· Let stand for one hour and then bring to 62° in 15 min. by placing in a water bath regulated at 65°; the sample may be swirled several times to aid solution. Finally mix by inversion, let stand 15 min. and place in a water bath at 10° controlled at ±0.1°. Chill for 17 hours. Determine jelly strength on Bloom Gelometer (2)* adjusted for a 4 mm depression and to deliver 200 gms shot/5 seconds (±5 gms), using the ½" plummet."

10. LEATHERS

No additions, deletions, or other changes.

11. TANNING MATERIALS

No additions, deletions, or other changes.

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 following details for alcohol by immersion refractometer, *Methods of Analysis*, 5th ed., Chapter XIV, section **5(c)**, were omitted from the 6th edition and should appear under section **14.5** (p.151) as **(c)**, and are as follows:

(c) *By immersion refractometer.*—Verify the percentage of alcohol as determined under **(a)** or **(b)** by ascertaining the immersion refractometer reading of the distillate and obtaining the corresponding percentage of alcohol from Tables **44.24** and **44.25**."

(2) The following official, first action (*This Journal*, **30**, 67 (1947)) methods for malt were made official, final action:

Sampling, section **14.39** (p. 158).
Preparation of sample, section **14.40** (p. 159).
Bushel weight, section **14.41** (p. 159).
1,000 kernel weight, section **14.45** (p. 160).

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* Reference number refers to the selected references, Methods of Analysis, 6th Ed., p. 106.

Moisture in caramel malt and black malt, section **14.52** (p. 161).
Diastatic power, section **14.61** (p. 166).

(3) The following official, first action (*This Journal,* **30**, 67 (1947)) methods for beer were adopted as official, final action:

Extract of original wort, section **14.7** (p. 151).
Real degree of fermentation or real attentuation, section **14.8** (p. 152).
Apparent degree of fermentation or apparent attenuation, section **14.9** (p. 152).
Total acidity—Indicator Titration Method, section **14.10** (p. 152).
Total acidity—Potentiometric Titration Method, section **14.11** (p. 152).
H-ion concentration (*p*H)-Electrometric Method, section **14.12** (p. 153).

(4) The following official, first action (*This Journal,* **30,** 67 (1947)) methods for hops were adopted as official, final action:

Sampling, section **14.80** (p. 170).
Physical examination, section **14.81** (p. 170).
Preparation of sample for chemical analysis, section **14.82** (p. 171).
Moisture, section **14.83** (p. 172).
Resins, section **14.84–14.89,** inclusive (p. 172).

(5) The following official, first action (*This Journal,* **30**, 67 (1947)) methods for brewing sugars and sirups were adopted as official, final action:

Extract, section **14.90** (p. 174).
Non-extract (apparent water), section **14.91** (p. 174).
Fermentable extract (b) rapid fermentation method, section **14.92** (p. 174).
Protein, section **14.93** (p. 175).
Iodine reaction for unconverted starch, section **14.95** (p. 175).
Acidity, section **14.96** (p. 175).
H-ion concentration (*p*H), section **14.97** (p. 176).
Ash, section **14.98** (p. 176).
Total reducing sugars, Munson-Walker method, section **14.99** (p. 176).

(6) The following official, first action (*This Journal,* **30**, 67 (1947)) methods for wort were adopted as official, final action:

Specific gravity, section **14.103** (p. 176).
Original extract or original gravity, section **14.104** (p. 176).
Fermentable extract, section **14.105** (p. 176).
Iodine reaction, section **14.106** (p. 177).
Total acidity, section **14.107** (p. 177).
H-ion concentration (*p*H), section **14.108** (p. 177).
Color, section **14.109** (p. 177).
Protein, section **14.110** (p. 177).
Total reducing sugars, section **14.111** (p. 177).

(7) The official Milos test for caramel, **14.35** (p. 158) was deleted, first action.

15. WINES

(1) The official Milos test for caramel, section **15.38** (p. 188), was deleted, first action.

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

CARAMEL

Mathers Test

Preliminary.—Before applying the following tests, the sample to be analyzed should be filtered through ordinary laboratory filter paper in order to remove suspended particles and sediment. The sediment or particles could also be eliminated by centrifuging. Use the clear portion for analysis.

Mather's Test.—To 10 ml of the above filtered wine or spirits previously introduced into a Babcock cream bottle or any convenient small centrifuge bottle, add 1 ml of pectin soln (made by dissolving 1 g of pectin in 75 ml of water and adding 25 ml of alcohol for preserving it. Shake well before using.) Add to the material to be tested in the centrifuge bottle about 3 drops of concentrated HCl and fill bottle with alcohol (ca 50 ml or more). Shake well and centrifuge for 5 min. or more and decant carefully the supernatant liquid off of the gelatinous residue. Dissolve the residue in the bottle by adding 10 ml water and shaking well. To this residue dissolved in water add about 3 drops of concentrated HCl and 50 ml or more of alcohol; shake well and again centrifuge. Repeat this process until the upper alcoholic layer is quite clear and colorless. After the final decantation of the water-white supernatent alcohol, the gelatinous residue is dissolved in 10 ml of hot water. A clear brown soln indicates caramel coloring. To further confirm caramel, add 1 ml of the following reagent (made by dissolving 1 g of 2,4-di-nitro-phenyl-hydrazine in 7.5 ml of conc. sulphuric acid and bringing the volume up to 75 ml with 95% ethyl alcohol; kept in a glass-stoppered bottle in which it will stay clear and stable for several months) to the residue dissolved in the 10 ml of hot water. Stand the bottle in a beaker of boiling water for 30 min. In the presence of substantial quantities of caramel a precipitate forms almost at once. Smaller amounts show as a precipitate before the 30 min. are up. Even the smallest amount of caramel will show a precipitate under the conditions described, but if the analysis has been conducted as described above, no precipitate will appear if caramel is absent. In order to be sure a precipitate has formed, pour the hot test soln from the bottle on a small filter paper and wash any residue with hot water. A reddish brown precipitate will be clearly seen on the filter. This precipitate, although amorphous, is quite characteristic and will always be the same if caramel is present. A low-power microscope affords a fine examination of the precipitate. If caramel is found to be present by the above test, check the sample as follows:

Confirmatory Test

Place 10 ml of wine or spirits in a small centrifuge bottle (Babcock cream bottle), approximately neutralize with 2% KOH soln, add 2 ml of 5% $ZnCl_2$ soln, and 2 ml of 2% KOH soln. Shake thoroughly and centrifuge for about 5 or more min. Decant the supernatent liquid carefully from the residue and add hot or boiling water. Shake thoroughly to wash precipitate, centrifuge, and again wash with hot water. Repeat this process until the upper aqueous water layer is quite colorless. Any amount of hot water may be used. To this well-washed residue add 50 ml or more of 85% alcohol containing 0.5% HCl. Shake well and centrifuge and decant off the upper liquid from any residue that remains below. If caramel is present a thin brown layer will be at the bottom of the centrifuge bottle. Add another portion of 85% alcohol containing 0.5% HCl, and again centrifuge. Repeat this process until the upper supernatant liquid is quite clear and colorless. To facilitate this washing of the caramel or any residue, the centrifuge bottle containing the 85% alcohol with 0.5% HCl may be dipped for a few min in the beaker of boiling water. After the final washing with acid alcohol and decanting off the alcoholic wash liquid, the residue, if any is present, is dissolved in 10 ml of water. A brown solution indi-

cates caramel. To this brown soln, add 1 ml of the 2,4-di-nitro-phenyl-hydrazine reagent in the manner described above. If no precipitate appears after 30 min., no caramel is present. If the precipitate is difficult to see in the orange-colored test soln, pour it on the filter paper immediately after the 30 min boiling, and a 30 min cooling period is over.

(3) The directions of the tentative confirmatory tests for caramel, section **15.39** (p. 189) were changed by—

(a) Deleting the last sentence of paragraph 1 beginning "If there . . ." and paragraphs 2, 3, 4 and 5;

(b) substituting therefor the following—

To the brown solution in the Babcock or other test bottle add 1 ml of 2,4-dini-tro-phenyl-hydrazine reagent and place bottle in beaker of boiling water and heat the bottle and its contents therein for 30 min, turn off the heat and allow the bottle to cool with the water for 30 min and examine; a brown precipitate confirms the presence of caramel.

16. DISTILLED LIQUORS

(1) The official modified Marsh test for caramel, section **16.39** (p. 302), was deleted, first action.

(2) The following test was adopted as tentative for distilled liquors and for cordials and liqueurs:

CARAMEL

Fulton Test

REAGENTS

(a) *Acetone reagent.*—Acetone, 500 ml; amyl alcohol, 200 ml; ethyl acetate, 200 ml; syrupy H_3PO_4, 50 ml; water, 50 ml.

(b) *Sodium di-hydrogen phosphate.*—NaH_2PO_4, 25 g; water, 100 ml.

DETERMINATION

To ca 5 ml of spirits add 10 ml of the acetone reagent and 3 ml of the NaH_2PO_4 soln, shake and let stand. Color in the lower layer indicates caramel.

(3) The official Milos test for caramel, section **16.41** (p. 202) was deleted, first action.

(4) The tentative method for alcohol by volume, section **16.46(b)** (p. 203), was changed by adding "except calibrate pycnometer at 15.56° and measure sample at that temperature."

(5) The Mathers test for caramel, see (2) and (3) under **15, Wines,** above, was adopted for distilled liquors and for cordials and liqueurs, as official, first action.

(6) The tentative method for caramel, section **16.60** (p. 204), was changed to drop reference to sections **16.39** and **16.41**.

17. BAKING POWDERS AND BAKING CHEMICALS

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

(2) The following method was adopted as tentative:

RESIDUAL CARBON DIOXIDE IN BAKING POWDERS

GASOMETRIC METHOD

Place 1.7 g of baking powder in a clean, dry, 250 ml, wide-mouthed sohxlet extraction flask (flask A 17.5). Add 20 ml distilled water. Put flask *on the cover* of a water bath (single or multiple) in which boiling water is kept at a constant level of 2 inches below top of bath. The water in the bath should be boiling vigorously all through the determination and bath heated with gas or electricity. The opening in the cover of bath into which flask is set must be 3 inches in diameter. This prevents the flask from touching water, keeping it at a definite distance above. Evaporate contents of flask until there is no visible evidence of moisture in residue or inside surface of flask. If set-up is functioning properly, sample should be completely dry in 1½ to 2 hours. Leave the flask on water bath for 2 hours. Add 10 ml of distilled water and let stand until flask is at room temperature (ca 1 hour).

Determine carbon dioxide with Chittick apparatus as in **17.6**, using correction factors in section **44.30**. Shake the flask vigorously until further shaking produces no increment in the reading.

(3) The official method for available carbon dioxide, section **17.9** (p.210) was changed, first action, deleting "**17.8** from **17.6**" and substituting "the result from the tentative gasometric method from **17.6**"

18. COFFEE AND TEA

No additions, deletions, or other changes.

19. CACAO BEAN AND ITS PRODUCTS

The following method was adopted as tentative.

TANNINS AND PIGMENTS

DETERMINATION

Place 25 g of sweetened products (such as milk chocolate, light sweet chocolate, skim, buttermilk, or malted milk chocolate) in a 250 ml centrifuge bottle. Extract three times with 100 ml of ether, shaking well, centrifuging, and decanting each time. Expel most of the residual ether by passing a moderate current of air through the bottle for ca 10–15 min. Add 40 ml of water, measured closely by graduate, to the bottle, stopper, and shake well for a minute or more to thoroughly disperse the contents of the bottle. Now add 40 ml of 1% sodium oxalate soln and again shake well. (If no milk solids are present, 80 ml of water can be used instead of the water and sodium oxalate.)

Place the bottle in the centrifuge and whirl for 10 min. or more at about 1800 r.p.m. Remove and decant the supernatant liquid into a 125 ml separatory funnel. Again add 20 ml of 0.5% sodium oxalate (equal volumes water and 1% Na oxalate) to the centrifuge bottle, shake well, and centrifuge as before.

Place 55 ml of absolute alcohol, 60 ml of acetone, and 130 ml of ethyl ether (all measured by graduate) in a 500 ml Erlenmeyer flask. Add also 28–30 g of salt (NaCl) and 1 ml of glacial acetic acid. Add the contents of the separatory funnel gradually in a small stream (requiring about 5 min time) to the contents of the flask while shaking the latter with vigorous rotation. Close the stopcock on the funnel and pour in the liquid of the second extraction from the centrifuge bottle. Shake funnel to rinse and add this extract also in a similar manner (shaking) to the contents of Erlenmeyer flask. Reserve the contents of the centrifuge bottle for further treatment. Stopper and shake the Erlenmeyer and if it contains a milk protein precipitate, pour

the contents of the flask into centrifuge bottles, rinse flask with a wash of mixed ether, alcohol, and acetone in proportions used above. Centrifuge 10 min. and carefully decant the liquids into a 500 ml separatory funnel, retaining the precipitate in the bottles.

Rinse the bottles with about 30 ml of the wash of the three mixed solvents, pour out he contents of bottle on a Büchner funnel containing a Whatman No. 54 filter paper, and filter with suction. Add filtrate to contents of 500 ml separatory funnel. Draw off the lower layer and filter the top remaining layer through a plug of cotton into an 800 ml beaker. Wash funnel once with mixed solvent.

Measure 125 ml of absolute alcohol in a graduate. Add a portion of it to the bottle containing the residue reserved above. Stopper and shake well to disperse the residue and transfer it to a 300 ml Erlenmeyer flask, using all the alcohol. Add 2 ml conc. HCl to the flask, place on the steam bath, heat to the boiling point and 1 minute longer. Remove and filter on a 7-cm Büchner funnel using a CS&S No. 589 blue ribbon paper. Rinse the flask and wash the filter with 20 ml of absolute alcohol and with 25 ml of acetone.

Transfer the alcoholic filtrate quantitatively to the 800 ml beaker containing the previous extract. Evaporate the extracts on an electric hot plate to about 200 ml, add 75 ml of water, and continue boiling to about 75 ml. Add more water (40–50 ml) and continue boiling until all alcohol is driven off, adding water again if necessary. Transfer the liquid and any sediment quantitatively to a 250 ml beaker and boil down to ca 35 ml. Cool and pour the contents into a 50 ml graduate and note volume. Decant into a 200–250 ml flask and rinse beaker and graduate with small portions of water sufficient to make the total volume to 50 ml. Transfer any residue to the flask. Add 4 ml conc. HCl and 10 ml of 40% formaldehyde soln (Stiasny's reagent) to the contents of flask. Place the flask under a condenser and reflux for 30 min. Cool to room temperature and filter off the precipitate on a tared gooch crucible. Transfer the precipitate and wash with two 15 ml portions of wash soln (5 ml conc. HCl and 10 ml 40% formaldehyde added to each 100 ml H_2O). Then wash once with 10 ml of water. Dry the precipitate in the oven at 100°C., cool, and weigh. Obtain weight and per cent of precipitated tannins and pigments.

20. CEREAL FOODS

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

PHOSPHORUS IN CEREALS AND CEREAL PRODUCTS

REAGENTS

$Mg(NO_3)_2$ soln.—Dissolve 8 g MgO in HNO_3(1+1), avoiding excess of the acid; add a little MgO in excess, boil, filter from the excess MgO, Fe_2O_3, etc, and dilute to 100 ml.

Molybdate soln.—section 2.7 (a), p. 21.

DETERMINATION

(a) Transfer 1.0 g of sample to a size #3 porcelain casserole (140 ml capacity), add 3 ml $Mg(NO_3)_2$ soln, mix well, using a small glass rod, clean off the rod with a small piece of filter paper and place in the casserole. Drive off most of the moisture by drying in the oven at 100°C for about 2 hours, transfer to a cold muffle and ignite at 550°C until a white or gray ash is obtained (about 6–8 hours). Cool, cover with watch glass, take up with 10 ml HCl (1+4), add 5 ml conc. HCl, rinse off watch glass, and evaporate to dryness on steam bath, add 5 ml conc. HCl and 50 ml H_2O, heat 15 min on the steam bath, filter into 100 ml volumetric flask, cool and make to volume. Pipet 50 ml into 300 ml Erlenmeyer flask, neutralize to litmus with NH_4OH,

make just faintly acid with HNO_3, dilute to 75–100 ml, add ca 15 g NH_4NO_3, and proceed under section **2.12**, p. 23, beginning "add sufficient molybdate soln to insure complete precipitation . . . " or

(b) Transfer 5.0 g of sample to a size No. 000 porcelain evaporating dish (35 ml capacity), mix well with 0.5 g Na_2CO_3, ash at 550°C until gray ash, cool, cover with watch glass, take up with 2 ml HCl (1+4), add 5 ml conc. HCl, rinse off watch glass, evaporate to dryness, add 5 ml conc. HCl and 10 ml H_2O and heat about 10 min on steam bath, filter into 100 ml volumetric flask, cool, make to volume. Pipet 10 ml aliquot in 300 ml Erlenmeyer flask and proceed as above beginning "neutralize to litmus with NH_4OH . . . " Report results as % P.

(2) The official dry ashing method for iron, sections **20.9–20.12** (pp. 238–239), was adopted as official, final action, for enriched macaroni products, degerminated, bolted, and whole corn meals.

(3) The wet ashing method for iron, *This Journal*, **30**, 71 (1947), was made official, final action.

(4) The tentative method for benzoyl peroxide bleach in flour, section **20.53** (p. 253), was deleted as applied to wheat flour but retained as applicable to rye flour and the title changed to Benzoic Acid in Rye Flour.

(5) The following method was adopted as tentative:

BENZOIC ACID IN WHEAT FLOUR

Place 50 g of flour in (preferably) glass-stoppered flask, add 30–40 glass beads (about 6 mm diam.), 0.1 g powdered iron, 100 ml ether or isopropyl ether. Allow to stand a few min. shake with a rotary motion and add slowly (preferably dropwise) 2.5 ml HCl from a Mohr pipet. Allow to stand overnight. Shake well with rotary motion, allow the flour to settle a few minutes, and decant through a Büchner funnel (100 mm) fitted with filter paper moistened with ether into a 500 ml suction flask. Add 50 ml ether, shake, and allow to settle a few minutes, decant as before, repeat twice more, transferring the whole contents to the funnel following the last addition. Transfer through a large funnel into a 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 a 125 ml Erlenmeyer flask, repeat with two more 20 ml portions of 5% $NaHCO_3$. Add to this soln 0.3 g decolorizing C (Nuchaw W), shake and filter (11 or 12.5 cm 589 white ribbon, S&S) into 125 ml Erlenmeyer flask, wash flask and filter with about 20–25 ml H_2O, add 2.0 ml H_2SO_4 (1+1) dropwise to avoid foaming out of the flask. (The soln should be definitely acid to litmus paper.) Transfer to a 125 ml separatory funnel, rinse out the flask with 12 ml ether and add to the 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 out the flask each time with the ether. After each extraction draw off the aqueous soln into the same 125 ml Erlenmeyer flask and transfer the ether to a Pyrex test tube (about 50 ml capacity, 25 mm diam. and 150 ml length) and add 2 ml 10% NaOH. Hold top of tube firmly against the hand and shake vigorously, insert a piece of copper wire (1 mm diam.×200 mm) into the tube, evaporate the ether very slowly on the steam bath. Remove copper wire. Place the tubes into a beaker of boiling water and evaporate nearly to dryness, add 0.5 ml 30% H_2O_2, followed by another 0.5 ml as soon as the foam condition permits. Continue evaporation until there is no apparent moisture in the tube. (The introduction of a gentle air blast into the tube hastens the 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 the tube, heat

20 min. in a gently boiling water bath, immediately cool under tap, add 6 ml water while continuing to cool. Then slowly add 15 ml conc. NH_4OH with continuous shaking under the tap to keep soln cool. Add 2 ml of 6% hydroxylamine hydrochloride soln, stir, place in 65° water bath 5–6 min, stirring occasionally. Cool under tap, filter into similar tube and observe color of filtrate. Red or definitely pink color indicates the presence of benzoic acid. In case the color is not definite enough to indicate the presence of benzoic acid in a light bleach flour, repeat the analysis as above, except use 100 g flour, 200 ml ether, and 5 ml HCl.

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

(6) The tentative method for soybean flour, sections **20.77–20.79**, and **20.82**, were changed to read as follows:

20.77 Moisture, "see **20.2** or **20.4**, with the exception that a 5 g sample be dried at 130° for 2 hours.

20.78 Ash, "see **27.9**."

29.79 Nitrogen, "Proceed as directed under **2.26** using 10 g K_2SO_4 or Na_2SO_4 and 0.7 g Hg or its equivalent in Hg with the option of using sodium alizarin sulfonate."

20.82 Oil or Petroleum Benzine Extract, "see **31.07** except that ca 2 g full fat soy flour or 5 g low defatted flour be extracted for 5 hours."

(7) The method for total solids in entire loaf of bread (raisin bread and bread containing raisins and fruits) section **20.84(b)**, p. 261, official, first action, *This Journal* **30**, 72 (1947), was adopted as official, final action.

21. COLORING MATTERS

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

Intermediates

ANILINE

REAGENTS

Sodium nitrite soln.—Dissolve 79 g of $NaNO_2$ in 1 liter of water.

Sulfamic acid soln.—Dissolve 97 g of NH_2HSO_3 in 1 liter of water.

Coupling soln.—In a 500 ml wide-mouthed flask dissolve 0.1 g of 1-(4 sulfophenyl) 3-methyl-5-pyrazolone in 2 ml of ca 2.5 *N* NaOH soln and add 50 ml of H_2O and 10 grams of trisodium citrate. Cool to 10°C or below and hold at that temp. until used. This reagent must be freshly prepared for each determination.

DETERMINATION

Place 10 g of the dye, 200 ml of H_2O, and 5 g of trisodium citrate in a 500 ml round bottom flask equipped with a standard-taper neck. Connect the flask to a condenser with a suitable connecting tube. Distill 100 ml into a 100-ml graduated cylinder containing 1 ml of conc. HCl. Regulate the rate of distillation to require ca 1 hour.[1]

[1] In the distillation of aniline from water insoluble colors, varying amounts of foaming and entrainment are encountered; in such cases, a trap may be used. The presence of a few particles of water insoluble color in the distillate, however, does not affect the results.

Place the distillate in a 500 ml Erlenmeyer flask (washing out the graduate into the flask with a few ml of water), cool in ice to below 10°C and add 1 ml of the $NaNO_2$ soln. Mix well and let stand in the ice bath for 30 min. Then add 2 ml of the sulfamic acid soln, mix well, wash down the sides of the flask with a little H_2O cooled in ice. Let stand in ice 2 to 3 min, then pour into the flask containing the coupling soln.

Let stand at least one hour, heat on a steam bath for ½ hour, then heat to boiling and titrate the yellow color with 0.1 *N* $TiCl_3$. A 1% soln of FD&C Green No. 2 may be used as an indicator; in this case, a correction for indicator blank must be made. 1 ml of 0.1 *N* $TiCl_3$ = 0.0023 grams aniline.

(2) The tentative method for tartrazine, section **20.125** (p. 268), was changed by substituting "0.1 ml" for "1 ml" in 3rd line from end, and adopted as official, first action.

22. DAIRY PRODUCTS

(1) The following method was adopted as official, first action, for fluid milk and cream, cheddar type cheese, and soft uncured cheeses, and as tentative for other types of cheese, ice cream mix, sherbet mix, chocolate-flavored milks and skim milk, butter, sweet-cream and cultured buttermilks, fermented milks, goats' milk, cheese whey, and concentrated milks.

PHOSPHATASE TEST FOR PASTEURIZATION

REAGENTS

1. *Buffers*:

a. *Barium borate-hydroxide buffer.*—Dissolve 25.0 g of C. P. barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$—fresh, not deteriorated] in distilled water and dilute to 500 ml. In another flask or cylinder dissolve 11.0 g of C. P. boric acid (H_3BO_3) and dilute to 500 ml. Warm each to 50°C, mix the two together, stir, cool to approximately 20°C, filter, and stopper the filtrate tightly (*p*H 10.6).

The buffer thus prepared is designated as the 25–11 buffer, the figures indicating the grams per liter of each of the respective reagents. Modifications in the quantities of these two reagents, necessary in preparing the appropriate buffers for testing various products, are indicated in Tables 1 and 2.

b. *Color development buffer.*—Dissolve 6.0 g of sodium metaborate ($NaBO_2$)[1] and 20 g of sodium chloride in water and dilute to 1 liter with water (*p*H 9.8).

c. *Color dilution buffer.*—Dilute 100 ml of color development buffer **1-b** to 1 liter with water.

d. *Standard borax buffer*, 0.01-molar, for checking *p*H meter, *p*H 9.18 at 25°C.[2] Dissolve 0.9603 g of pure borax (Bureau of Standards Sample 187) in distilled water (distilled recently or freshly boiled and cooled) and dilute to 250 ml. Keep stoppered tightly.

2. *Buffer substrates*:

a. *For evaluating pasteurization.*—Dissolve 0.10 g of phenol-free crystalline disodium phenyl phosphate[3] in 100 ml. of the appropriate (Tables 1 and 2) barium borate-hydroxide buffer **1-a.**

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[1] Obtainable from Amend Drug and Chemical Company, Inc., 117 East 24th Street, New York 10 N.Y.

[2] All *p*H values reported herein were determined at 25° C or corrected to that temperature.

[3] Obtainable, relatively pure, from Applied Research Institute, 2 East 23rd Street, New York 10, N.Y.

b. *For quantitative results with raw milk and raw-milk products.*—Dissolve 0.20 g of the phenol-free crystalline disodium phenyl phosphate in 100 ml of the appropriate (Tables 1 and 2) barium borate-hydroxide buffer **1-a.**

3. *Protein precipitants:*

a. *Zinc-copper precipitant for milk.* Dissolve 3.0 g of zinc sulfate ($ZnSO_4 \cdot 7H_2O$) and 0.6 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water and dilute to 100 ml with water. The precipitant thus prepared is designated as the 3.0–0.6 precipitant.

b. *Zinc-copper precipitant for unripened cheese.* Dissolve 6.0 g of zinc sulfate and 0.1 g of copper sulfate in water and dilute to 100 ml with water. This precipitant is designated as the 6.0–0.1 precipitant.

c. *Zinc precipitant for ripened cheese and for butter.* Dissolve 6.0 g of zinc sulfate in water and dilute to 100 ml with water. This precipitant is designated as the 6.0 precipitant.

The quantities of the respective reagents to use in preparing the precipitants for testing other products, not mentioned under "Protein precipitants" above, are indicated in Tables 1 and 2.

4. *BQC* or *2,6-dibromoquinonechloroimine solution* (*Gibbs' reagent*)—Dissolve 40 mg of BQC[4] powder in 10 ml of absolute ethyl or methyl alcohol and transfer to a dark-colored dropper bottle. This reagent remains stable for at least a month if kept in the ice tray of a refrigerator. Do not use it after it begins to turn brown.

5. *Other reagents:*

a. *Copper sulfate, 0.05 percent, for standards.*—Dissolve 0.05 g of copper sulfate in water and dilute to 100 ml.

b. *Butyl alcohol.*—Specify n-butyl alcohol, boiling point 116–118°C. To adjust the *p*H, mix 50 ml of the color development buffer **1-b** with a liter of the alcohol.

6. *Phenol standards:*

a. *Stock soln.*—Weigh accurately 1.0 g of pure phenol, transfer to a liter volumetric flask, dilute to a liter with water, and mix. One ml contains 1 mg of phenol. Use this stock soln to prepare standard solns. It is stable for several months in the refrigerator.

b. *Preparation of standards.*—Dilute 10.0 ml of the stock soln **6-a** to a liter with water, and mix. One ml contains 10 micrograms (0.00001 g; 10 mmg, or 10 units) of phenol. Use this standard soln to prepare more dilute standard solns; *e.g.*, dilute 5, 10, 30, and 50 ml to 100 ml with water to prepare standard solns containing 0.5, 1.0, 3.0, and 5.0 mmg or units of phenol per ml, respectively. Keep standard solns in the refrigerator.

In a similar manner, prepare from the stock soln as many more concentrated standard solns as may be needed, containing, for example, 20, 30, and 40 units per ml.

Measure appropriate quantities of the phenol standard solns into a series of tubes (preferably graduated at 5.0 and 10.0 ml) to provide a suitable range of standards as needed, containing 0 (control or blank), 0.5, 1.0, 3.0, 5.0, 10.0, etc., to 30 or 40 units. To increase the brightness of the blue color and improve the stability of the standards, add 1.0 ml of 0.05% copper sulfate soln **5-a** to each.

Add 5.0 ml of color dilution buffer **1-c** and add water to bring the volume to 10.0 ml. Add 4 drops (0.08 ml) of BQC[4], mix, and allow to develop for 30 min. at room temperature. If the butyl alcohol extraction method is to be used in the test, extract the standards as described under "Conducting the test."

Read the color intensities with a photometer, subtract the value of the blank from the value of each phenol standard, and prepare a standard curve (straight line). When the standards are to be used for visual comparisons they should be stored in a refrigerator.

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[4] Obtainable from Applied Research Institute.

TABLE 1.—*Phosphatase test modifications for different kinds of cheese and cheese of different ages*

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg /0.25 g.
Cheddar, granular, stirred curd, hard cheese	<1 wk.	25-11[b]	6.0-0.1[c]	3
	1 wk.–1.5 mo.	25-11	6.0[d]	3
	1.5–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Washed curd, soaked curd, Colby	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
Swiss, Gruyère	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Brick, Muenster	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
Edam, Gouda	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	2–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Blue mold, blue	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	26-11	6.0	3
	1–4.5 mo.	27-11	6.0	3
	>4.5 mo.	28-11	6.0	3
Camembert, Limburger	<1 wk.	25-11	6.0-0.1	4
	1 wk.–1 mo.	25-11	6.0	4
	1–2 mo.	26-11	6.0	4
	>2 mo.	27-11	6.0	4
Monterey	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	25-11	6.0	3
	>2 mo.	26-11	6.0	3
High moisture Jack	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2.5 mo.	25-11	6.0	3
	>2.5 mo.	26-11	6.0	3
Provolone, pasta filata	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3

TABLE 1.—(*continued*)

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg /0.25 g.
Parmesan, reggiano, monte, modena, Romano, asiago old	<1 wk.	25-11	6.0-0.1	3
	1 wk.–2 mo.	26-11	6.0	3
	2–6 mo.	27-11	6.0	3
	6 mo.–1 yr.	28-11	6.0	3
	>1 yr.	29-11	6.0	3
Asiago fresh	Same as Cheddar			
Asiago medium	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	1–3 mo.	26-11	6.0	3
	>3 mo.	27-11	6.0	3
Gorgonzola	Same as blue			
Cottage,[e] cook cheese, koch kaese	Dry	25-11	6.0-0.1	1
	Moist	25-11 (8+2)[f]	4.5-0.1	1
Cream cheese		25-11 (7+3)	4.5-0.1	3
Semi-soft cheese	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1 mo.	25-11	6.0	3
	>1 mo.	26-11	6.0	3
Soft ripened cheese	<1 wk.	25-11	6.0-0.1	4
	1 wk.–1 mo.	25-11	6.0	4
	>1 mo.	26-11	6.0	4
Nokkelost, kuminost, sage cheese	<1 wk.	25-11	6.0-0.1	3
	1 wk.–1.5 mo.	25-11	6.0	3
	1.5–4 mo.	26-11	6.0	3
	>4 mo.	27-11	6.0	3
Pasteurized process, past. proc. pimiento, past. proc. with fruits, meats, etc.	Soft, mild	25-11	6.0	3
	Medium firm	26-11	6.0	3
	Firm, sharp (incl. Swiss, Gruyère)	27-11	6.0	3
Past. proc. cheese foods; past. proc. cheese foods with fruits, meats, etc.	Same as pasteurized process			
Past. proc. cheese spreads; past. proc. cheese spreads with fruits, meats, etc.	Soft, high moisture, incl. cream spreads	25-11	6.0	3
	Less soft, incl. Blue	26-11	6.0	3

TABLE 1.—(continued)

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
				mmg/0.25 g.
Cold pack, club; cold pack cheese foods; cold pack cheese foods with fruits, meats, etc.	Mild to medium flavored, soft	26-11	6.0	3
	Sharp, firm	27-11	6.0	3

[a] Values higher than those shown indicate under-pasteurization.
[b] Grams $Ba(OH)_2 \cdot 8H_2O$ and H_3BO_3 respectively, per liter.
[c] Grams $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ respectively, per 100 ml.
[d] Grams $ZnSO_4 \cdot 7H_2O$ per 100 ml.
[e] See also more sensitive modification in text (p. 90), alternative.
[f] Eight parts of 25-11 buffer plus 2 parts of water.

TABLE 2.—*Phosphatase test modifications for various dairy products other than cheese*

PRODUCT	QUANTITY OF SAMPLE	BUFFER FOR OPTIMUM pH (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[a]
Milk:				mmg
Fresh	1 ml.	25-11[b] (5+5)[c]	3.0-0.6[d]	2/0.5 ml.
Old or slightly sour	1 ml.	25-11	6.0[e]	2/0.5 ml.
Cream:				
Fresh	1 ml. or 1 g.	25-11 (5+5)	3.0-0.6	2/0.5 ml. or 0.5 g.
Old or slightly sour	1 ml. or 1 g.	25-11 (8+2)	4.5	2/0.5 ml. or 0.5 g.
Ice cream mix	1 ml.	25-11 (8+2)	4.5-0.1	2/0.5 ml.
Sherbet mix	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.
Chocolate drink	1 ml.	25-11 (8+2)	4.5-0.1	2/0.5 ml.
Butter	1 g.	18-8	6.0	2/0.5 g.
Sweet buttermilk	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.
Cultured buttermilk and fermented drinks:				
Medium acid	1 ml.	25-11	6.0	2/0.5 ml.
Very acid, pH <4.5	1 ml.	26-11	6.0	2/0.5 ml.
Goats' milk[f]	3 ml.	27-11	7.5-0.1	1/1.5 ml.
Cheese whey	1 ml.	25-11 (5+5)	3.0-0.6	2/0.5 ml.

[a] Values higher than those shown indicate under-pasteurization.
[b] Grams $Ba(OH)_2 \cdot 8H_2O$ and H_3BO_3, respectively, per liter.
[c] Five parts of 25-11 buffer plus 5 parts of water.
[d] Grams $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively, per 100 ml.
[e] Grams $ZnSO_4 \cdot 7H_2O$ per 100 ml.
[f] Four-hour incubation period; use 7.0 ml. of filtrate and add 3.0 ml. of color development buffer **1-b**.

PHOTOMETRIC DETERMINATION

To read the color in aqueous soln, use a filter with maximum light transmission in the region of 610 mμ wave length.

To read the color in butyl alcohol, extract the color as described above and centrifuge the sample for 5 min. to break the emulsion and to remove the moisture suspended in the alcohol layer. A Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice a section ¼ inch thick from a rubber stopper of suitable diameter to fit into the bottom of the centrifuge cup. Glue together two cork stoppers of appropriate diameter, bore through the center a hole of proper size to hold the tube snugly, and insert the double cork section into the cup. After centrifuging, remove nearly all of the butyl alcohol by means of a pipet with a rubber bulb on the top end. Filter the alcohol into the photometer cell and read with a filter with maximum light transmission in the region of 650 mμ wavelength.

If more than approximately 4 ml of butyl alcohol is required for the photometer used, conduct the test in a larger tube and extract the color, in both the test and the standards, with the necessary quantity of butyl alcohol rather than with 5 ml specified above.

SAMPLING

1. Milk and other fluid products.—Mix the product well, pour several ml into a small tube, stopper the tube, and keep it in a refrigerator.

2. Hard cheese: Take a sample from the interior with a *clean* Roquefort trier, place in a small tube, stopper the tube, and keep it in a refrigerator.

3. Soft and semi-soft ripened cheese.—Harden the cheese by chilling it in the freezing chamber of a refrigerator. Taking special precautions to avoid contaminating the sample with phosphatase that may be present on the surface, use either of the following methods for sampling:

(a) Cut a portion from the end of the loaf or from the side of the cheese, extending in at least 2 inches if possible or to a point somewhat beyond the center in the case of a small cheese. Cut a slit ¼ to ½ inch deep at least halfway around the portion and midway between the top and bottom. Break the portion into two parts, pulling it apart so that it breaks on a line with the slit, being careful not to contaminate the freshly exposed, broken surface. Remove the sample from the freshly exposed surface at or near the center of the cheese.

(b) Remove the surface of the area to be sampled—*e.g.*, the end and the adjacent sides—with a clean knife or spatula, to a depth of ¼ inch. Clean the instrument and hands with hot water and phenol-free soap and wipe them dry. Remove the freshly exposed surface to a similar or greater depth, and repeat the cleaning. Then take the sample from the center of the freshly exposed area, preferably at or near the center of the cheese in the case of a small cheese.

4. Process cheese, spreads, butter, and other non-fluid products.—Take the sample from beneath the surface with a clean knife or spatula.

5. Ice cream and sherbet.—Melt the portion removed and allow it to remain melted for an hour or longer before testing, testing it as a fluid product.

Avoid the use of samples contaminated with mold.

6. Preservation.—If a preservative is necessary, for liquid products add 1 to 3 per cent of chloroform; for solid products put 1 to 3 ml of chloroform in the container, cover with a plug of cotton, insert sample and stopper container tightly. *Label preserved samples: "Poison, preservative added."*

CONDUCTING THE TEST

The chemical principles involved in the detection and measurement of milk phosphatase activity are the same for all dairy products. Some modifications,

described below, have been found necessary for different dairy products, because of their differences in physical properties, compositions, and especially buffering capacities.

Cheese:

Step 1. Weigh, on a *clean* balance pan or watch glass, a 0.50-g sample (preferably in duplicate) and place in a culture tube 16 or 18×150 mm. Similarly, weigh another sample and place in a tube as a control or blank. If the cheese is sticky, weigh the sample on a piece of wax paper about 1×1 inch and insert the paper with the sample into the tube. Macerate the blank and the test with a glass rod about 8×180 mm.

Step 2. Add *to the blank* 1.0 ml of the appropriate (Table 1) barium buffer **1-a** (without substrate added), macerate with the rod, leave the rod in the tube, heat for about a minute to at least 85°C (185°F) in a beaker of boiling water with the beaker covered so that the entire tube is heated to approximately 85°C, cool to room temperature, and macerate again with the rod.

Step 3. Add *to the test* 1.0 ml of barium buffer substrate **2-a** or **2-b** (Table 1) and macerate.

From this point, treat the blank and the test in a similar manner.

Add 9.0 ml of the appropriate barium buffer substrate **2-a** or **2-b** (total, 10.0 ml added), and mix. The rod may be left in the tube during incubation; if it is removed at this point, cut a piece of filter paper ca 1×1 inch, wrap and hold it tightly around the rod, rotate the rod while withdrawing it from within the tube so as to wipe the rod clean, insert the paper with the adhering fat into the tube, and stopper the tube.

Step 4. Incubate in a water bath at 37–38°C for 1 hour, mixing or shaking the contents occasionally.

Step 5. Place in a beaker of boiling water for nearly a minute, heating to approximately 85°C (use a thermometer in another tube containing the same volume of liquid), and cool to room temperature.

Step 6. Pipet in 1.0 ml of the zinc precipitant **3-c** for ripened cheese, or the zinc-copper precipitant **3-b** for unripened cheese, and mix thoroughly (*p*H of mixture, 9.0–9.1).

Step 7. Filter (5-cm funnel, 9-cm Whatman No. 42 or No. 2 paper recommended), and collect 5.0 ml of filtrate in a tube, preferably graduated at 5.0 and 10.0 ml.

Step 8. Add 5.0 ml of color development buffer **1-b** (*p*H of mixture, 9.3–9.4).

Step 9. Add 4 drops[5] of BQC, mix, and allow the color to develop for 30 min at room temperature.

Step 10. Determine the intensity of blue color by either of two methods:

a. *With a photometer:* Read the color intensity of the blank and that of the test, subtract the reading of the blank from that of the test, and convert the result into phenol equivalents by reference to the standard curve described under "Phenol standards." The butyl alcohol extraction method ordinarily is unnecessary when using a photometer.

b. *With visual standards:* For quantitative results in borderline instances, *e.g.*, tests yielding 0.5 to 5 units of color, extract with butyl alcohol **5-b**. Add 5.0 ml of the alcohol and invert the tube slowly several times, centrifuge if necessary to increase the clearness of the alcohol layer, and compare the blue color with the colors of standards in the alcohol.

With samples yielding more than 5 units, compare the colors in aqueous tests with those of aqueous standards.

Step 11. *Dilution method for quantitative results:* In tests that are observed to be

[5] For merely detecting under-pasteurization in testing unripened cheese, 2 drops are sufficient, provided the visual standards likewise are prepared with 2 drops.

strongly positive during color development—*e.g.*, 20 units or more—in which 4 drops of BQC may be much less than sufficient to combine with all of the phenol, pipet an appropriate proportion of the contents into another tube, make up to 10.0 ml with color dilution buffer **1-c**, and add 2 drops more of BQC in the case of an unripened product or 4 drops in the case of a ripened product. With each test, dilute and treat the blank in the corresponding manner. Dilute each strongly positive test thus until the final color is within the range of the visual standards or photometer. Allow 30 min. for color development after the last addition of BQC, and make the reading at the end of the 30-min. period. To correct, multiply by 2 for a 5+5 dilution, by 10 for a 1+9 dilution, and by 50 for a 1+9 followed by a 2+8 dilution.

Alternatively, to reduce the amount of yellow off color, add 2 instead of 4 drops of BQC after each dilution, and allow the color to develop. Then test the completeness of color development by adding a third drop. Repeat the dilution procedure until the addition of an extra drop does not cause any further increase in the amount of blue color.

Step 12. *Calculation and evaluation of result:* When using 0.5 g of solid sample and adding a total of 11.0 ml of liquid, multiply the value of the reading by 1.1 to convert it to units of color or phenol equivalents per 0.25 g of cheese. The result, if desired, may be converted to phenol equivalents per 1 g. by multiplying by 4.4. Evaluate the result by comparing it with the criteria of pasteurization in Table 1.

Milk and other fluid products:

Step 1. Pipet a 1.0-ml sample (preferably in duplicate) into a tube and pipet 1.0 ml into another tube as a control or blank. In testing cream, the sample may be weighed (1.0 g) if desired; in testing goats' milk, pipet in a 3.0 ml sample (Table 2).

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2," and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml of barium buffer substrate **2-a** or **2-b** (Table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, substituting the appropriate precipitant (Table 2) in Step 6, and, for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in Step 9.

Step 12. *Calculation and evaluation of result:* When using 1.0 ml of fluid sample and adding 11.0 ml of liquid (total liquid 12.0 ml, 5.0 ml of filtrate used), multiply the value of the reading by 1.2 to convert it to phenol equivalents per 0.5 ml of sample. If desired, the result may be converted to phenol equivalents per 1 ml by multiplying by 2.4. Evaluate the result by comparing it with the criteria of pasteurization in Table 2.

Butter:

Step 1. Weigh, on a piece of wax paper about 1×1 inch on a balance, a 1.0-g sample (preferably in duplicate) and insert the paper with the sample into the tube. Similarly, weigh another sample and place in a tube as a control or blank.

Step 2. Heat the *blank* to the temperature indicated under "Cheese: Step 2." and cool to room temperature. From this point, treat the blank and the test in a similar manner.

Step 3. Add 10.0 ml of barium buffer substrate **2-a** or **2-b** (prepared with 18–8 barium buffer, Table 2), stopper the tube, and mix.

Steps 4 to 11, inclusive. Follow the directions given for the corresponding steps under "Cheese" above, mixing the contents frequently and thoroughly during incubation, substituting the appropriate zinc precipitant (Table 2) in Step 6, and,

for merely detecting under-pasteurization, using 2 rather than 4 drops of BQC in Step 9.

Step 12. *Calculation and evaluation of result:* When using 1.0 g of butter and adding 11.0 ml of liquid, multiply the value of the reading by 1.1 to convert the result to phenol equivalents per 0.5 g of butter. Evaluate the result by comparing it with the criterion of pasteurization in Table 2.

MODIFICATIONS FOR DIFFERENT CHEESES AND OTHER DAIRY PRODUCTS

Different dairy products, and different kinds of cheese and cheeses of different ages, have different buffering capacities, and therefore some of them require modification of concentrations of the reagents. The modifications of the barium buffer needed to produce optimal *p*H conditions during incubation (9.85–10.20), and of the precipitant to yield uniformly clear filtrates and to minimize interference during color development under optimal *p*H conditions (9.3–9.4), are specified in Tables 1 and 2.

With some samples, especially cheese samples of unknown history, slight deviations from the optimal *p*H range may occur, but such deviations do not very materially affect the results. For example, *p*H values as low as 9.6 or as high as 10.35 during incubation have been found to result in an average decrease of not more than 20 per cent below the maximum in the quantity of phenol liberated. The use of the 25–11 buffer substrate with samples for which the 27–11 buffer substrate is specified yields *p*H values not lower than 9.8.

A trace of cloudiness in the filtrate, following the use of the precipitant as prescribed, indicates that the concentration of barium hydroxide in the buffer was not sufficiently great, *i.e.*, that the buffer substrate was not sufficiently alkaline. For example, the 25–11 buffer diluted 5+5 with water, for use with fresh milk, may yield a cloudy filtrate if used with old milk having a *p*H below approximately 6.0, or with milk that has soured, and the test should be repeated with a more concentrated buffer and precipitant. Likewise, the 25–11 buffer, for use with unripened cheese, may yield a cloudy filtrate if used with ripened cheese, indicating that the concentration of the buffer used was not sufficient. Increasing the concentration of zinc sulfate in the precipitant also eliminates turbidity of the filtrate.

In testing cheese of unknown history or age, information as to the percentage of solids, especially the nonfat solids, is useful as an indication of the correct buffer to use; cheese with a relatively high percentage of nonfat solids generally requires the use of a relatively concentrated buffer to adjust the *p*H of the mixture correctly.

For precise quantitative results on unknown samples, adjust the *p*H to 10.0–10.05 for the incubation.

Cottage cheese curd is heated in the presence of considerable acid during manufacture, and therefore its phosphatase values are comparatively low. Alternatively, to increase the sensitivity of the test on cottage cheese, apply the following modifications: Use a 1.0-g sample, 27–11 buffer substrate, 2-hour incubation, 6.0–0.1 precipitant, and a pasteurization criterion of 2 units per 0.5 g.

Phosphatase activity is much less in goats' milk than in cows' milk. The details of a modification designed to increase the sensitivity of the test applied to goats' milk are specified in Table 2.

To test concentrated milk products, reconstitute the product with water to its original concentration of milk solids and test in the manner specified for the original product.

To test for the presence of microbial phosphatase, which is indicated by blue color in the blank prepared as directed above, repeat the determination, adding 1 ml of the appropriate barium buffer (without substrate) to the blank and heat it for

5 min. in boiling water in a covered beaker. If the blank treated thus is negative, it indicates that the blue color in the original blank was due to microbial phosphatase, *i.e.*, a "false positive" sample.

PRECAUTIONS

The length of time that the crystalline disodium phenyl phosphate and the BQC powder will remain stable can be increased greatly by keeping them in the freezing chamber of a refrigerator.

The glassware, stoppers, and sampling tools should be scrupulously clean, and it is desirable to soak them in hot, running water after cleaning.

The bottles containing solid barium hydroxide and the barium buffer must be kept stoppered tightly to prevent absorption of carbon dioxide.

Phenolic contamination from plastic closures on reagent bottles has been encountered, and therefore the use of plastic closures should be avoided. Rubber stoppers should not be used in flasks in which butyl alcohol is stored. Glass or cork stoppers should be used.

(2) The tentative methods for phosphatase test for pasteurization, sections **22.43–22.57**, were deleted.

(3) The following was adopted as tentative as a rapid screening method.

MOISTURE IN CHEESE

Weigh 2–3 g of prepared sample into moisture dishes with tight fitting covers. Partially dry on the steam bath with lids removed and then insert in a forced draft oven which has come to equilibrium at 130 ± 1°C. Dry for 1.25 hours (with covers entirely off), cover tightly, remove from oven, cool, and weigh.

(4) The following method was adopted as tentative.

BUTTER

Preparation of Sample—Mechanical Stirrer Method

EQUIPMENT

(a). A cylindrical jar holder mounted on the shaft of a hand centrifuge or similar apparatus for rotation of the jar on a vertical axis by means of a crank and transmission mechanism (See Fig. 1). The sample container must be cylindrical.

(b). A common steel spatula with wooden handle and fairly stiff blade at least 1¼" wide by 5" long with blade shaped to fit the jar as indicated in Fig. 2.

PREPARATION

Warm the sample in the closed sample jar to a temperature of about 25°C, avoiding liquefaction of any portion to the extent that oily and aqueous phases separate. The most easily managed samples are those that have been warmed to room temperature overnight, but it is not essential that the entire contents of the jar be thus uniformly tempered, provided all parts are at least in a plastic state. In principle the sample should be warmed only enough to permit initiation of mixing.

Place the jar in the jar-holder and wedge it in position with shims of corrugated pasteboard. Remove the cover and scrape off any butter adhering thereto with the back of the spatula blade. Immerse the blade in the jar with the edge against the side, holding it at a slight angle from the vertical and the plane of the blade at a slight angle to the radius. Operate the crank with the other hand, slowly at first, increasing speed as lumpiness decreases. In a short time the lumpiness disappears rather abruptly, and from this point on tilt the spatula occasionally on its horizontal

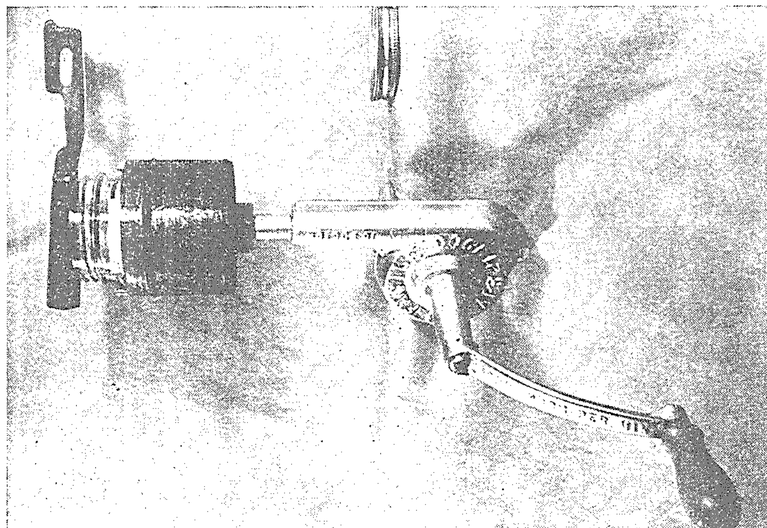

Fig. 1.—Mechanical stirrer for preparation of butter samples showing special blade for rapid mixing.

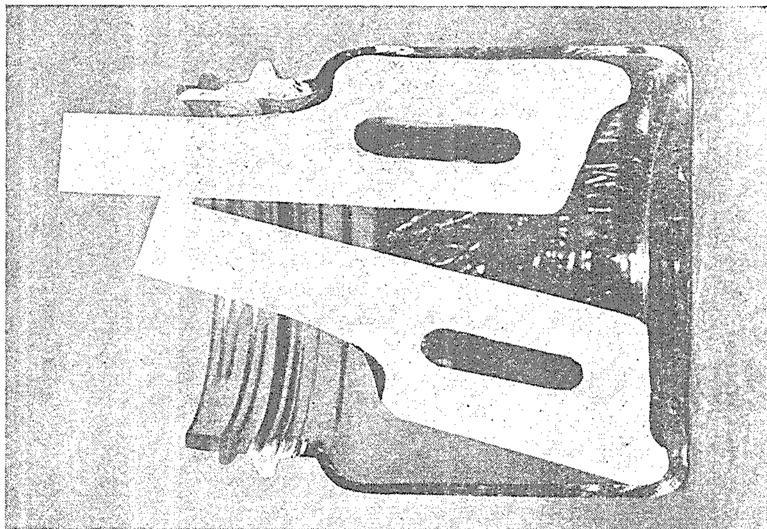

Fig. 2.—Special blade in two positions facilitating mixing action.

axis as illustrated in Fig. 2, move it back and forth to bring the toe of the blade a little past the axis of rotation, then up and down against the side of the jar, so twisted on its vertical axis as to throw the material first to the side and then to the center of the jar. After lumpiness disappears a mixing time of one minute is normally adequate. When mixing has been completed wipe off the blade against the mouth of the jar and replace the cover promptly. Start the analysis without undue delay—in no case later than one hour after mixing.

(5) The following editorial change was made in the method for fat in ice cream and frozen desserts, section **22.149** (p. 343), line 4.

After "60" add "for 20 min with occasional shaking" and add "cool and" before "Proceed."

(6) The following editorial change was made in the official method for added water in milk:

Section **22.28(a)** (p.311), line 8, after "suspected" add "Scale readings are identical on Bausch & Lomb refractometers except those bearing serial numbers 4000 to 10000, in which case readings of 38.6 and 39.6 correspond, respectively to 39 and 40 on the Zeiss instrument (Zeiss scale = B&L scale times 1.0092)."

Section **22.29(a)** (p.312), line 3 after "H_2O" add "Scale readings are identical on Bausch & Lomb immersion refractometers except those bearing serial numbers 4000 to 10000 in which case a reading of 37.9 corresponds to 38.3 on the Zeiss instrument."

Section **22.30** (p.312), line 5 after "H_2O" add "Scale readings are identical on Bausch & Lomb immersion refractometers except those bearing serial numbers 4000 to 10000, in which case a reading of 35.6 corresponds to 36 on the Zeiss instrument."

(7) The following editorial changes were adopted in section **22.33** (p. 314) of the cyroscopic method for added water in milk.

Insert "depression" after "point" line 6, p. 316.

Delete "depression" in line 9 and in 3rd line from end of method, p. 316.

(8) The sediment test, sections **22.40–22.42**, was dropped.

(9) The following method from "Standard Methods of Milk Analysis," American Public Health Association, was adopted as tentative.

SEDIMENT IN FLUID MILK

A. COLLECTION OF SAMPLE

(a) *Mixed Sample Method.*—Use 1 pt. samples. A retail pint bottle is satisfactory. Thoroly mix the milk in bulk containers before removal of the test portion. Where quart or other sizes are involved, including 10 gal. cans, transfer with a small strainer all floating extraneous matter, such as flies, hairs, large chunks of dirt, etc., if any are present, to the mounted disc. B (g). Avoid contamination of the milk with dirty stirrers or by any other means.

(b) *Off-bottom Method.*[1]—Use 1 pt. samples but do not disturb the bulk milk

[1] J. Amer. Public Health Assn., 37, 728 (1947).

except as needed to insert the tester carefully to the bottom of the container and remove therefrom the 1 pt. portion, C (b). Where quart, or other sizes, are involved, including 10 gal. cans, transfer with a small strainer all floating extraneous matter, such as flies, hairs, large chunks of dirt, etc., if any are present, to the mounted disc, B (g). Avoid contamination of the milk with dirty stirrers or by any other means.

B. APPARATUS AND MATERIALS

The tester should be of simple construction, easily cleaned and quickly adjustable between samples so as to permit the sanitary removal of the used disc and the replacement therein of the unused disc. Before use, check the tester for duplicability of results, B (e). Milk must not by-pass the filter disc.

(a) *Sediment testers for mixed sample method.*—Pressure gravity or vacuum types may be used.

(b) *Sediment testers for off-bottom method.*—Single unit type for intake of 1 pt. of milk on the up stroke of the plunger and discharge through the disc on the down stroke, or, two-unit type, one for removal of 1 pt. of milk from the bottom of the can and the other to filter the sample. Sampling device for the off-bottom method must be designed to permit insertion to the bottom of the milk can. Milk enters the tester through an opening measuring 1 in. in diameter.

(c) *Cotton sediment discs.*[2]—Provide special lintine cotton discs, diameter 1¼ in., for use over a flat wire screen in the tester so as to expose a filtration area at the center measuring 1⅛ in. in diameter. Testers and discs usually can be purchased from dealers in dairy supplies.

(d) *Preparation of standard sediment discs.*—Make a uniform mixture of oven dried (100°.) materials which meet the following screening specifications:

	Per cent
Cow manure, 40 mesh	53
Cow manure, 20 mesh, retained on 40 mesh	2
Garden soil, 40 mesh	27
Charcoal, 40 mesh	14
Charcoal, 20 mesh, retained on 40 mesh	4

Moisten 2 gm of the above mixture with 4 ml of 1 % aerosol soln or other suitable wetting agent, and 46 ml of 0.75% gum soln such as gum arabic, and then make up to 100 ml with a 50% by weight sucrose soln. Mix thoroly, transfer a 10 ml portion (200 mg of standard sediment) with a large tip, graduated pipette to a flask and make up to a liter with 50% by weight sucrose soln. When thoroly mixed, each ml contains 0.2 mg of sediment. Add definite volumes of the sediment mixture to ¾ pt. of filtered milk. Mix thoroly and pass the mixture thru a standard sediment disc with a filtering area measuring 1⅛ in. in diameter. Wash the container promptly with ¼ pt. of filtered milk. For a permanent record, mount and spray the disc with 40% formaldehyde or with a soln containing 2.5 g menthol and 2.5 g thymol made up to 100 ml with alcohol. Following the above method, prepare a series of discs containing the sediment remaining from 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, and 14.0 mg of the standard mixture. Identify each disc on the permanent record with the amount of sediment in milligrams used to prepare the pad.

(For the purpose of comparison, the entire series of discs may be used. It will usually be found more convenient to select a few discs denoting the variations in grade which are applicable to the particular investigation being made. Where grading charts are prepared and where reports are made, the chart and report must indi-

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[2] J. Milk Tech., 7, 260 (1944).

cate whether the mixed sample or off-bottom method has been used. If standards are to be handled or to be used for any appreciable length of time, place them under glass, transparent plastic sheets, or other suitable materials. In using standards, the sediment discs of the sample tested should be graded to the nearest standard sediment disc whether the actual amount of sediment is above or below the standard. Gross pieces of material, such as whole flies, large chunks of manure, etc., should be disregarded in grading the pads, but should be plainly listed in connection with each pad on which they occur.)

(e) *Checking sediment testers.*—Because of mechanical variations in sediment testing devices, check each tester frequently for duplicability of results as directed below.

To check the sediment testing devices, proceed as follows: Measure the actual amount of milk delivered to make sure that 1 pt. is withdrawn and passes thru the disc. Thoroly mix 2 gm of the oven-dried mixture described in B (d) with 4 ml of 1% wetting agent soln and 46 ml of 0.75% gum soln, such as gum arabic, and then make up to 100 ml with a 50% sucrose soln. Mix thoroly and transfer 10 ml with a large tip, graduated pipette to 10 gal of clean filtered water in a clean milk can. After thoroly agitating the mixture, remove a 1 pt. portion with a clean pint measuring device and filter the pint mixture, water, and sediment, thru a $1\frac{1}{8}$ in. diameter area of a standard lintine disc mounted in a Büchner, or other suitable funnel, of proper size. After thoro agitation again, remove a pt. sample with the sediment testing device from the same can and pass it thru the lintine disc in exactly the same manner as used in milk testing. This procedure is to be followed for testers used for mixed sample and off-bottom methods. Repeat this operation several times with the tester to determine whether or not all discs obtained in this manner are comparable with the disc obtained by filtering thru a Büchner funnel as described above.

(f) *Photograph of sediment disc standards.*—Photographic standards may be used as a guide in grading sediment pads. Preferably, use actual discs as directed in B (d). A photograph showing the complete range, as there described, is obtainable at $1.50 each from the American Public Health Association, 1790 Broadway, New York 19, N. Y. Do not use photographs which have become faded, stained, soiled, or otherwise damaged.

(g) *Use of discs.*—Discs may be mounted on special size paper or placed individually in transparent waxed envelopes. Place the disc on the paper or in the envelope while still moist with milk. As the milk dries, it serves as an adhesive. To prevent decomposition, observe spraying treatments in B (d). Spray the discs with a 40% formaldehyde soln or with a soln containing 2.5 gm menthol and 2.5 gm thymol, made up to 100 ml with 95% alcohol. Do not use glue to affix the disc to the paper. If the discs become detached, moisten with a few drops of water and remount. Protect discs from contamination.

C. DIRECTIONS

(a) *Mixed Samples.*[1]—Pass a 1 pt. sample, taken from the well mixed milk, thru a properly adjusted, firm cotton lintine disc held in correct position in the sediment tester. Milk varies considerably in its rate of flow through the lintine discs. Pasteurized milk may be more difficult to filter than raw milk. Other factors influencing the rate of flow are the temperature, fat content, the degree of clumping of the fat globules, stage of lactation, the presence of mastitic milk, and the amount of sediment in the sample.

Remove disc from tester, mount and identify. Grade by comparison with standard discs as provided in B (d). Always indicate on report whether pad is graded

when wet or when dry. The character of the sediment may be determined by microscopic examinations.

(b) *Off-bottom Samples.*—For either type of off-bottom tester, the pt. sample is taken not more than ¼ in. off the bottom of the unstirred can of milk while the sampling device is moved diametrically across the bottom of the can. Take the sample on the up stroke of the plunger, synchronizing the withdrawal of a full pint with the movement of the head of the tester once completely across the bottom of the can. To do this, push the head of the tester away as the plunger is pulled up in the tube to suck in the pint sample. Proceed as directed in C (a) after "Remove disc from tester. . . ."

23. EGGS AND EGG PRODUCTS

No additions, deletions, or other changes.

24. FISH AND OTHER MARINE PRODUCTS

The following method was adopted as tentative.

INDOLE IN SHRIMP, OYSTERS, AND CRABMEAT

APPARATUS AND REAGENTS

Color Reagent.—Dissolve 0.4 g p-dimethylaminobenzaldehyde in 5 ml acetic acid and mix with 0.92 ml phosphoric acid and 3 ml HCl. The purity of the p-dimethylaminobenzaldehyde exerts a strong influence on the intensity of the reagent blank. The yellow commercial reagent must be purified as follows:

Dissolve 100 g p-dimethylaminobenzaldehyde in 600 ml dil. HCl (1+6). Add 300 ml water and precipitate the aldehyde by slowly adding 10% NaOH soln with vigorous stirring. As soon as the precipitated aldehyde appears white, stop the addition of the NaOH, filter and discard the precipitate. Continue the neutralization until practically all of the aldehyde has been precipitated. The neutralization should not be carried to completion since the last 4–5 g of aldehyde are inclined to be colored. Filter and wash the precipitate with water until the washings are no longer acid. Dry the aldehyde, which should be practically white, in a desiccator.

Acetic Acid.—C. P. If this reagent gives a pink color with the color reagent, purify by distilling 500 ml in all glass still with 25 g $KMnO_4$ and 20 ml H_2SO_4.

Chloroform.—U.S.P.

Sodium Sulfate.—Saturated aqueous solution.

Hydrochloric Acid.—Dilute 5 ml conc. HCl to 100 ml with water.

Standard Indole Soln.—Weigh accurately 20 mg indole into a 200 ml vol. flask and dilute to mark with alcohol. Keep stock solution under refrigeration and do not use after two weeks.

Distillation Apparatus.—Use a separate steam generator for each unit. Steam generator may be made from 1 liter Erlenmeyer flask and connected to all-glass steam distillation apparatus with minimum use of rubber tubing. Distillation flask (capacity not less than 500 ml) is connected to straight bore condenser through a spray trap. A 500 ml Erlenmeyer flask is an effective receiver. Foil covered rubber stoppers may be used in absence of all-glass apparatus. (Unprotected natural or synthetic rubber connections and stoppers cause variable distillation blanks.)

Note: Guard against traces of chlorine in the distilled water since they may partly or entirely inhibit the development of the indole color.

PREPARATION OF SAMPLE

Crabmeat:

Weigh 25 g or 50 g of drained crabmeat (depending upon the amount of indole expected) into a Waring blendor, add 80 ml water and mix for several min. until a

smooth homogeneous mixture is obtained. Transfer quantitatively to the distillation flask, using a minimum of wash water to rinse the mixing chamber.

Oysters:

Weigh 50 g of drained oyster meats into a Waring blendor, add 80 ml water and mix until a smooth homogeneous mixture is obtained. Transfer quantitatively to the distillation flask, using a minimum of wash water to rinse the mixing chamber.

Shrimp:

Weigh 25 g or 50 g of peeled raw or cooked shrimp (depending upon the amount of indole expected) into a Waring blendor, add 80 ml of alcohol and mix for several min. until the shrimp are finely divided, and transfer quantitatively the mixture to the distillation flask, using a minimum quantity of alcohol to rinse the mixing chamber.

DETERMINATION

Connect flask to steam distillation apparatus and gently apply steam. Care should be exercised until the distillation is well started in order to avoid excessive foaming which is encountered occasionally. Apply sufficient heat to the distillation flask to maintain 80–90 ml volume. Collect 350 ml distillate in ca 45 min. If alcohol was used in the preparation of the sample, collect 450 ml of distillate. Wash the condenser with a small amount of alcohol and allow to drain into receiving flask containing the distillate.

Transfer the distillate to a 500 ml separatory funnel, add 5 ml dilute HCl and 5 ml sodium sulfate soln. Extract successively with 25, 20, and 15 ml portions of chloroform. Shake each portion vigorously for at least one min. Combine and wash the 25 and 20 ml chloroform extracts in a 500 ml separatory funnel with 400 ml water, 5 ml sodium sulfate soln and 5 ml dil. HCl. Save wash water. Filter the combined extracts through a plug of cotton into a dry 125 ml separatory funnel. Wash the 15 ml portion, using the same wash water and combine with the other portions in the 125 ml separatory funnel.

Add 10 ml of the color reagent to the combined extracts, shake vigorously for exactly two min. and allow acid layer to separate as completely as possible. Drain until 9.0 ml have been secured.

Fill to 50 ml mark with acetic acid, mix well, transfer the soln to a suitable photometer cell and measure color photometrically at 560 mμ. The color soln may be diluted with acetic acid, containing 9.0 ml color reagent per 50 ml of soln, provided blanks are determined at the same dilutions.

Prepare a standard curve by the above procedure by steam distilling a series of freshly prepared diluted solutions of standard indole soln. Determine the distillation blank in a like manner, omitting addition of indole.

25. FLAVORING EXTRACTS

(1) The official method for alcohol, section **25.2** (p.365), was changed by deleting from "or" to "25.9" and substituting "but measure the sample used at 15.56° in a pycnometer (Fig. 1 or Fig. 2, p. 192–3) calibrated at that temperature."

(2) The tentative method for alcohol in ginger extract, section **25.6** (p. 376), was changed to read "Proceed as directed in **16.6(b)**."

26. FRUIT AND FRUIT PRODUCTS

No additions, deletions, or other changes.

27. GRAINS AND STOCK FEEDS

(1) The following editorial change was adopted in section **27.59** (p. 419): Insert "Cool, make to measured volume of 50 or 100 ml and mix" before the last sentence beginning "Compare" line 11.

(2) The following method was adopted as tentative:

CALCIUM AND PHOSPHORUS

PREPARATION OF SAMPLE

(a) Proceed as directed under **12.5** to end of paragraph.

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

DETERMINATION

Calcium.—With soln prepared as above, proceed as directed in section **27.47** beginning "Pipet 25 ml . . . ," line 4.

Phosphorus.—With soln prepared as above, proceed as directed in section **2.12(a)**.

(3) The following method was adopted as tentative:

FAT IN FISH MEAL

Weigh 4–5 g of the meal to the nearest 0.01 g into an alundum or paper extraction thimble, cover with light layer of cotton, and extract in a continuous extractor for 16 hours with acetone.

At the end of this extraction period, distill off acetone until volume in flask is 10–15 ml. Transfer this oil soln to a 100 ml tared beaker washing flask free of all oil with fresh acetone and evaporate with a current of warm air. A convenient method is to place flask on a grill (*e.g.*, over a steam radiator) in front of a small electric fan.

When no moisture or acetone can be observed, place beaker in a vacuum oven at 80°C and apply a vacuum of 24 to 25 inches for 1 hour. Transfer to desiccator, cool, and weigh.

Transfer the extracted meal residue from the thimble to a 150 ml beaker. Remove any remaining solvent by heating on warm grid and then add 60 ml of 4 *N* HCl. Digest for one hour at or near the boiling point on a hot plate, occasionally stirring with a glass rod, adding water as necessary to maintain volume in the beaker. (Thoro removal of all acetone is necessary preliminary to this digestion, for otherwise vaporization of solvent will carry meal particles over side of vessel on to hot plate.) Filter through a 12.5 cm fluted filter. Wash residue on filter until free of acid, using methyl red indicator on portions of filtrate to follow progress of washing. Place filter and meal in a 150 ml beaker and dry in air oven at 80–90°C for one hour. Transfer filter paper and contents to a thimble and extract 16 hours with acetone. Remove solvent and weigh extract as above. The sum of the weights of extracts represents total fat.

(4) The following editorial changes were made in the official method, sections **27.28–27.30**, for crude fiber, p. 408:

27.28

(a) Delete "Contains" substitute ".255 *N*."

(b) Delete "Contains" substitute ".312 *N*."

(c) Add to paragraph:

"Gooch grade, medium fiber, acid washed and ignited is usually satisfactory but should be tested for chemical stability and filtering speed before use."

27.29

(c) Delete "appreciable," line 1. Add "Retention can be tested by running filtrate thru Gooch" after "rapid," line 2.

27.30

Lines 8 and 9—delete "ca every 5 min. in order to mix charge thoroly" and substitute "frequently until sample is thoroly wetted."

(5) The official method for crude protein, section **27.10** (p. 405), was changed to read "Determine N as directed under **2.24**, **2.25**, or **2.26**. With products containing 30% of protein or over, proceed as directed under **2.26**. Multiply results by 6.25."

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

No additions, deletions, or other changes.

31. OILS, FATS, AND WAXES

(1) The S.P.A. method for unsaponifiable residue, section **31.40** (p. 504) was made official, final action.

(2) The F.A.C. method for unsaponifiable residue, sections **31.37–31.39** (p. 504) was deleted, final action.

(3) The method for squalene, sections **31.41–31.43** (p. 505), was adopted as official, final action.

32. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) The following method was adopted as tentative:

VOLATILE FATTY ACIDS IN BAKERY PRODUCTS

PREPARATION OF SOLUTION

(a) *Air-Dried Bread.*—For analysis of the air-dried product prepare sample as directed in section **20.83**.

(b) *Fresh Bread or Cake.*—For analysis of the fresh product or for analysis of cake, which is often difficult to air-dry without spoilage, pass the sample through a meat grinder, equipped with a ⅛″ hole plate, and reduce to a finely divided condition by rubbing through an 8 mesh sieve. Proceed with the analysis promptly.

DETERMINATION

Transfer 25 g of air-dried or 35 g of fresh sample to a 250 ml volumetric flask. Add 100 ml of H_2O and mix by swirling until all particles are wet and any lumps are completely broken up. Add 25 ml of ca normal H_2SO_4, shake for 2 min and let stand for ½ hour, shaking occasionally to stir up the particles. Do not allow

bread to clog in the neck of flask. This can be prevented by avoiding too vigorous shaking. Wash down with a small amount of water if necessary. Add 15 ml of 20% W/V phosphotungstic acid, shake for 2 min. and make to volume. Transfer mixture to centrifuge bottle and centrifuge for 10 min., at 1000–1500 r.p.m. Disregard turbidity. Decant (should be about 180 ml) and pipet 150 ml to a 500 ml flask equipped with water condenser for refluxing. Add ca 1.0 gm Ag_2SO_4 and heat for 5 min. after boiling begins. Cool flask under running water to room temperature—reflux connected—wash down condenser, transfer contents of flask to a 200 ml vol flask and make to volume. Mix in a 400 ml beaker with 3–5 gm of filter cell, stir and filter (S.S. #589 15 cm preferred). Pour back to give best possible clearness. Difficulty in obtaining a clear filtrate indicates insufficient Ag_2SO_4. Test for excess Ag by allowing a few drops of filtrate to flow into a test tube containing about 5 ml of 5% NaCl in 1+3 HNO_3. If excess of Ag is not indicated, add more Ag_2SO_4 (0.2 gm), shake and filter on a new paper.

Transfer 150 ml of the chloride-free filtrate to the standard distillation flask and proceed as directed under section **24.11**.

Collect only the two initial portions of distillates (50 ml and 200 ml) unless the ratio of their titers is less than the standard C ratio for propionic acid by more than 0.1. In this case collect two additional 200 ml portions of distillate for calculation of acids higher than propionic.

Determine formic acid in composite of distillates and correct the titrations for the titer contributed by this acid. Using the prescribed aliquots, the results in terms of mg of acid per 100 grams of sample is calculated from the determined ml of 0.01 *N* acid in the distillation by means of the following factors: Formic acid—4.09; acetic acid—5.33; propionic acid—6.58.

(2) The following method was adopted as tentative:

THIOUREA IN ORANGE JUICE

REAGENTS

(a) *Grote's reagent.*—Dissolve 0.5 g of sodium nitroprusside in 10 ml of H_2O in a 50 ml Erlenmeyer flask. Weigh out 0.50 g of hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) and 1 g of $NaHCO_3$. Add the $NH_2OH \cdot HCl$ to the nitroprusside soln, give it a single whirl, then add immediately the 1 g of $NaHCO_3$ with no agitation. Allow the reaction to proceed without any molestation 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 solution composed of 5 ml reagent **(d)**, 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 temperature for 5–10 hours to age the solution. 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 4 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 200 mg of the pure chemical in water and dilute to 200 ml.

(d) *Dilute thiourea soln.*—Dilute 5 ml of reagent **(c)** to 100 ml with water.

APPARATUS

Siphon.—**Insert two bent glass tubes in a two-hole cork or stopper, one termina-**

ting 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 diameter as the tube part way through 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 for 10 min. at about 1800 r.p.m. and remove. Cap the end of the siphon which is inserted in the bottle and lower it thru the top layer into the lower aqueous layer. Then push off the cork cap with a glass rod. Lower the tube to the bottom of the bottle with cork in mouth and blow in the short tube to start the flow of liquid. Carefully siphon off as much of the lower layer as possible, and control the rate of flow by pressing on the rubber connection. Stop the flow when material from center emulsion layer begins to enter the tube. Add a teaspoonful of filtercel to the siphoned liquid, stir well, and filter on a Büchner funnel with suction, using a Whatman No. 54 or 41 H filter. Warm flask and contents to about 40°C. (on steam bath) and again apply suction to remove ether.

Measure 50 ml of the filtrate into a 150 ml beaker and boil down to about 20 ml. Add 0.5 g MgO and stir until the liquid has a *p*H of about 8.0, as shown by universal indicator paper. Immediately transfer the material quantitatively to a 25 ml volumetric flask, make to the mark, and mix. Filter off the excess MgO immediately, preferably by suction, and pipet 20 ml of the filtrate into a Majonnier flask containing 4 g of salt. Stopper and shake to dissolve salt.

Extract the sample in the tube alternately, first with a 50 ml portion of isoamyl alcohol and then with 50 ml of isoamyl alcohol saturated with water. Shake thoroly each time for 1 min. or longer. Centrifuge in appropriate cups at about 700 or 800 r.p.m. If necessary, add a few glass beads to the tube to bring the interface just to the bottom of the neck after the first extraction. Decant the isoamyl alcohol layer each time into a 500 ml separatory funnel. Make a total of seven extractions in this manner, four with the straight isoamyl alcohol, and three with this solvent saturated with water. (Prepare water-saturated solvent by shaking well about 300 ml with an excess of water, drawing off excess water and filtering the alcohol layer.) Extract the combined alcohol extracts in the funnel four times by shaking well with 120 ml portions of water. Draw off the first aqueous extract into a 400 ml beaker and receive the three succeeding extractions in a separate 600 ml beaker. Add to the contents of the 600 ml beaker 0.1 ml of sirupy (85%) phosphoric acid. Evaporate the contents of the beakers separately by boiling until about 30 ml remains in each. Transfer and combine the contents of the beakers in a glass stoppered 100 ml graduated cylinder. Rinse the beakers in succession two or three times with about 4 ml portions of water. Add 0.4 g of Na citrate, shake to dissolve, and make to a total volume of 80 ml, stopper, and mix.

DETERMINATION

Pipet 10 ml of the final sample soln into a test tube about $\frac{7}{8}$ inch in diameter. Prepare standards containing 0, 1, 2, and 4 ml of dilute thiourea soln (reagent **d**) and make to a total volume of 10 ml with 0.6% Na citrate soln. Add 1 or 2 drops of acetic acid (1 + 5) to each tube and 1 ml of diluted Grote's reagent to each tube with stirring or shaking. Allow the tube to stand 45 min. at 20–25°C. or 15 min. at 45°C. and read the colors (absorption) in a photometer at 610 millimicrons. Construct a graph from the standards, plotting photometer reading or extinction against parts

per million of thiourea. (Each ml of standard represents 10 p.p.m. The reading from the sample soln gives directly from the graph the parts per million of thiourea extracted.) Multiply this figure by 1.1 to obtain true thiourea in sample, since only 91% of that originally present is extracted by the procedure.

THIOUREA IN FROZEN PEACHES

REAGENTS

See (a), (b), (c), and (d) of above method for orange juice.

(e) *Sodium bisulfite.*—Prepare a soln containing 2.5 g of $NaHSO_3$ per liter.

PREPARATION OF SAMPLE

Weigh 200–400 g of the frozen sample on a rough balance (0.1–0.2 g sensitivity) into a tared 800 ml beaker. (Cut the contents of a 1 lb. package into quarter or eighth portions and select alternate portions for the determination and keep the remainder as a reserve sample. Several packages can be composited in this manner if desired.)

Immediately weigh into the beaker containing the sample an amount of $NaHSO_3$ soln equal to half the weight of the sample. (See Note 1). Stir the contents of the beaker and pour into a blendor (Waring or other suitable type), drain well, and comminute in the blendor for 20–30 seconds. Return the diluted comminuted sample to beaker.

Weigh 150 g of this blended material to accuracy of 0.2 g and transfer to a 250 ml volumetric flask and dilute to about 200 ml with water. Remove most of the air by adding about 4 drops of hexyl alcohol and inserting a two-hole stopper (No. 0) carrying a small bent glass tube and another straight tube extending about 3 inches into the flask, the end of which is drawn to small bore (near capillary size). Apply gentle suction to the bent tube and shake flask with rotation. The bore of the small tube should be large enough so that reduction of pressure is not too great. If the froth rises in the neck, release vacuum for a moment. Then continue with suction and rotation until most of air is removed, add 20 ml more of sulfite soln and then dilute to the 250 ml mark with water. Mix well and pour about 165 ml into a 250 ml centrifuge bottle. Add 50 ml of ethyl ether to the contents of the bottle, rotate a few times, then stopper and shake, open once to release pressure, than shake vigorously for 1–1.5 min. (If preferred, divide the liquid in vol. flask between two centrifuge bottles and extract each with about 30 ml of ether, etc.)

Place bottle in centrifuge and whirl at about 1800 r.p.m. for about 10 min. Carefully pour off a little of the top ether layer into a beaker, then inclining the bottle, push the sludge cake toward the bottom of the bottle with a glass rod and pour the liquid contents on a cotton filter in a Bunsen funnel. To prepare filter place a small pledget of cotton in the apex of a 85–100 mm funnel, then insert a piece of absorbent cotton of half thickness (split sheet), about 3½ inches in diam.

Pipet 100 ml of the lower aqueous filtrate into a 200 ml volumetric flask. (Squeeze the cotton on the side of the funnel with a rod if necessary to obtain sufficient filtrate.) Add gradually to the contents of the flask (from a separatory funnel), with constant shaking, sufficient acetone to bring the contents to the 200 ml mark. As the surface of the liquid enters the neck of flask, stopper and mix by inverting a few times before making to mark. Mix contents, cool to room temperature (in bath if desired), make to mark again, and mix well. (Note: Acetone causes some rise in temperature.) Let precipitate separate and pour contents of flask into a 250 ml centrifuge bottle. Add a spoonful of filtercel, stopper, and shake well, then centrifuge about 8 min. at about 1800 r.p.m. Decant off the supernatant liquid and filter if unclear. Measure 125 ml of the clear liquid into a 250 ml beaker, add several glass beads and boil off the acetone on the steam bath. Then boil down to about 35 ml

on the hot plate, remove and cool to room temperature. Add dropwise a 15% soln of NaOH until basic and about 2 drops in excess. Add acetic acid (1+5) with stirring until just acid and add 2 drops in excess.

Transfer the liquid quantitatively to a 50 ml volumetric flask and make to mark with water and mix. Pour the contents of the flask into a small beaker or flask (100–125 ml), add a spoonful of filtercel, and mix well by stirring or stoppering and shaking. Filter the liquid on a 12.5 cm folded filter (E. & D. No. 195 is suitable). Pour through filter again if not clear. The filtrate or final sample soln is designated F. S.

DETERMINATION

Pipet 10 ml of soln F. S. into a 6-inch test tube. For expected amounts of thiourea below 20 p.p.m., prepare standards containing 0, 1, and 2 ml portions of soln **(d)** (5 mg thiourea per 100 ml). For amounts from 20 to 50 p.p.m., prepare standards containing 0, 2, and 4 ml of standard soln **(d)**. Add 0.6% Na citrate to the tubes to make the standards to 10 ml volume, then add 1 drop acetic acid (1+5) to each tube (samples and standards). Place a stirring rod in each tube and stir up and down to mix, leaving rod in tube. Place the tubes in a bath or a room maintained at a temperature of 20–25°C.

Add with stirring 1 ml of recently diluted Grote's reagent **(b)** to each tube. Allow the tubes to stand at the above temperature for 60 min. and read the colors (blue) of each tube in a photometer, using a 1-inch cell and a filter centering at about 610 millimicrons. Designate the reading of the sample as X. Construct a lineal curve from the standard readings, plotting parts per million of thiourea (1 ml stand. = 10 p.p.m.) against photometer readings. Extrapolate the curve made with 2 and 4 ml portions of standard soln for amounts up to 50 p.p.m.

A correction of the reading (X) obtained above is necessary since the soln naturally contains some color before the reagent is added. To do this make readings of the soln F. S. with no added reagent in the same cell and also of distilled water. Obtain the difference of (F. S.) reading − distilled water reading = **(d)** and subtract **(d)** from the reading X above. X − d = R = corrected reading. From the reading (R) obtain the thiourea in p.p.m. in the sample soln (F. S.) by use of the curve. Multiply the thiourea thus found by the factor 1.065 to correct for the volume increase due to ether and obtain the true thiourea content of the original sample. (See Note 2). Repeat the determination (color development) on a smaller aliquot (1–5 ml) for quantities greater than 50 p.p.m.

NOTE 1

Two hundred g is sufficient sample to be representative and should be used where a portion is to be reserved. The unused portion of the sample should be maintained in a frozen condition. It is necessary to add the sodium bisulfite soln immediately to the frozen sample before blending to prevent losses of thiourea due to attack by the enzymatic systems present. Blending in the Waring blendor whips air throughout the material and if the enzymes are not inactivated, large losses of thiourea are likely to occur. The enzymes can also be inactivated by plunging the frozen sample into boiling water and boiling 3 or 4 min. The action of the enzymes is slow in the frozen condition where the material is in unbroken cakes or chunks.

NOTE 2

The correction factor 1.065 is to compensate for the solubility of ether in the aqueous soln. Measurement of 100 ml of soln is made after extraction with ethyl ether. The latter has considerable solubility in aqueous solutions. In previous methods the presence of NaCl in the soln reduced the solubility of the ethyl acetate to a large extent and correction was not necessary for this reason.

(3) The following tests were adopted as tentative.

QUALITATIVE TESTS FOR PRESENCE OF THIOUREA IN ORANGE JUICE

(A) Using the Pentacyanoammonioferrate

Reagent.—Preparation according to Fearon.

Dissolve 10 g of sodium nitrosoferricyanide (nitroprusside) in 40 ml of conc. NH_4OH soln (sp. gr. 0.88) and keep at about 0°C. until all the nitrosoferricyanide has decomposed. This is shown when a few drops of the mixture no longer give a red color when added to a solution of creatinine in *N* NaOH. Decomposition is complete by the end of 24 hours. Remove the precipitate by filtration and precipitate the residual pentacyanoammonioferrate in the soln by addition of absolute ethanol until no further precipitate appears. Collect the precipitate, wash with absolute ethanol until free of ammonia, and dry in vacuo over sulfuric acid. Keep the solid reagent in a desiccator over $CaCl_2$ in the dark.

Prepare a 1% soln of the solid in distilled water, expose it to light and air for a day, and then store in a brown glass bottle in the dark. The reagent is now ready for use, gains in potency for several weeks, and can be kept for about six months.

PREPARATION OF SAMPLE

Extract a volume of juice with about two thirds its volume of ethyl ether, centrifuge, and separate lower layer. Stir in some filtercel and filter with suction. Keep vacuum on for a short time and agitate to remove most of ether.

To about 5 ml of the extracted sample add 5 drops of the reagent above. Note color. If a blue color does not develop, add about 0.1 *N* iodine soln a drop at a time, shaking after each drop. Usually, about 5 drops are necessary to develop maximum color (blue green). Excess iodine tends to reduce the color.

(B) Qualitative Test with Grote's Reagent

Reagent.—Use that given above in quantitative method for orange juice **(a)**, p. 100.

TEST

Use extracted sample prepared in (*A*) above.

To about 5–10 ml of prepared sample add .02 *N* iodine soln dropwise until a drop remains and does not disappear for some time. Add a ml or so of diluted Grote's reagent. A blue green or blue color develops rather gradually in the presence of thiourea.

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

DETERMINATION OF MONOCHLORACETIC ACID

(Applicable to carbonated beverages and fruit juices containing 5–150 mg CH_2Cl COOH in 150 ml).

APPARATUS

Continuous extractor **(39.112, Fig. 15)**.—Make the outer part 45 cm long from 43 mm tubing with side tube issuing 25 cm above the bottom, and fitted with standard taper, drip tip, joint 24/40. Make the inner tube 40 cm long from 12 mm tubing. Use a 250 ml conical flask with suitable joint to hold the overflowing ether extract. Any equally efficient means of extraction may be used.

REAGENTS

Silver nitrate soln.—(1 ml = ±5 mg CH_2Cl COOH.) Dissolve 9 grams of $AgNO_3$ in water and dilute to 1 liter.

Ammonium sulfocyanate soln.—(1 ml = ±5 mg CH_2Cl COOH.) Dissolve 4.03 g

of NH_4CNS in water and dilute to 1 liter. Standardize against pure NaCl soln, 3.093 g per liter, which contains 1.8762 g of Cl (equivalent to 5 g of monochloroacetic acid, which contains 1.8764 g of Cl).

Ferric indicator.—A saturated soln of ferric ammonium alum.

DETERMINATION

In the outer part of the continuous extractor, place such a quantity (not over 150 ml) of carbonated beverage, apple juice, grapefruit or orange juice as contains 5–100 mg of CH_2Cl COOH. Dilute if necessary to 150 ml, add 3–5 ml of H_2SO_4, mix, and extract with ether 2–3 hours. Tilt the extractor in such a way as to drain as much as possible of ether into the flask; disconnect the flask, add 25 ml 1 *N* NaOH in excess of that required to make the water layer alkaline to litmus paper after shaking, shake, and evaporate the ether on the steam bath until only about 25 ml of liquid remains, hastening the process by passing a current of air into the mouth of the flask. Digest on the steam bath for 2 hours or boil under a reflux condenser for ½ hour. Add 50 ml of water, 15 ml of HNO_3, and a known volume of the $AgNO_3$ soln in excess. Shake ½–1 min, add the ferric indicator, and titrate the excess Ag with the NH_4CNS soln. In the titration, add NH_4CNS soln carefully until the pink color formed fades slowly on mixing. At this point shake the soln for ca 30 seconds and filter thru a folded filter into a second flask. When the first flask is empty wash down its walls with ca 50 ml of H_2O and add this to the filter after the soln has all gone thru. When the wash water has passed thru, complete the titration. In the same way, titrate a quantity of $AgNO_3$ soln equal to that added to the sample. The difference between the two titrations is a measure of the CH_2Cl COOH.

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

QUATERNARY AMMONIUM COMPOUNDS

I. Ferricyanide Method.

(For commercial preservatives)

REAGENTS

Buffer.—Dissolve 130 g of sodium acetate in water, add 42 ml of acetic acid and make up to 500 ml.

Ferricyanide solution.—Dissolve 6.6 g of $K_3Fe(CN)_6$ in water and dilute to 1 liter.

Zinc sulfate solution.—Disolve 20 g of $ZnSO_4 \cdot 7H_2O$ in 180 ml of water.

Thiosulfate solution.—Dissolve 5 g of $Na_2S_2O_3 \cdot 5H_2O$ in water, dilute to 1 liter and standardize (1 ml of 0.02 N = 0.02142 g alkyldimethylbenzylammonium chloride, molecular weight 357.

Approximation of Quaternary Ammonium Salt Content

Pipet 1 ml of buffer, 2 ml of ferricyanide solution, and 20 ml of water into each of 4 small conical flasks. Pipet into them, respectively, 0.5 ml, 1.0 ml, 2.0 ml, and 4.0 ml of sample, mix and filter. Now add 2 ml of sample to each filtrate, mix, and observe.

DETERMINATION

Into a 100 ml Kohlrausch flask[1] pipet such an aliquot of sample as contains about 0.5 g of quaternary ammonium salt as indicated in Table 1, dilute if necessary to make the volume 50 ml, add 5 ml of buffer and mix. Then add from a pipet 30 ml of ferricyanide solution, rotating the flask during the addition. Dilute to the mark

[1] In case of a solution containing approximately 0.5 g of the compound per 100 ml, pipet 100 ml into a 200 ml volumetric flask, use 10 ml of buffer, and make the back titration upon 100 or 150 ml of filtrate.

TABLE 1.—*Approximation of content of alkyldimethylbenzylammonium chloride (mol. wt. 357)*

	SAMPLE ADDED			
	A 0.5 ML	B 1.0 ML	C 2.0 ML	D 4.0 ML
Per cent				
8.4 or more	No ppt.	No ppt.	No ppt.	No ppt.
5	Ppt.	No ppt.	No ppt.	No ppt.
2.5	Ppt.	Ppt.	No ppt.	No ppt.
1.25	Ppt.	Ppt.	Ppt.	No ppt.
1 or less	Ppt.	Ppt.	Ppt.	Ppt.

with water and mix. After ½ hour, filter, discarding the first 10–15 ml of filtrate. Pipet 50 ml of filtrate into a 500 ml conical flask, add 100 ml of water and 1–2 g of solid KI. Rotate until the salt is dissolved; add 10 ml of HCl (1+1), mix, and let stand for 2 min. Add 10 ml of zinc sulfate soln, mix, and titrate with thiosulfate soln, adding starch indicator when the iodine color fades to a tinge of yellow. Run a blank determination including all of the above operations but substituting water for the sample. Calculate the quaternary ammonium salt content from the difference in the two titrations.

II. Method for Bottled Beverages Containing Fruit Juices

APPARATUS

Centrifuge having a head about 12 inches in diameter equipped for accommodating 100 ml portions of sample.

Steam distillation apparatus (see **25.77**): Use 500 or 1000 ml distillation flask fitted with spray tube reaching to within 1 or 2 cm of the bottom of flask (all connections standard taper joints), and with plug for steam inlet (to be used during the early part of the distillation).

Photometer: fitted with color filter 610 mμ and suitable cell.

REAGENTS

Sodium carbonate solution.—Dissolve 5 g Na_2CO_3 in 500 ml of H_2O.

Bromophenol blue.—Use the solid reagent.

Bromophenol blue solution.—Dissolve 40 mg of the dry compound in warm H_2O, cool, and dilute to 100 ml.

Sodium sulfate.[2]—Anhydrous granular (not powdered) reagent grade.

Solvents.—Ethylene chloride; petroleum ether.

DETERMINATION

(a) *Extraction.*—Mix thoroly, and measure out 50 ml of sample in a graduated cylinder. Filter on a 7 cm Büchner funnel and make the filtrate to 100 ml with water (Soln A). Place the filter paper in a 400 ml beaker and extract with small portions of alcohol until no more color is extracted and the paper remains white. Transfer the alcoholic extract to a 500 ml distilling flask, add 10 mg of bromophenol blue, 2 ml of HCl (1+1) and 100 ml of H_2O. Steam distil as before, collecting a volume of distillate which is at least 100 ml greater than the volume of alcohol in the extract. Cool the

[2] Mallinckrodt Sodium Sulfate Anhydrous granulated A.C.D. was found to be satisfactory.

residue, transfer to a separatory funnel and wash with 40 ml, 30 ml, and 30 ml portions of petroleum ether; pipet 50 ml of ethylene chloride into the separatory funnel and shake for 3–4 min. Let stand until clear and draw off the lower layer into a second separatory funnel containing 10 ml of sodium carbonate soln, and shake for 3–4 min. When the two layers separate, observe the lower one. If blue, a quaternary base is present. Judge from the depth of color whether or not it is suitable for reading in the photometer. If so, draw off the lower layer into a glass-stoppered flask containing 1–2 g of anhydrous granular Na_2SO_4 and, after 30 min, read as before in a suitable cell using a filter centering at 610 mμ.

If the color is too deep, acidify the contents of the second separatory funnel with 1 or 2 ml of HCl (1+1), shake until the contents become yellow, and return to the first separator. Pipet a second 50 ml portion of ethylene chloride into the first separator, shake 3–4 min and let stand until the lower layer is clear. (If the sample is known to contain over 1 mg of quarternary ammonium compound in 100 ml, add the entire 100 ml of ethylene chloride in the first instance.) Now follow the directions for (c) "*Measurement of Color.*"

Meanwhile, pipet a suitable aliquot (first try 5 ml) of soln A into a separatory funnel, add 3 ml of bromophenol blue soln and 1 ml of HCl (1+1); and proceed as above beginning "pipet 50 ml of ethylene chloride * * *."

(b) *Preparation of the Standard Curve.*—Standardize a 1% soln of the quaternary ammonium compound to be determined, using the ferricyanide method. By carrying out the procedure given below, ascertain the maximum and minimum concentrations of the chemical which produce, in 50 ml of ethylene chloride, colors of a density suited to the color-measuring instrument to be employed. Now prepare a set of three or more standards containing, in 50 ml quantities of the quaternary ammonium compound covering the range between these two points. (If the neutral wedge photometer is to be used, 0.0 mg, 0.1 mg, 0.2 mg, and 0.25 mg per 50 ml are suitable standards.)

Pipet 50 ml of each standard into a series of separatory funnels, add to each 3 ml of bromophenol blue solution and 1 ml of HCl. Pipet 50 ml of ethylene chloride into each and shake for 2–3 minutes. When clear, draw off each lower layer into a series of funnels containing 10 ml of sodium carbonate solution, and shake for 2–3 min. Let stand until clear, draw off into a glass-stoppered flask containing 1–2 g of granular anhydrous Na_2SO_4 and, after 30 minutes, read in the color-measuring instrument, using the same (or a similar) cell and a light filter centering at 610 mμ (a 1″ cell was used in the neutral wedge photometer). If the instrument reads in terms of per cent transmission, the proper conversion to color density must be made. If, however, it reads directly in color density, or in scale readings which are proportional to the density of color (as in the case with the neutral wedge photometer), the readings may be plotted directly against the concentrations employed.

(c) *Measurement of color.*—Treat each ethylene chloride extract *separately*[3] as follows: Introduce 30–50 ml of the extract into a separatory funnel containing 10 ml of sodium carbonate solution. Shake for 3–4 min. When clear, draw off the lower layer into a glass-stoppered flask containing 1–2 g of anhydrous granular Na_2SO_4. After 30 min read in a suitable cell using a filter centering at 610 mμ.

If the color is too deep for accurate reading, estimate a suitable volume of the extract to be used as an aliquot; pipet this volume into a 50 ml volumetric flask and fill to the mark with ethylene chloride. Shake the mixture with sodium carbonate solution, separate, dry, and read as above. From the quantities of quaternary ammonium compound found in the extracts from the liquid and the solid materials, calculate to mg per 100 ml of fruit juice.

[3] When the two ethylene chloride extracts were united for this step, lower results were obtained.

III. Method for Beer

Place 100 ml of decarbonated beer in the steam distillation flask; add 10 mg of bromophenol blue and 2 ml of HCl (1+1). Steam distil, collecting about 200 ml of distillate. Cool the residue, transfer to a separatory funnel, wash with 100 ml and then with 50 ml of petroleum ether. Then proceed as directed in the method for Bottled Beverages Containing Fruit Juices, beginning "pipet 50 ml of ethylene chloride, ..."

IV. Method For Table Sirup

Weigh 20 g of sample, transfer to a 100 ml volumetric flask, mix thoroly, and make to volume with water. Pipet an aliquot of the soln into a separatory funnel; add 5 ml of bromophenol blue soln, 1 ml of HCl (1+1) and proceed as directed in the Method for Bottled Beverages Containing Fruit Juices, beginning "pipet 50 ml of ethylene chloride, ..."

33. SPICES AND CONDIMENTS

(1) The tentative method for caramel in vinegar, section **33.78** (p. 553), was deleted.

(2) The following method for starch in mayonnaise and salad dressing was adopted as official, first action.

STARCH IN MAYONNAISE AND SALAD DRESSING

REAGENTS—*See* **33.41**

DETERMINATION

Determine the total acidity of the prepared sample by **33.51.** Place 4–5 g of the prepared sample in a 500 ml Erlenmeyer flask, add the calculated quantity of 0.1 *N* NaOH to neutralize the acid in the weight of sample taken, and add 100 ml of the $CaCl_2$ soln from a pipet. Stopper the flask and swirl gently until all large lumps of dressing are broken up. Continue as in the method for starch in mustard products, **33.42,** beginning "Add glass beads. Connect to reflux condenser . . . ." Calculate percentage of starch from the formula:

$$\% \text{ Starch} = \frac{\text{g. dextrose} \times 0.9\ (100 - A) \times 8}{\text{weight of sample}}$$

where A = ml 0.1*N* NaOH used to neutralize acidity of the sample.

(3) The tentative method for starch in mustards, *This Journal,* **30,** 75 (1947), was adopted as official, first action, after the following changes:

(a) Delete the sentence "Filter . . . funnel," lines 10 and 11, and substituting "Filter thru an 11 cm circle of absorbent cotton ca ½ cm thick placed in a 60° funnel."

(b) Adding "several glass beads and" between "Add" and "water," line 16.

(c) Adding "as possible" after the word "dispersed," line 17.

(d) Correcting "or" to "on" in parenthesis, last line.

(e) Changing "0.8" to "8.0" in the numerator of the formula for calculating the percentage of starch.

(4) The official method for copper-reducing substances in prepared mustard, section 33.40 (p. 545) was deleted, final action.

34. SUGARS AND SUGAR PRODUCTS

(1) The following method was adopted as tentative:

UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

*Method of Java Sugar Experiment Station**

REAGENTS

(a) *Baker's yeast, free from starch.*—Fleischmann's, sold in packages of 1 lb. each by Standard Brands, Inc., was found suitable. It keeps fresh for a few days if kept in the refrigerator.

(b) *Neutral lead acetate soln.*—Dissolve 20 g $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ in H_2O to 100 ml.

(c) *Anhydrous potassium oxalate.*

(d) *Soxhlet soln.* See section **34.33** (a) and (b).

(e) *Potassium iodide soln.*—Dissolve 20 g KI in H_2O to 100 ml.

(f) *Dilute sulfuric acid.*—5 volumes of water plus 1 volume of conc. H_2SO_4.

(g) *Standard thiosulfate soln.*—0.1 *N*.—See sections **43.28** and **43.29**.

FERMENTATION

Transfer 12 g of blackstrap molasses (or 8 g High-test molasses) to a 500-ml volumetric flask, using in all 75 ml of H_2O. Add 25 g of coarsely chopped, fresh baker's yeast and mix thoroughly with the molasses soln. Close the flask with a stopper provided with a delivery tube the other end of which dips about 1 cm below the surface of H_2O in a beaker; or use any other type of fermentation trap. Place the flask in a water bath kept at 30°C. and allow to ferment for at least 4 hours, shaking the flask from time to time. When fermentation is complete dilute with H_2O, clarify with 15 ml of neutral $Pb(C_2H_3O_2)_2$ soln, make to the mark at 20°C., add a teaspoonful of Filter-Cel, shake well and filter, discarding the first runnings. Delead the entire filtrate with ca 0.5 g of anhydrous $K_2C_2O_4$ and filter again with the aid of Filter-Cel. Test the filtrate for Pb. If necessary add more oxalate and refilter.

DETERMINATION

Transfer 25 ml of the final filtrate to a 250-ml Erlenmeyer flask, mix with 20 ml of combined Soxhlet soln, and wash the wall of the flask down with 5 ml of H_2O, making 50 ml in all. Add a few pieces of ignited pumice stone and place the flask on a wire gauze covered with an asbestos plate which has a hole in the center, slightly smaller than the bottom of the flask. As the source of heat use either a Bunsen burner or preferably an electric heater with temperature control. Heat to boiling in 3 min and boil gently for exactly 2 min longer. Close the flask immediately with a stopper provided with a Bunsen valve and cool quickly under the water tap to prevent reoxidation. Add 15 ml of KI soln and then 10 ml of dilute H_2SO_4. Titrate the liberated I at once with 0.1 *N* $Na_2S_2O_3$ soln, adding starch indicator toward the end of the titration.

Run a blank with 75 ml of H_2O instead of molasses soln, adding yeast, etc., as described. Deduct the titer of the sample from the titer of the blank, and find the mg invert sugar corresponding to the difference, from the table. The result, divided by 6 (in the case of High-Test molasses, by 4) gives directly the percent of unfermentable reducing substances in the molasses, in terms of invert sugar.

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* "Methoden van Onderzoek bij de Java-Suikerindustrie," 6th ed. (1931), p. 365.

Milligrams of Invert Sugar Corresponding to Milliliters of 0.1 N Thiosulfate

0.1 *N* THIOSULFATE	INVERT SUGAR	0.1 *N* THIOSULFATE	INVERT SUGAR
ml	*mg*	*ml*	*mg*
1	3.2	14	47.3
2	6.4	15	50.8
3	9.7	16	54.3
4	13.0	17	58.0
5	16.4	18	61.8
6	19.8	19	65.5
7	23.2	20	69.4
8	26.5	21	73.3
9	29.9	22	77.2
10	33.4	23	81.2
11	36.8	24	85.2
12	40.3	25	89.2
13	43.8	—	—

(2) The following changes were adopted in sections **34.18** (p. 560), as official, final (special action).

Change **34.18(a)** to **34.18(b)**; delete the second paragraph beginning "Verification" and the third paragraph beginning "According"; delete in sixth paragraph on page 561, line 4, "at 20° before reading is made." and substitute "at the same temperature (preferably 20°) when the observation is made," and delete "recently" in next to last line; delete in eighth paragraph, page 561, "basic acetate of lead . . . " and substitute "dry basic lead acetate (Horne's Dry Lead, or **34.19(c)**)"; delete ninth paragraph beginning "Whenever reducing"; delete tenth paragraph beginning "After" and substitute "Bring the soln exactly to mark at proper temp., and after wiping out neck of flask with filter paper, add minimum amount of dry basic lead acetate (**34.19(c)**), shake to dissolve, pour all the clarified sugar soln on rapidly acting, air-dry filter. Reject at least first 25 ml of filtrate and use remainder, which must be perfectly clear, for polarization."

Change **34.18(b)** to **34.18(c)** delete "Ventzke" lines 2 and 3 and substitute "Herzfeld-Schönrock"; delete "Bureau of Standards" lines 4, 5, and 6 and substitute "International Sugar."

(3) The following new subsection was adopted as official, final (special action), as section **34.18(a)**.

"The saccharimeter scale shall be graduated in conformity with the International Sugar Scale adopted by the International Commission for Uniform Methods of Sugar Analysis. Rotations on this scale shall be designated as degrees sugar (°S).

The basis of calibration of the 100° point on the International Sugar Scale is defined as the polarization of the normal solution (26.000 grams of pure sucrose dissolved in 100 ml solution and polarized at 20° in a 200 mm tube, using white light and the dichromate filter as defined by the Commission). This solution, polarized in room or cabinet, temperature of which is also 20°, must give saccharimeter reading

of exactly 100°S. Temperature of the sugar soln during polarization must be kept constant at 20°.

The following rotations shall hold for the normal quartz plate of the International Sugar Scale:

Normal Quartz Plate = 100°S = 40.690° ± 0.002°.
(λ = 5461A) at 20°.
1° (λ = 5461A) = 2.4576°S.

Normal Quartz Plate = 100° S = 34.620° ± 0.002°.
(λ = 5892.5A) at 20°.
1° (λ = 5892.5A) = 2.8885°S.

According to determination of Bates and Jackson (7)* at the National Bureau of Standards, the Herzfeld-Schönrock scale reading for 26 grams of pure dry sucrose under the above conditions is 99.89_5. The scale reading has been redetermined by Balch and Hill (8)* and by Zerban and Hardin (8)* who found values of 99.907° and 99.912°, respectively. Average value of these independent investigations is 99.90_5°. The value 99.90° was adopted by the International Commission in 1932.

In the case of existing instruments graduated on the Herzfeld-Schönrock scale, it shall be permitted either to change the saccharimeter scale or to use a weight of 26.026 g in 100 ml."

(4) The following change was adopted as section **34.19** (p. 562) as official, final (special action).

Delete in subsection **(a)** last sentence in parenthesis and substitute "(This reagent is not recommended for clarifying products of low purity, due to error caused by volume of precipitate)"; delete in subsection **(c)** last sentence in parenthesis and substitute "(Unless in excess, dry basic lead acetate does not cause a volume error)"; delete subsection **(e)** and substitute the following:

"**(e)** To remove excess lead used in clarification add to clarified filtrate anhydrous K or Na oxalate in small quantities, until test for Pb in filtrate is negative; refilter."

(5) The "**(e)**" in section **34.23(a)** (p. 565) line 3, was deleted, official, final (special action).

(6) The reference "Int. Sugar Jour. **35**, 19 (1933); **39**, 32s (1937)" was added to selected reference (6) p. 594.

35. PROCESSED VEGETABLE PRODUCTS

No additions, deletions, or other changes.

36. VITAMINS

(1) The following method was adopted as alternate tentative.

CAROTENE

EXTRACTION

(a) *Hays and dried plants.*—Grind the sample to pass a 40-mesh sieve. Weigh accurately a 2-g sample (4 if carotene content is low) and place in the flask of an extractor (Goldfisch, Bailey-Walker, or ASTM is suitable if no thimble is used). Add 30 ml of a 3:7 mixture of acetone and commercial hexane (therefore 30% acetone) to the flask and reflux the contents at a rate of 1–3 drops per second for one hour.

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* Reference numbers refer to selected references, *Methods of Analysis*, 6th Ed., p. 594.

After cooling to room temperature, decant or filter the extract into a 100 ml volumetric flask, wash the residue with hexane, and dilute the soln to volume. The soln, which now contains 9% acetone, is chromatographed.

CHROMATOGRAPHY

Prepare a chromatographic column with a 1:1 mixture of activated magnesia (Micron brand No. 2642, Westvaco Chlorine Products Company, Newark, California) and diatomaceous earth (Hyflo Supercel, Johns-Manville, Chicago, Illinois). A suitable chromatographic tube can be made from a Pyrex test tube 22 mm O.D. and 175 mm long by sealing a smaller tube (ca 10 mm O.D.) in the bottom. To prepare the chromatogram place a small plug of glass wool or cotton inside the tube, add the loose adsorbent to a depth of 15 cm, attach the tube to a suction flask, and apply the full vacuum of a water pump. Use a flat instrument such as an inverted cork mounted on a rod to gently press the adsorbent and flatten the surface. The packed column should be about 10 cm deep. Place 1 cm. layer of anhydrous sodium sulfate above the adsorbent.

With the vacuum continuously applied to the flask pour the extract into the chromatographic column and use 50 ml of a 1:9 mixture of acetone and hexane to wash the carotene into the adsorbent and develop the chromatogram. Keep the top of the column covered with a layer of solvent during the entire operation. This is conveniently done by clamping an inverted volumetric flask full of solvent above the column with the neck 1–2 cm above the surface of the adsorbent.

The carotenes pass rapidly through the column and the entire eluate is collected. Bands of xanthophylls, carotene oxidation products, and chlorophylls should be present in the column when the operation is complete. Transfer the eluate, which has been reduced in volume by loss of vapor through the water pump, to a 100 ml volumetric flask and dilute to volume with a 1:9 mixture of acetone and hexane. The carotene content of this soln is determined photometrically.

PHOTOMETRY

Determine the density of the solution as soon as possible with a spectrophotometer at 436 mμ or with some other instrument which is provided with a suitable filter system, such as the Klett photometer with a No. 44 filter, or the Evelyn photoelectric colorimeter with a 440 filter. These instruments are first calibrated with solns of beta-carotene of high purity as shown by the characteristic absorption curve (See Beadle and Zscheile, *J. Biol. Chem.*, **144**, 21 (1942), for characteristic curve). A calibration chart is prepared and the density of the soln to be determined is then converted into carotene concentration by referring to the chart.

When determinations are made with the Beckman spectrophotometer at 436 mμ, the specific absorption coefficient (*alph* = 196) is used in the formula

$$C = \frac{\log \frac{I_0}{I} \times V \times 1000}{196 \times L \times W}$$

where C is concentration of carotene in parts per million contained in the original sample, V is the final volume of the eluate at time of reading, L is length of cell in cm, and W is the weight of sample. Report results as parts per million of beto-carotene.

(2) The tentative fluorometric method for vitamin B (thiamine), sections **36.24–36.26** (p. 608), was adopted as official, first action.

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 for ephedrine was adopted as official, first action.

EPHEDRINE

(a) *Oily inhalants containing oxazolidines (products from the reaction of ephedrine with carbonyl compounds).*—Accurately weigh or otherwise measure out a sample containing ca 100 mg of ephedrine. (In the case of most inhalants, the sample size will be about 10 ml. Although a sample containing as little as 20 mg of the drug may be used, if necessary, the larger sample should be employed if practicable.) Rinse it quantitatively into a 125 ml Erlenmeyer flask with portions of reagent grade benzene totalling about 5 ml, add 10 ml. of 5% sulfuric acid, and frequently agitate and swirl the mixture while it is boiled gently for 10 min. The boiling is best done on a hot plate and should be conducted carefully to avoid superheating, bumping, and loss of sample. Cool the flask, transfer the contents to a 125 ml separatory funnel, and rinse the Erlenmeyer with portions of benzene totalling about 1½ times the volume of sample in order to remove all oily matter from the flask. (It is desirable that the Erlenmeyer be provided with a lip in order to facilitate the quantitative transfer of the contents to the funnel.) Shake the funnel containing the acid and benzene rinsings, drain off the acid layer into a second separatory funnel, and swirl the first funnel vigorously to force down additional acid and insure more complete separation of the phases. Drain into the second funnel any acid layer which further separates, and extract the benzene-oil phase with three 5-ml portions of water which have been previously used to rinse the flask. Swirl the first funnel each time after the main portion of water has been drained into the second. (In the transfer of ephedrine from organic solvent to aqueous phase, and *vice versa,* shakeouts should be conducted for at least 1 min and in as vigorous manner as possible without causing troublesome emulsions.) Wash the acid soln of ephedrine sulfate with 3 ml of chloroform, and discard the chloroform washings. Make the soln alkaline to litmus with 20% sodium hydroxide (about 2.5 ml is required), then add 0.5 ml in excess and extract the ephedrine by shaking *vigorously* with six 15-ml portions of U.S.P. chloroform. If more than 50 mg of ephedrine is present, filter the extracts through a pledget of cotton into a tared 100 ml beaker previously dried at 110° and cooled in a desiccator. After the fourth extraction, rinse the filter funnel and its tip with chloroform (allowing the rinsings to drain into the beaker), float 5 drops (0.2 ml) of conc. hydrochloric acid on the surface of the combined extracts, and evaporate on the steam bath in a current of air until the beaker can easily accommodate the remaining extracts. To test for complete extraction, shake the alkaline phase with a seventh and an eighth 15-ml portion of chloroform, filter these through cotton into a small beaker, float 2 drops of conc. hydrochloric acid on the surface, and evaporate to dryness on the steam bath in a current of air. If a *crystalline* residue results, combine it with the main extracts by using a little reagent grade methanol for the transfer, and repeat the test if considered necessary. Continue the evaporation of the main extracts until the volume of liquid has been reduced to about 1 or 2 ml. Then cautiously heat on the bath, but without the air current, until the odor of hydrogen chloride has disappeared, and the residue of ephedrine hydrochloride is apparently dry. Heat the beaker in an oven at 110° C. for ½ hour, cool in a desiccator, and weigh: Weight of residue X 0.8192 =

weight of ephedrine base. If less than 50 mg of ephedrine is present in the sample, carry out the extraction of ephedrine to completion as described above, and evaporate the filtered extracts in an untared beaker until the chloroform, but not the excess hydrochloric acid, has been removed. Direct a fine stream of redistilled reagent grade methanol around the inside of the beaker to dissolve the ephedrine salt, and immediately repeat the process with a stream of chloroform. Transfer the methanol-chloroform soln to a tared 20 ml beaker, previously dried at 100° and cooled in a desiccator, and repeat the methanol and chloroform rinsings until the ephedrine hydrochloride has been quantitatively transferred. Evaporate the soln on a steam bath, in a current of air, until the salt begins to crystallize. Continue the removal of solvent by cautious heating alone, to avoid loss from crepitation, until the residue is apparently dry and there is no odor of hydrogen chloride. Dry and weigh as previously described.

(b) *Oily inhalants or petroleum jelly preparations containing free ephedrine only.* If the product is an oil, quantitatively transfer a suitable sample (see **a**) to a 125 ml separatory funnel with the aid of portions of benzene totalling about 1½ times the volume of sample, and extract the mixture with 5 ml of 10% sulfuric acid, then with four 5-ml portions of water, swirling the funnel each time as in **(a)**, and continue the assay as described in that paragraph beginning at the point "Wash the acid solution of ephedrine sulfate. . . . "

If the product is a petroleum jelly preparation, dissolve the sample in sufficient benzene to obtain a soln of suitable fluidity (30 ml should be sufficient for a sample of 10 g) and proceed with the assay as described for oily inhalants.

(c) *Water-soluble jellies.*—If the product is a thin jelly of viscosity similar to that of the N. F. VIII preparation, accurately weigh a sample of about 10 g. If it is a thick jelly, reduce the size of the sample (which should contain 20–100 mg of the alkaloid) to about 5 g to diminish the possibility of emulsion formation during the ether extractions. Transfer quantitatively to a separatory funnel with the aid of water, and dilute with the latter until the volume of jelly plus water is about 20 ml. Render the mixture slightly alkaline to litmus with 20% sodium hydroxide, add an additional one-half ml, and quantitatively extract the ephedrine with U.S.P. ether according to the usual three-funnel technic. In each shakeout use a volume of ether equal to that of the aqueous phase, and swirl the funnel vigorously after draining off the bulk of the aqueous layer in order to obtain an efficient separation of the two phases. If equilibrium is attained in each shakeout, 5 or 6 will be sufficient. Extract the ether with 5 ml of 10% sulfuric acid and four 5-ml portions of water in the same manner as described for the extraction of the benzene-oil mixture in **(b)**, and continue the assay from this point according to the directions of that section.

(d) *Syrups.*—Use a 10 ml sample, measured by means of a volumetric flask, and proceed as directed under **(c)**.

(e) *Solutions of ephedrine salts.*—Use an accurately measured volume of sample containing about 100 mg of ephedrine and carry out the assay as directed under **(c)**, except that if the sample amounts to less than 20 ml it need not be diluted to this volume before extracting.

(f) *Tablets and capsules.*—Accurately weigh a sample of the capsule contents or finely powdered tablets preferably equivalent to about 100 mg of the drug. Transfer to a separatory funnel containing 20 ml of water and basify with ca 0.5 g of anhydrous sodium carbonate. Proceed with the assay as described under **(c)**, beginning with the ether extractions, but basify the acid extracts with anhydrous sodium carbonate instead of 20% sodium hydroxide. Use ca 0.5 g in excess of that required to make the soln alkaline to litmus.

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

SPIRIT OF CAMPHOR

REAGENT

2, 4-dinitrophenylhydrazine.—Dissolve 2 g of 2, 4-dinitrophenylhydrazine in 20 ml of 1+1 cold H_2SO_4 by shaking in a glass-stoppered flask; add 35 ml of water, mix, cool, and filter.

DETERMINATION

Dilute an accurately measured quantity of spirit of camphor with aldehyde-free alcohol so that the soln contains ca 0.2 g of camphor per 10 ml. Pipet 10 ml of the dilution into a 125 ml pressure bottle containing 50 ml freshly prepared dinitrophenylhydrazine reagent. Close pressure bottle, immerse it in a beaker of water, and heat on steam bath 4 hours. (Temperature of pressure bottle should be maintained ca 75°C.)

Cool the bottle and contents to room temperature, then transfer contents to a beaker using 100 ml 1+11 H_2SO_4. Allow to stand overnight at room temp. Collect the precipitate on a tared Gooch crucible, wash with 10 ml 1+11 H_2SO_4, then with 75 ml cold water to remove the acid. Dry at 100°C. The weight of precipitate ×.458 = weight of campher.

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

QUINACRINE HYDROCHLORIDE (ATABRINE)

REAGENTS

Sodium acetate-acetic acid mixture.—Dissolve 2.5 g sodium acetate and 1 ml glacial acetic acid in sufficient H_2O to make 45 ml.

Dilute HCl.—Add sufficient H_2O to 15 ml conc. HCl to make 80 ml.

KI soln.—Dissolve 16.5 g KI in sufficient H_2O to make 100 ml.

DETERMINATION

Transfer the accurately weighed sample, equivalent to ca 0.25 gm quinacrine hydrochloride, to a 100 ml volumetric flask with the aid of 45 ml of the sodium acetate-acetic acid mixture. Shake thoroly to dissolve the quinacrine hydrochloride. Allow to settle and add exactly 50 ml 0.1*N* $K_2Cr_2O_7$. Fill to the mark with distilled water and mix well. Allow to stand 10–15 min and filter thru a dry filter, rejecting the first 15 ml of filtrate. Measure exactly 50 ml of the subsequent filtrate into a glass-stoppered flask. Add 80 ml of the dilute HCl and 20 ml of the KI soln. Stopper the flask and mix by gentle swirling. Allow to stand for 5 min. and titrate the liberated iodine with 0.1*N* $Na_2S_2O_3$ adding starch indicator when the end point is neared.

1 ml 0.1*N* $K_2Cr_2O_7$ is equivalent to 0.00842 g of $C_{23}H_{30}OCln_3 \cdot 2HCl \cdot 2H_2O$.

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

DEMEROL

Distillation Method

APPARATUS

An all-glass apparatus was assembled, using pieces made with standard taper (24/40) ground glass points. It consisted of a 500 ml round bottom, short neck flask into which fitted an adapter with attached separatory funnel (Ace catalog, 5270). A distilling head (Scientific Glass Apparatus Co., J-1500) fitted into this adapter. A connecting adapter (Ace, 5125) was used to connect the distilling head to a straight inner-tube, water cooled condenser. Another adapter, attached to the bottom of the

condenser, was long enough to dip below the surface of the liquid in a 1,000 ml Erlenmeyer flask. Other apparatus could be set up which would probably work equally as well. The essential features are provision for adding liquid during the distillation and a distillation head efficient enough to prevent any spray of the calcium carbonate mixture from carrying over into the receiving flask.

REAGENTS

Methyl red indicator. See **39.8(b)**.

Sodium hydroxide soln. 0.02 *N* NaOH standardized against sulfamic acid using methyl red indicator.

Sulfuric acid soln., 0.02 *N* H_2SO_4 standardized against the 0.02 *N* NaOH using methyl red indicator.

Powdered calcium carbonate.

PREPARATION OF SAMPLE

Weigh a counted number of not less than 20 tablets and reduce them to a fine powder without appreciable loss.

DETERMINATION

Weigh accurately an amount of the powder equivalent to ca 100 mg of Demerol. Wash it into a 500 ml round bottom flask with ca 25 ml of water. Introduce into flask ca 1 gm of powdered $CaCO_3$ and connect the flask to the distillation apparatus described above. Place 1-liter Erlenmeyer flask, containing 20 ml of 0.02 *N* H_2SO_4 under condenser in such manner that the adapter on end of condenser will be below surface of the acid. Thru separatory funnel introduce into flask 100 ml of water. Heat until only ca 25 ml of liquid remains in flask. Without interrupting distillation, add a second 100 ml of water slowly enough that distillate does not suck back into distillation flask. Continue distillation until this portion has distilled over. In like manner, distill over a third 100 ml of water. Then add, thru the separatory funnel, 10 ml of ethyl alcohol. When most of alcohol has distilled over, add and distill over a fourth 10 ml portion of water. Disconnect condenser from distillation apparatus and rinse inside of condenser and adapter which dipped into the standard acid, catching rinsings in a receiving flask. Bring collected distillate to a vigorous boil to remove any dissolved CO_2, cool, and titrate excess acid with 0.02 *N* NaOH, using methyl red as indicator. Each ml of 0.02 *N* H_2SO_4 = .005673 gm of demerol hydrochloride $C_{15}H_{21}O_2N \cdot HCl$.

Extraction Method

REAGENTS

See under Distillation Method.

PREPARATION OF SAMPLE

Same as for distillation method.

DETERMINATION

Weigh accurately a portion of powder, equivalent to ca 0.1 gm of demerol and macerate it with 10 ml of distilled water and 1 ml of normal H_2SO_4 for 2 hours. Decant liquid thru small filter into separatory funnel. Macerate residue with 5 ml of distilled water for 20 min, filter thru same filter and wash residue and filter with small portions of distilled water. Saturate the soln with NaCl, then add 5 ml of normal NaOH and extract with 25 ml of ether. Draw off aqueous layer into another

separator, and repeat extraction of aqueous layer in similar manner 6 times, using 20 ml of ether each time. Wash combined ether extracts with two 5-ml portions of distilled water, then extract this water with 10 ml of ether and add this ether to main ether extract. Extract ether soln first with 20 ml of 0.02 *N* H_2SO_4, accurately measured, then successively with 10 ml and 5 ml of distilled water. Combine the H_2SO_4 and water extracts in beaker and warm on water bath until odor of ether is no longer perceptible. Cool soln and titrate excess acid with 0.02 *N* NaOH, using methyl red indicator. Each ml of 0.02 *N* H_2SO_4 is equivalent to .005673 gm of demerol hydrochloride, $C_{15}H_{21}O_2N \cdot HCl$.

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

DIHYDROCODEINONE

Weigh a quantity of the powdered sample to contain ca 0.065 g of dihydrocodeinone. Transfer to a separator and dissolve in 15 ml of H_2O. Make alkaline to litmus with dilute NH_4OH. Extract with $CHCl_3$, using 30, 20, 20, and 10 ml portions. Filter into beaker and evaporate the combined $CHCl_3$ extracts on water bath to ca 5 ml, using a current of air to assist evaporation. Add 20 ml 0.02*N* H_2SO_4 and evaporate the remainder of the $CHCl_3$. Titrate the excess acid with 0.02 *N* NaOH, using methyl red as indicator. 1 ml of 0.02 *N* H_2SO_4 = 0.00707 g of dihydrocodeinone hydrochloride, $C_{18}H_{21}O_3N \cdot HCl \cdot H_2O$.

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

THIOURACIL

Weigh an amount of sample equivalent to 0.25 gm (±0.040 gm) thiouracil and transfer to a 100 ml volumetric flask. Add ca 50 ml of distilled water and then add 3 ml of 5% NaOH. Shake for a few min. Dilute to volume with distilled water, shake, filter thru a dry filter, and transfer 50 ml of the filtrate to an iodine flask. Add 25 ml of 0.5 *N* standard KBr-$KBrO_3$ and 10 ml of 10% hydrochloric acid, and let stand for exactly 15 min. Then add 10 ml of 15% KI soln and let stand for exactly 5 min. Titrate the iodine with 0.1 *N* $Na_2S_2O_3$ and take the first disappearance of the yellow (or blue) color as the end point. The use of starch as an indicator in this titration is optional. One ml of 0.1 *N* KBr-$KBrO_3$ is equivalent to 0.001282 g of thiouracil ($C_4H_4ON_2S$).

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

OINTMENT OF BENZOIC AND SALICYLIC ACIDS

Transfer an accurately weighed sample, ca 2.5 g, into a separatory funnel, add ca 50 ml of ether and swirl until dissolved. Completely extract with saturated soln of $NaHCO_3$, using 15, 15, 10, and 10 ml portions, or more. Extract the combined $NaHCO_3$ soln with 10 ml of $CHCl_3$ and discard the latter. Acidify with HCl and extract with $CHCl_3$ ether (2+1) until the benzoic and salicylic acids are completely extracted. Filter extracts into a 250 ml beaker thru filter moistened with $CHCl_3$. Evaporate to ca 5 ml on a steam bath using a current of air, then continue spontaneously.

Dissolve residue in about 20 ml of diluted alcohol (about 50%), carefully titrate with 0.1 *N* NaOH using phenolphthalein, record the volume, and add an excess of ca 2 ml. Completely evaporate the alcohol on a steam bath using a current of air; evaporation from ca 50 ml volume to 5 or 10 ml is sufficient. (Alcohol consumes bromine.)

Transfer remaining titration liquid and washings to 100 ml volumetric flask, cool to room temperature, and fill to mark with water. Mix thoroly. Pipet a 25 ml aliquot into an iodine flask, add 25 ml of water, exactly 25 ml of 0.1 *N* KBr-$KBrO_3$, and ca 5 ml of HCl. Swirl mixture repeatedly during 30 min. Carefully add 5 ml of KI soln (ca 10%), shake well and in about one min. titrate with 0.1 *N* $Na_2S_2O_3$ using starch indicator.

Calculate salicylic acid from the 0.1 *N* KBr-$KBrO_3$ consumed, 1 ml = 0.0023 g. Calculate benzoic acid from the difference between the 0.1 *N* NaOH titration value and the 0.1 *N* NaOH equivalent of the salicylic acid found in sample taken. 1 ml of 0.1 *N* NaOH = 0.01221 gram of benzoic acid or 0.01381 gram of salicylic acid.

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 tentative method for rodent excreta in corn meal, section **42.32** (p. 781), was made official, first action.

(2) The official method for molds in tomato products, section **42.57** (p. 788), was changed, official, final action, as follows:

(a) Deleting beginning "mix," line 3, par. 1, to end of paragraph.

(b) Substituting "add H_2O to make a mix having a total solid content such as will give an immersion refractometer reading at 20° of 45.0–48.7 or an Abbé refractometer reading at 20° of 1.3447–1.3460."

(c) Introducing a reference in the selected references, Chapter 42, applying to this change, as follows: "National Canners' Assoc. Bul., 27–l, revised Feb. 1941, Table 3, p. 58."

(3) The tentative method for manure fragments in dairy products, section **42.11** (p. 774), to be changed by substituting "up to" for "ca" in line 3.

(4) The word "examination" in line 1 of the method for dried mushrooms, *This Journal*, **30**, p. 105, was changed to "contamination."

43. STANDARD SOLUTIONS

(1) The official, first action, method for sodium thiosulphate, sections **43.28–43.29** (p. 809), was adopted as official, final action.

(2) The official method for standardization of potassium permanganate, section **43.18** (p. 807), was deleted, first action.

(3) The following method for standardization of potassium permanganate was adopted as official, first action.

STANDARDIZATION

Transfer 0.3 g of dried (105°) sodium oxalate (National Bureau of Standards standard sample) to a 600-ml beaker. Add 250 ml of diluted sulfuric acid (5+95) previously boiled for 10–15 min and then cooled to 27°±3°.

Stir until the oxalate has dissolved. Add 39 to 40 ml of 0.1*N* potassium permanga-

nate at a rate of 25 to 35 ml per minute while stirring slowly. Let stand (about 45 seconds) until the pink color disappears. If the pink color should persist because the permanganate is too strong, discard, and begin again, adding a few ml less of the $KMnO_4$ soln. Heat to 55 to 60°C, and complete the titration by adding permanganate until a faint pink color persists for 30 seconds. Add the last 0.5 to 1 ml dropwise with particular care to allow each drop to become decolorized before the next is introduced.

Determine the excess of permanganate required to impart a pink color to the solution. This can be done by matching the color by adding permanganate to the same volume of the boiled and cooled diluted sulphuric acid at 55 to 60°C. This correction usually amounts to 0.03 to 0.05 ml.

(4) Add the following reference in selected references, Chapter 43, as applying to method in (3): *J. Research Natl. Bur. Standards,* **15,** 493 (1935), Research Paper No. 843.

(5) In the method for thiocyanate, paragraph on reagents (*This Journal,* **30,** 105, 1947), the reference (4) is as follows: (4) See Volumetric Analysis, Kolthoff and Furman, Vol. II (1929).

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

BROMIDE—BROMATE

REAGENTS

Bromide-bromate soln.—Weigh out about 2.8 grams of potassium bromate and 12 grams of potassium bromide into a one liter flask. Fill with boiled distilled water.

Arsenious oxide soln.—Prepare standard arsenious oxide by the method given in **43.19** and **43.20.**

STANDARDIZATION

Measure 40 ml of the standard arsenite soln from a buret into a 300 ml Erlenmeyer flask. Add 10 ml HCl and 3 drops of methyl orange (**43.12(a)**). Titrate with the standard bromide-bromate solution until one drop, or less, causes the color of the methyl orange to fade completely. The soln should be swirled constantly and the last ml added dropwise with swirling between each drop.

(7) The method for thiocyanate soln, *This Journal,* **30,** 105 (1946), was adopted as official, final action.

ERRATA AND EMENDATIONS, METHODS OF ANALYSIS, A.O.A.C. 1945

Supplemental to list as published, *This Journal,* 30, 60 (1947)

Section	*Page*		
15.5	182		Raise section number, **15.5,** to aline with "Glycerol in dry wines." In same paragraph change "allowering" to "allowing."
16.3	191	line 1	Change "HO_2" to "H_2O."
19.16(a)	227	line 7, par. 2	Change "ca 20° (below 25°)" to "20° or lower."
20.125	268	3d line from end	Change "1 ml" to "0.1 ml."
27.59	419	line 11	Insert the words "Cool, make to measured volume, and mix." before "Compare."
	521	Reference 25	Delete "82."
42.62	790	Title .1	Insert reference (6) before (7).

ANNOUNCEMENTS

The 62d Annual Convention of the Association of Official Agricultural Chemists will be held October 11 to 13, inclusive, at the Shoreham Hotel, 2500 Calvert Street, N. W., Washington 8, D. C.

As the Shoreham Hotel will provide the halls and other facilities for the meeting it is urged that members and visitors secure their accommodations at this hotel if possible and make it their headquarters. Twin bed double rooms are $8.00 and $9.00 per day. A limited number of single rooms may be available at $6.00 and $7.00. All rooms are with private bath. The rates are on the European plan. It is suggested that reservations be secured well in advance of the meeting, by direct communication with the hotel.

NOTICE TO SUBSCRIBERS

The Association urgently needs a substantial number of copies of Vol. 29, No. 1, the Feb. 15, 1946, issue of its *Journal;* and, in addition, all four numbers, Vols. XXIV and XXV; Vol. XXVII, No. 4 (November, 1944); and Vol. 28, No. 2 (May, 1945). 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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CORRECTIONS—NOVEMBER JOURNAL

In the paper on "Factors Affecting the Availability of Ammoniated Superphosphates. Part II," by William H. Ross, J. Richard Adams, John O. Hardesty, and Colin W. Whittaker, published in the preceding number of *This Journal,* **30,** 624 (1947), in Table 10, p. 634, last figure in the last column should read "−27" instead of "+27."

CONTRIBUTED PAPERS

THE USE OF THE SODIUM-CHLORINE RELATIONSHIP FOR THE DETECTION OF SODIUM NEUTRALIZED NON-FAT DRY MILK SOLIDS

By WILLIAM HORWITZ (Food and Drug Administration, Federal Security Agency, Minneapolis, Minnesota)

The presence of neutralizers in non-fat dry milk solids can be detected by the alkalinity of ash method as developed by Hillig (1). The reliability of this method is supported by the work of Kunkel and Combs (2) and it has been adopted as official by the A.O.A.C. (3). The chief disadvantage of this procedure is a lack of precision since a large excess of acid must be added to the ash and 95 per cent of it is back titrated to arrive at the alkalinity value.

The use of the mineral constituents of milk ash as an index of neutralization is mentioned by Davies (4). The phosphorus content of milk is reported to be a straight line function of the calcium content, and, likewise, the sodium content is a straight line function of the chlorine content. The purpose of this work was to obtain additional data to support the use of the sodium:chlorine ratio (5) as an index of sodium neutralization in non-fat dry milk solids.

METHODS

The methods used in this study were:

Ash.—Methods of Analysis, section **22.99**.

Alkalinity of Ash.—Methods of Analysis, section **22.100**.

Chlorine.—Methods of Analysis, section **23.22**. Open Carius on 2 g sample, using 10 ml 0.1*N* $AgNO_3$ and back titration with 0.05*N* thiocyanate.

Sodium —Methods of Analysis, section **12.21**. Magnesium uranyl acetate on the sulfated ash from 2 g sample. Solution made up to 100 ml after precipitation of phosphates, filtered, and 50 ml aliquot used for the determination. It was later determined that the use of the sulfated ash was unnecessary since the direct ash (*Methods of Analysis,* **22.99**) gave similar results.

SAMPLES

A total of 104 samples divided into three groups were examined for sodium and chlorine in this study. The first group consisted of 15 samples of authentic dry milks prepared by Hillig in 1941 (1). Nine of these samples were unneutralized; six were neutralized. The second group consisted of 58 samples which had previously been examined for neutralization in this laboratory. Fifteen of these samples were considered neutralized by the alkalinity of ash determination. The Kunkel-Combs criterion of the alkalinity of the ash of 130 ml 0.1*N* HCl/100 g sample was used as the

base line in this study for determining whether a given sample was to be classified as neutralized. The third group consisted of 31 samples of unknown history which were collected for this study from ordinary commercial channels. Two samples of this group showed an abnormal sodium to chlorine ratio and the presence of a neutralizer was confirmed by alkalinity of ash determinations. The other 29 samples of this group were not examined for alkalinity of ash and, for brevity, they will be referred to as normal or as unneutralized, with the understanding that they are normal only with respect to sodium neutralization and that the possibility that some of these samples may have been neutralized with other than sodium compounds can not be ruled out.

RESULTS

Figure 1 shows the individual values plotted with per cent sodium as the abscissa and per cent chlorine as the ordinate. All of the data are expressed

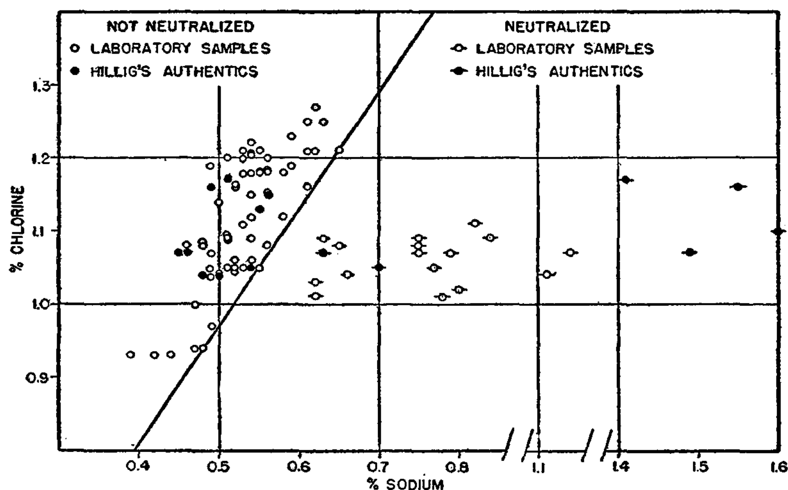

FIG. 1.—The sodium and chlorine contents of 104 non-fat dry milk solids samples. The sodium axis is omitted from 0.86 to 1.08 per cent sodium and from 1.16 to 1.38 per cent sodium without omitting any data or changing the scale.

on an *as is* basis, since the ratio of the two components is independent of the method of expression of the results. For convenience, the sodium axis has been broken at two points without eliminating any data or changing the scale. The circles represent normal samples with an alkalinity of ash value of less than 130. The solid circles represent the values obtained on the authentic unneutralized samples prepared by Hillig. A similar representation is used for the neutralized authentic material prepared by Hillig and the neutralized commercial samples, *i.e.*, having an alkalinity

of ash greater than 130. The average sodium to chlorine ratio of the 81 unneutralized samples is 0.47 with a standard deviation of 0.03. The maximum ratio is 0.54. The minumim ratio of the neutralized samples studied is 0.58.

The equation of the line which marks the high sodium boundary of the normal samples in figure 1 is:

$$\% \text{ Na} = 0.62\% \text{ Cl} - 0.10.$$

This equation may be used instead of the ratio sodium:chlorine to determine the presence of a sodium neutralizer. If the per cent chlorine in the sample (*as is* basis) is substituted into the equation, the calculated per cent sodium should be equal to or greater than the actual sodium content of the sample as determined by analysis if the sample is to be classified as normal (or non-sodium neutralized). If the actual sodium content is greater than the calculated sodium content, then the dry milk was prepared from a sodium neutralized skim milk.

This method of approach to the problem of detecting the presence of neutralizers can easily be extended to calcium neutralized dry milks, if encountered, since the ratio of calcium to phosphorus is 1.3 (6). The magnesium content of normal milk is so low (7) that a direct analysis for this constituent would be sufficient to establish its presence in excessive quantities.

The value of this method of detecting neutralized dry skim milk lies in the fact that it is based upon definite chemical constants which can be determined with a relatively high degree of accuracy and precision as compared with the empirical alkalinity of ash determination. It has the disadvantage that the time involved is considerably greater than that required for the alkalinity determination.

SUMMARY

The sodium and chlorine contents of (1) 23 sodium neutralized and (2) 81 normal or non-sodium neutralized dry skim milks were determined. The ratio of sodium to chlorine in the normal samples was 0.47 with a standard deviation of 0.03. If the per cent sodium is plotted as the abscissa against the per cent chlorine as the ordinate, all of the sodium neutralized samples lie to the right of the line represented by the equation: $\% \text{ Na} = 0.62\% \text{ Cl} - 0.10$. All of the normal or non-sodium neutralized samples lie to the left of this line.

ACKNOWLEDGEMENT

The assistance of Dona S. Clark is gratefully acknowledged.

BIBLIOGRAPHY

(1) Hillig, F., *This Journal*, **22**, 495 (1939); **23**, 468 (1940); **24**, 556, 744 (1941); **25**, 253, 610 (1942); **28**, 205 (1945).

(2) Kunkel, R. W., and Combs, W. B., *J. Dairy Sci.*, **28**, 219 (1945).
(3) A.O.A.C., *Methods of Analysis*, 6th Ed., **22.99**, **100**.
(4) Davies, W. L., *J. Soc. Chem. Ind.*, **57**, 246 (1938).
(5) Jones, T. S. G., and Davies, W. L., *Biochem. J.*, **29**, 978 (1935).
(6) Bulletin 905, Revised 1946, American Dry Milk Institute, Inc., 221 North LaSalle Street, Chicago 1, Illinois, page 5.
(7) Davies., W. L., "The Chemistry of Milk," D. Van Nostrand Co., New York, Second Edition, 1939.

SERUM METHODS FOR ADDED WATER IN MILK

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The Association of Food, Dairy, and Health Officials of the South Central States at a recent conference adopted a resolution that a study be made of the official method for added water in milk involving the sour serum (*Methods of Analysis*, 6th Edition, **22.29**). The Association found need for further information as to the reliability of this method for detecting added water in amounts of less than thirteen per cent. Since so wide a departure from accuracy as reported by the South Central officials, if true for any considerable percentage of samples of normal milk, would effect a most generous tolerance for adulteration, it was thought a review of this and allied methods might be in order.

Our *Methods of Analysis*, Sixth Edition, describes three methods (**22.28**, **22.29**, and **22.30**) by which milk serum may be prepared for examination by means of the immersion refractometer. The first two of the methods also call for determination of ash in the serum as an aid in detection of added water. Leach and Lythgoe[1] made a study of the acetic serum method. Their report on thirty-two separate samples collected and examined in the routine of inspection gave the maximum immersion refractometer reading on the serum of 44.5, the minimum as 34.7, with an average of 42.1. From the determinations of total solids, fat, solids not fat and ash, the sample with the reading 34.7 appeared to be watered. Two samples had a refractometer reading below 39.0, which is the minimum reading for acetic serum obtained from normal milk, according to the present official method (**22.28**). Readings between 39.0 and 40.0 are suspicious and ash is to be determined on the serum when the reading is 40.0 or below. Only three samples in the group studied by Leach and Lythgoe gave a reading below 40.0. They also reported on twenty-two individual fancy Holstein and twelve individual common Holstein cows. The maximum immersion refractometer reading on the serum of the fancy cows was 44.5, the minimum 40.0, with an average of 42.7. The maximum immersion refractometer reading on the serum of the common cows was

[1] *J. Am. Chem. Soc.*, **26**, 1195 (1904).

43.1, the minimum 39.0, with an average of 41.6. Only three samples had a refractive index below 40.0.

Lewis I. Nurenberg[2] made a study of the refractometer readings obtained using the sour serum method and also on the amount of ash present in both the acetic and sour serum. In his report on 660 individual cows, there was not any refractometer reading below 38.3, which is the minimum reading for serum obtained from normal milk by means of simple souring, according to the present official method **(22.29)**. Only 5.2 per cent of the samples gave readings below 39.0. The refractometer readings ranged from 40.0 to 43.9 on 77.3 per cent of the samples. Nurenberg's report on 49 separate herds did not show any refractometer readings below 39.0 and only 4.1 per cent below 40.0. In his report on the ash obtained on the sour serum from the milk of 504 individual cows, there were none showing an ash below 0.730 gm/100 ml of serum, which is the minimum for normal milk, according to the present official sour serum method **(22.29)** and only 8.2 per cent below 0.740 gm/100 ml of serum. From 35 separate herds not any of the samples had a sour serum ash below 0.740 gm/100 ml, and only 3 samples (8.6%) were below 0.760 gm/100 ml. Nurenberg also published the results submitted by Leslie W. Ferris of the U. S. Bureau of Chemistry, who made a comparison of ash values obtained on the acetic serum and the sour serum of 40 herd samples. The maximum for the acetic serum ash was 0.877 gm/100 ml of serum, the minimum was 0.709 gm/100 ml, with an average of 0.769 gm/100 ml. Only 7 samples gave an ash below 0.715 gm/100 ml, which is the minimum for normal milk, according to the present official acetic serum method **(22.28)**. For the sour serum method, the maximum ash was 0.924 gm/100 ml, the minimum 0.671 gm/100 ml (later raised to 0.722 gm/100 ml upon further souring), with an average of 0.765 gm/100 ml. Nuremberg recommended the adoption of the current sour serum method and also the adoption of the minimum values for ash used in both the present acetic and sour serum methods.

Since it appeared that the sour serum method did not give accurate results, this brought suspicion on the other methods, so the three official serum methods, 22.28, 22.29 and 22.30, were included in this preliminary qualitative investigation. The sour serum method was modified by adding about 1 per cent (20 drops to 100 ml of milk) of buttermilk culture to aid the souring. It was determined that 1 per cent of culture was as effective as 2 per cent and that it was necessary to let the milk sour at least 40 hours at near 30°C for satisfactory results. Twenty-seven herd samples of raw milk were examined. Each sample was divided into five parts and systematic dilutions of 0, 5, 10, 15, and 20 per cent of water were used. The cryoscopic method was used only on the undiluted samples. The maximum, minimum, and mean values are given in Table 1.

[2] *This Journal*, **2**, 145 (1916).

An inspection of the minimum values in the above table shows that in the case of all three methods, more than 5 per cent of added water must be present before it would be indicated, if one accepts as correct the immersion refractometer readings given in the present methods. The minimum value for the acetic serum at 5 % dilution arouses suspicion. Five samples of the acetic serum at 5% dilution gave readings between 39.0 and 40.0. The mean values for all three serum methods do not indicate added water until more than 10 per cent is present.

Eight samples of the acetic serum at 10% dilution gave readings below

TABLE 1.—*A study of 27 herd samples of milk*

% ADDED WATER		IMMERSION REFRACTOMETER READING AT 20°C.			ASH GMS/100 ML OF SERUM		TRUE FREEZING POINT
		ACETIC SERUM	SOUR SERUM	COPPER SERUM	ACETIC SERUM	SOUR SERUM	
None	Max.	44.1	43.5	39.2	0.8324	0.8916	0.561
	Min.	40.3	40.0	37.0	0.7104	0.7608	0.530
	Mean	42.5	41.8	38.3	0.7751	0.8001	0.544
5	Max.	42.5	41.9	38.2	0.7940	0.8384	
	Min.	39.0	38.3	36.1	0.6732	0.7040	
	Mean	41.0	40.4	37.3	0.7352	0.7557	
10	Max.	41.0	40.5	37.1	0.7540	0.7916	
	Min.	37.8	37.5	35.1	0.6372	0.6536	
	Mean	39.6	39.0	36.3	0.6995	0.7172	
15	Max.	39.5	38.9	35.9	0.7020	0.7428	
	Min.	36.5	36.2	34.1	0.5920	0.6260	
	Mean	38.1	37.5	35.3	0.6584	0.6748	
20	Max.	37.8	37.5	35.0	0.6568	0.6984	
	Min.	35.1	34.9	33.3	0.5816	0.6052	
	Mean	36.7	36.1	34.3	0.6200	0.6362	

39.0. Five samples of the sour serum at 10% dilution gave readings below 38.3 and four samples of the copper serum at 10% dilution gave readings below 36.0.

This minimum value from Table 1 for the acetic serum ash in the undiluted milk is below the value given in the method, but the freezing point of the sample does not indicate added water. The mean values for the ash does not indicate added water until more than 5 per cent is present in both the acetic and sour serum methods. Three samples of the acetic serum at 5% dilution gave an ash below 0.715 gm/100 ml. Three samples of the sour serum at 5% dilution gave an ash below 0.730 gm/100 ml.

From the early work it appeared that it was the rule to use the lowest

figure that had been obtained for the immersion refractometer reading and the ash as the criteria in determining if added water was present. Using this as a basis, it would be possible for a milk with a fairly high refractive index and ash to contain at least 10 per cent added water before it could be detected.

It is believed that the lower limits for the immersion refractometer reading and for minimum serum ash in the present Official Methods should be raised. The sour serum method is objectionable for two reasons. First, it is impossible for pasteurized milk to sour spontaneously, and second, the statement "completely sour" is quite indefinite. If the milk is partially sour, the ash will probably be low because some of the calcium is precipitated with the casein[3] and not redissolved until the acidity approaches a maximum.

The copper serum method is the most rapid of the three from the standpoint of preparation of the serum and is desirable since it gives a narrow range of readings.

The cryoscopic method should be used to check the serum methods whenever added water is indicated. This is a rapid, accurate, and most reliable method.

STIRRER ACTUATED CONTINUOUS ETHER EXTRACTOR

By Morris L. Yakowitz and Herman J. Meuron

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A continuous extractor is described which employs a novel stirrer-pump to force the extracting solvent through the sample and absorbing solutions. The device has a rapid rate of flow and can be used with large or small volumes. The process is carried out at room temperature and the extracted material is not subjected to heat.

In removing an extractable substance from a solution by means of an immiscible solvent, the commonly used continuous extractor is that of Palkin (1) or Widmark (3), or a modification of one of these.

In the Palkin type continuous extractor, the extracted substance collects in a distilling flask from which the solvent is continuously distilled back into the vessel containing the sample. The extracted substance is subjected to heat at the boiling point of the solvent, and mixed solvents of greatly different volatilities cannot be used.

In the Widmark type continuous extractor, the solvent is in contact with the sample solution and an absorbing solution which changes the composition of the extracted substance so that it is no longer soluble in the

[3] Nurenberg, Lewis I., *Loc. cit.*

solvent. The extracted substance collects in the absorbing solution, and the extraction process can be carried out at any desired temperature including room temperature. However, the original Widmark apparatus (3) and the Pucher and Vickery modification (2) are slow.

The authors have devised an extractor like that of Widmark, in which the solvent is circulated from the compartment containing the sample solution, into a compartment containing the absorbing solution. In the authors' extractor, the movement of the solvent is produced by a novel stirrer-pump which gives a rapid rate of flow and causes the solvent actually to flow through both the sample solution and absorbing solution, resulting in a rapid rate of extraction.

The principle of the stirrer actuated extractor is shown in Figure 1, and a recommended model with dimensions is shown in Figure 2.

PRINCIPLE OF EXTRACTOR

In Figure 1, the left-hand vessel is divided into two compartments A and B, separated by a partition which has a hole at its center. A stirrer with vertical blades rotates just above the partition. The rotating stirrer causes the liquid in A to swirl and gives the liquid a deeply concave surface. This creates a hydrostatic head of pressure between the outlet on the sidewall and the inlet hole at the center of the partition. If the liquid in A is swirled rapidly enough to bring the concave surface down tangent to the partition, this external difference in pressure is equal to the height which the swirling liquid reaches on the sidewall, measured from the level of the partition. Actually, this pressure head is increased about 10–20 per cent by the centrifugal throw of the swirling liquid at the outlet hole. The maximum driving pressure which can be obtained with the stirrer pump is determined by the height of compartment A.

To operate, a suitable volume of sample solution is placed in compartment C to the level shown, the absorbing solution is placed in compartment B, and solvent is added until it has filled C to above its outlet and A is about half-filled. The stirrer is set in motion and when the swirling of liquid in A is great enough, solvent will flow out the side-tube from A, rise through sample solution in C, thence through absorbing solution in B, and back into the stirrer chamber, A.

If the sample solution in C is made alkaline while the absorbing solution in B is acidic, alkaline substances such as alkaloids will be removed from the sample solution and will collect in the absorbing solution. To extract organic acids from the sample solution it is necessary merely to reverse these conditions.

DESIGN OF PRACTICAL EXTRACTOR

The design and operation of the extractor shown in Figure 2 is based on the following practical considerations.

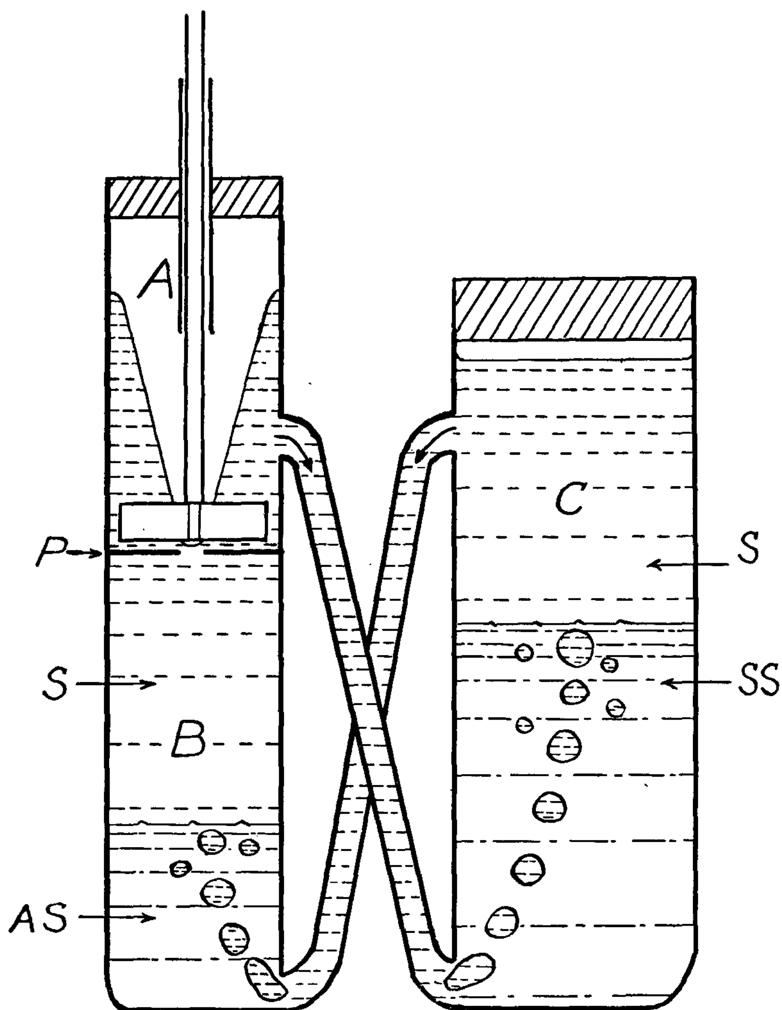

FIG. 1. Principle of Operation
A. Stirrer-pump compartment
B. Absorbing solution compartment
C. Sample solution compartment
P. Partition
S. Solvent
AS. Absorbing Solution
SS. Sample Solution

The pump must overcome the resisting hydrostatic head of pressure due to the difference in density of solvent and aqueous layers, and must also provide enough additional pressure to create a rapid rate of flow of solvent through the connecting tubes. The first resistance is a function of the differences in density between solvent and aqueous column. Thus, to force ether through 10 inches of water, the pump must furnish a minumim head of 4 inches of pressure before any ether will flow. In the apparatus shown in

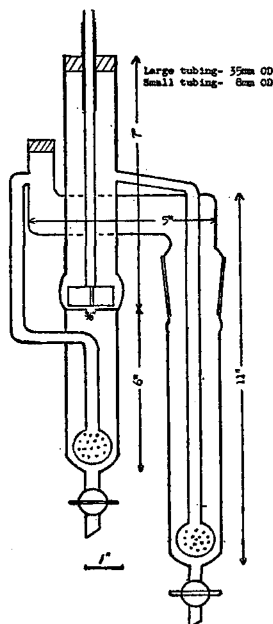

FIG. 2. Recommended Analytical Extractor

Figure 2, which has a total volume of 500 ml, the pump chamber has a height of 7 inches which allows it to force ether quite rapidly through a combined aqueous layer of up to 10 inches.

The solvent should pass through the aqueous layers in small droplets in order to achieve rapid exchange of dissolved matter, so some form of distributor is desirable where the solvent enters the aqueous portions. A satisfactory distributor may be made by blowing a small glass bulb and then blowing small holes in this bulb. Sintered glass discs offer too much resistance to the flow of liquid and are not satisfactory. A much better distributor can be made by sealing a porcelain Witte plate into Pyrex tubing, bent so that the droplets escape directly up into the solution. For solvents like benzene, whose droplets tend to coalesce when rising through water, a

platinum filter cone may be sealed into Pyrex tubing in place of the Witte plate.

There is a tendency for emulsions to form, so there must be a volume of solvent above both sample and absorbing solution to permit the breaking of such emulsions.

The outlet from the stirrer chamber should be about half way up the side of the chamber and approximately at the same level as outlet tube from vessel containing sample. This makes it possible to fill the sample

TABLE 1.—*Experimental results*

SAMPLE SOLUTION	ABSORBING SOLUTION	EXTRACTION TIME	RECOVERY
			Per cent
.1240 gm ephedrine hydrochloride in 100 ml of 1% NaOH, 1% NaCl solution.	25 ml of .2N · HCl	20 Min.	98.5
.1587 gm ephedrine hydrochloride in 150 ml of 1% NaOH, 1% NaCl solution.	25 ml of .2N · HCl	20 Min.	99.0
.1005 gm sodium oxalate in 100 ml of .2N · H_2SO_4.	25 ml of 2% $NaHCO_3$	90 Min.	99.0
.1005 gm sodium oxalate in 100 ml of .1N · H_2SO_4.	25 ml of 2% $NaHCO_3$	105 Min.	99
.129 gm citric acid in 150 ml of .1N · H_2SO_4.	40 ml of 2% $NaHCO_3$	2 Hours	25
.129 gm citric acid in 150 ml of .1N · H_2SO_4	40 ml of 2% $NaHCO_3$	4 Hours	40
.025 gm monochloracetic acid in 175 ml of 1N · H_2SO_4	30 ml of 1N NaOH	15 Min.	95
.025 gm monochloracetic-acid in 175 ml of 1N · H_2SO_4.	30 ml of 1N NaOH	15 Min.	95

vessel right up to its stopper when the stirrer chamber is half full, and yet allows emptying of the absorbing compartment without siphoning sample solution over.

The stirrrer chamber should be expanded into a slight bulge just above the partition which separates it from the absorbing compartment. This permits the four-bladed stirrer to rotate freely without striking the side-wall.

The apparatus is made in two pieces with a ground glass joint to permit use of various sizes of sample solution containers, and also to permit ease in cleaning apparatus. Each compartment is provided with a stop-cock to facilitate emptying.

The connecting tubing is made of relatively large diameter to decrease

resistance to flow and at the same time strengthen the apparatus.

The stirrer may be turned by any suitable device. A simple air-turbine, and a small, rheostat-operated fan motor have both been used. The cork stopper through which the bearing sleeve passes should have a small hole cut through it so that atmospheric pressure is maintained in the stirrer chamber. The well-fitting bearing may be lubricated with a few drops of heavy mineral oil.

EXPERIMENTAL

The apparatus was used to extract ephedrine, oxalic acid, citric acid, and monochloracetic acid, washed ether being employed as the solvent in each case. Results are shown in Table 1. In making these extractions, a stirrer speed of about 1500 r.p.m. was used, giving a flow of about 200 ml of solvent per minute.

LARGE VOLUME EXTRACTOR

The apparatus shown in Figure 3 was made to extract from a large container such as a 20 liter bottle. The stirrer compartment, absorbing solution compartment, and connecting tubing are separate pieces. The dropping funnel in the bottle is added to facilitate charging. This apparatus was used by the authors in making extractions from a 23 liter bottle (20 liters sample solution). The motion of the solvent droplets through the sample solution is usually enough to keep it well agitated.

A variation of this apparatus may be used to extract from a large open cylinder. The tubing leading from the stirrer chamber into the cylinder is bent to form an inverted U, allowing the solvent in the cylinder and and stirrer chamber to be at the same level.

Emulsions often form and the usual methods of breaking them should be tried. It has been our experience that a small motor or air-driven stirrer placed just above the sample solution, in the solvent, will break certain types of emulsions. If particles of emulsion are unavoidably carried into the absorbing solution, a further extraction from the absorbing solution (made acidic or basic as required) can be made using a smaller extractor, after the extraction from the original sample has been completed.

Using 20 liters of acidified water containing 22.9 mg of monochloracetic acid, 54.5% of the chloracetic acid was extracted after 2 hours operation. Using 2 liters of beer to which was added 176 mg monochloracetic acid, recovery was 100% after 1 hour of operation. The rate of circulation was approximately 500 ml of solvent per minute.

DISCUSSION

The apparatus as described has been tested with ether as the solvent and has been found to be a useful analytical tool. The large scale extractor should find use in extracting substances of biological origin which occur in small concentrations.

Experiments with various solvents have shown that there should exist a considerable difference in specific gravity, approximately .3 unit, between solvent and sample solution so that the two liquids may separate cleanly before the solvent enters the next compartment.

A considerable difficulty encountered is due to the formation of trouble-

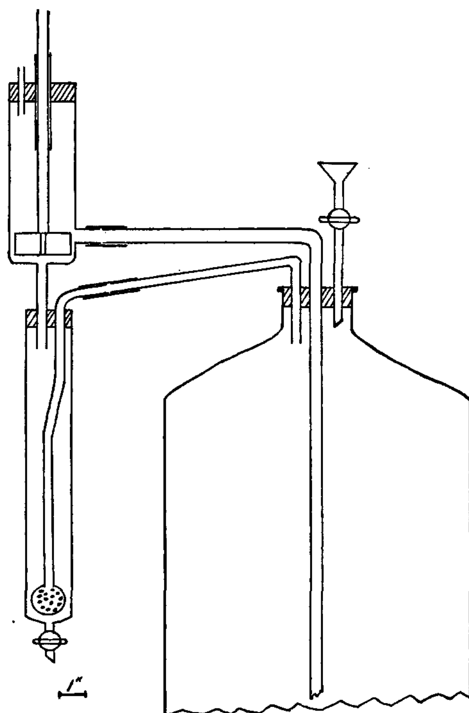

FIG. 3. Extraction Apparatus for Large Volumes

some emulsions which tend to separate slowly. Each such case presents its own problem and various expedients must be tried. A fine "spray" of sample solution droplets often forms in the solvent phase and is carried around by the circulating solvent, but ordinarily the effect of this is negligible.

Large variations in stirring speed during operations are undesirable and should be prevented.

The stirrer pump may be employed in a continuous extractor using solvents heavier than the sample solution, and the authors hope to describe such an extractor at a future date.

LITERATURE CITED

(1) PALKIN, S., MURRAY, A. G., and WATKINS, H. R., *Ind. Eng. Chem.*, **17**, 612 (1925).
(2) PUCHER, G. W., and VICKERY, H. B., *Ind. Eng. Chem., Anal. Ed.*, **11**, 656 (1939).
(3) WIDMARK, E. M. P., *Skand, Arch. Physiol.*, **48**, 61 (1926).

THE DETERMINATION AND IDENTIFICATION OF LACTIC AND SUCCINIC ACIDS IN FOODS*

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Numerous methods have been described for the determination of lactic acid, but practically all are not strictly specific, being based upon either the conversion to acetaldehyde (1, 2, 3, 4) or the color reaction with ferric chloride (5, 6). Previous methods for determining succinic acid in foods have depended for the most part upon ether extraction, followed by purification of the succinic acid by oxidation of contaminants (7). More recently, some excellent enzymatic methods have appeared (8, 9). Some of the above methods for lactic and succinic acids are very accurate and reliable for most purposes, but methods are needed by which these acids may be determined and *identified*, and that is the purpose for which the methods described below were developed. They furnish a means by which lactic and succinic acids may be quantitatively isolated and definitely identified in each sample analyzed.

The excellent specificity of the technique termed partition chromatography has been demonstrated in the analysis of the lower volatile fatty acids (10). The desirability of extending a method with such specificity to the separation of other acids which occur in food products, is obvious. A procedure utilizing this principle for separating a variety of polycarboxylic acids, including succinic acid, has already been used (11). Dilute sulfuric acid was used for the immobile solvent on silica gel and n-butanol-chloroform for the mobile solvent. Such a column required the use of an external indicator. Our experience, however, has demonstrated the convenience of an internal indicator on the partition column.

The method described below consists of five steps: (a) ether extraction (b) precipitation of the barium succinate from 80% alcohol, (c) isolation of lactic acid from the filtrate of (b), and of succinic acid from the insoluble fraction of (b) by partition chromatography, (d) titration of the acids with barium hydroxide, and (e) identification of the acids by microscopic examination of the barium salt of succinic acid or the zinc salt of lactic acid.

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‡ Contribution from the Food Division, W. B. White, Chief.

With modifications in the preparation of the sample, and separation of the acids from the sample in a form suitable for passage through the column, these procedures should be applicable to any food product; however, the procedures below are given for the determination of lactic acid in liquid or dried milks, and for the determination of succinic acid in liquid, frozen, or dried eggs.

METHODS

APPARATUS

Continuous extractor.—*Methods of Analysis, A.O.A.C.*, 1945, sec. **22.8** (p. 304).

REAGENTS

(a) *Solvent.*—20% tertiary butanol in chloroform V/V.

(b) *Glycerol indicator soln.*—150 mg of Alphamine Red-R dissolved in 100 ml of U.S.P. glycerol. This indicator is the ammonium salt of 3, 6 disulfo-beta-naphthalene-azo-N-phenyl-alpha-naphthylamine, also called $R\text{–}NH_4$ indicator, and is prepared according to Liddell and Rydon (12).

(c) *Dilute sulfuric acid* (1+1).

(d) *Concentrated sodium hydroxide soln.*—ca 40%.

(e) *Standard barium hydroxide soln.*—0.05 *N*.

(f) *Phenolphthalein indicator.*—1% in alcohol.

(g) *Silicic acid*[1]

(h) *Ammonium hydroxide.*—ca Normal.

(i) *Phosphotungstic acid soln.*—20%

(j) *Sulfuric acid.*—ca Normal.

(k) *Zinc sulfate.*—0.05 *N*.

(l) *Ammonium sulfate.*

(m) *Phenol red indicator.*—Rub 100 mg of phenol red in a mortar with 5.7 ml of 0.05 *N* NaOH until dissolved, then add sufficient H_2O to make the volume 100 ml.

Lactic Acid in Milk

PREPARATION OF SAMPLE[2]

(a) *Liquid, whole, or skim milks.*—Weigh 50 g into a 100 ml volumetric flask.

(b) *Dried whole or skim milks.*—Weigh 5 g into a 100 ml beaker and make into a smooth paste with small amount of water. Transfer contents of beaker to a 100 ml volumetric flask with about 50 ml of water.

To the mixtures add 6 ml of normal H_2SO_4 and mix, avoiding vigorous agitation. Add 5 ml of 20% phosphotungstic acid soln and make to mark with water. Shake and filter thru a folded filter paper.

PREPARATION OF SODIUM SALT OF LACTIC ACID[2]

Place 50 ml of filtrate in a continuous extractor, add 0.5 ml of 1+1 H_2SO_4 and extract with ether 3–4 hours. To the contents of the extraction flask add 20 ml of H_2O and evaporate the ether on the steam bath. Add one drop of phenolphthalein soln and make alkaline by dropwise addition of saturated $Ba(OH)_2$ soln. Transfer to a 110 ml volumetric flask, using about 70 ml of alcohol for the transfer. Heat almost to boiling on the steam bath, cool, and make to volume with alcohol. Filter thru a

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[1] Mallinckrodt's AR precipitated powder was used in this work.

[2] The preparation of sample, ether extraction, and precipitation of barium salts are described in *Methods of Analysis* (1945) secs. **22.8**, **22.12**, and **22.13**, respectively. They are repeated here for convenience only.

folded filter paper into a 250 ml beaker. Evaporate the filtrate to dryness on the steam bath. Add 20 ml of water and 3 or 4 drops of 1:1 H_2SO_4, stir well, and filter. Wash the beaker and filter with two 5 ml portions of water. Make the filtrate alkaline to phenolphthalein by dropwise addition of 40% NaOH. Evaporate to dryness.

PREPARATION OF THE PARTITION COLUMN

Place 20 g of silicic acid[3] in a mortar, add 5 ml glycerol indicator soln, 3.7 ml of H_2O and *N* NH_4OH, dropwise, sufficient to produce alkaline color to the indicator (1–3 drops). Mix, using a spatula to break up lumps. With pestle grind into a uniform powder. Make a slurry with 50 ml of 20% butanol-$CHCl_3$ solvent, and transfer with aid of a beaker to a glass tube 25×200 mm, one end of which has been constricted to an outlet 3 cm long and 5–6 mm in diam., plugged at the constriction with glass wool, and clamped in an upright position. Apply air pressure (3–5 lbs.) to the large end, forcing the excess solvent dropwise out of the small end (ca 20 ml). Release pressure as soon as the liquid disappears at the top of the gel. (Otherwise gel will dry and crack, becoming useless.) Add 1 ml of $CHCl_3$ containing 20 mg of acetic acid, and 2 ml of butanol-$CHCl_3$ solvent containing 20 mg of lactic acid. Apply pressure until the surface of the solvent just disappears into the gel. Fill the tube with solvent, place a 100 ml graduated cylinder beneath the outlet and apply pressure. Collect percolate until the lower edge of the blue band, second from the outlet end, reaches the constriction of the tube. The volume collected is the threshold volume (s) for lactic acid. (The first blue band to leave the column is the acetic acid.)

ISOLATION OF LACTIC ACID

To the dry residue of sodium lactate in the 50 ml beaker, add 2 ml of butanol-$CHCl_3$ solvent, 5 or 6 drops H_2SO_4 (1+1), mix well with glass rod, breaking up all lumps, and stir in 1 g of anhydrous Na_2SO_4.

Prepare a new partition column from the same batch of silicic acid, proceeding as directed under "Preparation of the Partition Column" above, down to and including the addition of the 1 ml of $CHCl_3$ containing 20 mg of acetic acid. Place a 100 ml graduated cylinder beneath the outlet of the tube. With a pipet, transfer the solvent containing the lactic acid to the column and apply pressure until the liquid disappears into the gel. Wash the beaker with three successive 1 ml portions of solvent, transferring to the column with the same pipet, and applying pressure each time until the liquid disappears. Fill the tube with solvent and apply pressure. A light placed behind, but not so close as to heat, the tube increases the visibility of the bands because of the translucency of the gel formed by glycerol and silicic acid.

Collect percolate until the bottom edge of the second band reaches the constriction of the tube, and discard. Place a 50 ml graduated cylinder beneath the outlet, and collect percolate until the second blue band has passed out of the column (not less than 35 ml).

With 2 mg of lactic acid or less, the second blue band may not be distinct enough to be visible. In such case remove and discard the percolate equal in volume to the threshold volume (s) for lactic acid established for the silicic acid being used. Place a 50 ml graduated cylinder beneath the outlet and collect 35 ml of percolate.

Transfer the 35 ml of percolate to a 125 ml Erlenmeyer flask with 20 ml of H_2O and titrate with 0.05 *N* $Ba(OH)_2$ soln (phenol red indicator). One ml 0.05 *N* alkali =4.5 mg lactic acid.

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[3] The optimum amount of silicic acid, for the quantities of glycerol and water used above, may vary with different lots of silicic acid. *See* Ramsey & Patterson, *This Journal*, p. 142.

IDENTIFICATION OF LACTIC ACID

Separate the aqueous layer in the titration flask. Add 0.05 *N* zinc sulfate equivalent to the $Ba(OH)_2$ used in the titration. Heat to boiling and boil for 5 min., stir in a small amount of Norite and filter. Evaporate to dryness on the steam bath. Add 10 ml of acetone, heat to boiling and decant. Repeat heating and decantation once more with acetone, and then once with alcohol. Add a small volume of water, and filter if the solution is not clear. Evaporate on the steam bath to a concentration of 5 mg of lactic acid per ml. Place a few drops of this solution on a microscope slide, allow the water to evaporate at room temperature and observe the crystals of zinc lactate. For amounts of lactic acid less than 5 mg, leave the solution in the beaker and allow the water to evaporate at room temperature. Place the beaker under the microscope and examine the crystals. Compare the crystals with those from a known solution of pure zinc lactate.

Succinic Acid in Egg Products

PREPARATION OF SAMPLE

(a) *Liquid eggs.*—Transfer 200 g to a tared 1500 ml beaker. With constant stirring, add 500 ml of H_2O, 75 ml of *N* H_2SO_4, and 125 ml of 20% phosphotungstic acid soln. Make contents to 1000 g by addition of H_2O, stir thoroly, and filter thru a folded filter paper.

(b) *Dried eggs.*—Weigh 50 g into a tared 1500 ml beaker and stir into a uniform paste with 100 ml of H_2O. With continuous stirring, add 600 ml of H_2O, 50 ml of *N* H_2SO_4 and 75 ml of 20% phosphotungstic acid soln. Make contents to 1000 g with H_2O, stir thoroly, and filter thru a folded filter paper.

PREPARATION OF SODIUM SALT OF SUCCINIC ACID

Evaporate 500 g of the filtrate in a 1000 ml beaker on a hot plate to ca 75 ml, and concentrate on a steam bath to ca 20 ml. Transfer to the continuous extractor by aid of 10 ml of H_2O. Add 23 g of $(NH_4)_2SO_4$ and 1 ml $H_2SO_4(1+1)$ to the soln in the extractor. Rinse the beaker with ether into the extractor, and extract with ether for $2\frac{1}{2}$ hours.

Add 20 ml of H_2O to the extraction flask and expel the ether on steam bath. Neutralize with saturated $Ba(OH)_2$ soln (phenolphthalein indicator), transfer to 110 ml volumetric flask with alcohol until volume is ca 90 ml. Heat almost to boiling on steam bath, cool, complete volume with alcohol and filter thru folded $12\frac{1}{2}$ cm filter paper. Place the paper and precipitate in a 50 ml beaker, add 20 ml of H_2O, 3 or 4 drops of $H_2SO_4(1+1)$, heat on steam bath ca 5 min., and filter. Wash the beaker and its paper with 3 successive 8 ml portions of H_2O, pour thru the filter, collecting filtrate and washings in a 50 ml beaker. Neutralize the filtrate with 40% NaOH soln (phenolphthalein indicator) and evaporate to dryness on steam bath.

ISOLATION OF SUCCINIC ACID

Prepare a partition column as described for lactic acid, using a soln of 20 mg of succinic acid in 2 ml of butanol-$CHCl_3$ solvent instead of the lactic acid soln to determine the threshold volume for succinic acid. Prepare another column, acidify the sodium succinate, and put the soln thru the partition column, in the same manner as described for "Isolation of Lactic Acid." Transfer the 35 ml percolate to a 150 ml Erlenmeyer flask with 20 ml of H_2O and titrate with 0.05 *N* $Ba(OH)_2$ soln (phenol red indicator). One ml N/20 alkali = 2.95 mg succinic acid.

IDENTIFICATION OF SUCCINIC ACID

Separate the aqueous soln in the titration flask, transfer to a 50 ml beaker,

evaporate to about 3 ml on the steam bath, and then allow to evaporate at room temperature until crystals appear. Compare the crystals with known barium succinate prepared by titrating about 25 mg of pure succinic acid with the $Ba(OH)_2$ soln, adding 3 or 4 drops of glycerol, and evaporating as directed above.

DISCUSSION

Methods for the determination of lactic and succinic acids in which partition chromatography is an essential step, have been developed. When lactic acid was added to samples of good powdered milk, which were shown to contain no lactic acid as measured by this procedure, the recoveries as shown in Table 1, range from 97 to 99 per cent. In order to determine if any lactic acid can be detected in fresh milk by this method, the acids extracted from 250 grams of milk were put on a column. That portion of the percolate which would have contained any lactic acid present gave a titration equivalent to 0.9 mg of lactic acid; but no crystals of the zinc salt could be identified by microscopic examination.

TABLE 1.—*Recovery of lactic acid added to dried whole milk (5 grams)*

LACTIC ACID ADDED	LACTIC ACID FOUND	RECOVERY
mg	*mg*	*Per cent*
6.5	6.5	100
13.0	12.7	97
26.0	25.6	98
39.0	38.6	99
52.0	51.0	98

When succinic acid was added to liquid and dried eggs, the recoveries, as shown in Table 2, were in the range 86 to 95 per cent No succinic acid was found in these eggs when they were analyzed by the same procedure.

When a mixture of α-hydroxybutyric, β-hydroxybutyric, lactic and glycolic acids was placed on this partition column, they separated and came off in the order named. The separation of β-hydroxybutyric and lactic acids was not complete; however, they did separate into two bands which would make a mixture of the two acids easily detectable.

When a mixture of acetic, formic, and lactic acids was placed on the column the acids came off in the order named. They separated into well-

TABLE 2.—*Recovery of succinic acid added to liquid and dried eggs*

PRODUCT	WT. SAMPLE	SUCCINIC ACID ADDED	RECOVERY
	grams	*mg*	*Per cent*
Dried eggs	50	29.5	87, 86
Liquid eggs	200	50.0	88
Liquid eggs	200	2.0	93
Liquid eggs	200	4.0	95

defined bands which makes complete separation possible. If these acids are placed on the column with α-hydroxybutyric and β-hydroxybutyric acids, the α-hydroxybutyric and acetic acids come off together, and the β-hydroxybutyric and formic acids come off together. Citric and malic acids cause no interference, since citric acid remains at the top of the column, and malic moves so slowly that it is hardly possible to wash it off at all. Succinic acid moves down the column at a rate slightly faster than lactic acid, but the difference in the threshold volumes of the two acids is not great enough for complete separation. These two acids may be separated by precipitation of the barium salts in 80 per cent alcohol; the precipitate may then be used for the determination of succinic, and the filtrate for the determination of lactic acid.

BIBLIOGRAPHY

(1) Friedmann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, **73**, 335 (1927).
(2) Troy, H. C., and Sharp, P. F., *Cornell Agri. Exp. Sta.*, Memoir 179–228 (1935)
(3) Mendel, B., and Goldscheider, I., *Biochem. Z.*, **164**, 163 (1925).
(4) Barker, S. B., and Summerson, W. H., *J. Biol. Chem.* **138**, 535 (1941).
(5) Hillig, F., *This Journal*, **20**, 130 (1937).
(6) Hillig, F., *Ibid.*, **25**, 253 (1942).
(7) Winton, A. L., and Winton, K. B., "The Analysis of Foods," pp. 219, 674 (1945).
(8) Weil-Malherbe, H., *Biochem. J.*, **31**, 299 (1937).
(9) Krebs, H., *Ibid.*, **31**, 2095 (1937).
(10) Ramsey, L. L., and Patterson, W. I., *This Journal*, **28**, 644 (1945).
(11) Isherwood, F. A., *Biochem. J.*, **40**, 688 (1946).
(12) Liddell, H. F., and Rydon, H. N., *Ibid.*, **38**, 68 (1944).

SEPARATION AND DETERMINATION OF THE STRAIGHT-CHAIN SATURATED FATTY ACIDS C_5 TO C_{10} BY PARTITION CHROMATOGRAPHY*

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The straight-chain saturated fatty acids C_5 to C_{10} can be separated and determined by the classical and time-honored method of distillation: steam distillation plus direct fractional distillation of the readily volatile acids, and then fractional distillation of the methyl or ethyl esters of the less volatile acids. (1) While refinements in apparatus and improvements in procedure have increased somewhat the reliability of this method, fractional distillation often does not produce sharp separations, is time con-

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suming, and requires a rather large sample. Therefore, it is obvious that there is a need for a rapid routine method for the analysis of these fatty acids. There are innumerable potential uses of such a method in research on fermentation, food decomposition, the composition of certain fats, and the composition of mixed esters and flavors, natural or synthetic.

The method presented in this paper for the separation and determination of the straight-chain fatty acids C_5 to C_{10} is a modification of the chromatographic partition method previously proposed for the lower volatile fatty acids C_1 to C_4 (2, 3). Whereas the fatty acids above butyric are only slightly soluble in water, their solubility in methanol is appreciable, and they are also soluble in aliphatic hydrocarbons such as isooctane. These two solvents were found to be satisfactory for use as the immiscible solvents in separating the fatty acids C_5 to C_{10} by partition chromatography. The silicic acid is almost saturated with methanol containing bromocresol green as the indicator, slurried with isooctane, poured into a suitable tube of the type normally used in chromatography, and allowed to pack down under pressure. Other hydrocarbons such as n-hexane and cyclohexane can be used in place of the isooctane but the bands on the column are less distinct with these solvents. When an isooctane solution containing the straight-chain acids: valeric, caproic, enanthic, caprylic, pelargonic, and capric is passed through this column, a separation into six well defined bands is obtained, analogous to that for the lower volatile fatty acids (2). The valeric acid band is found at the top of the column, the capric acid band at the bottom, and the others in order between these two. If as many as three 5-carbon acids (n-valeric, isovaleric, and α-methylbutyric), and three 6-carbon acids (n-caproic, isocaproic, and β-methylvaleric) are all present in a mixture, each group of acids with the same number of carbon atoms behaves as a single acid, *i.e.*, each group forms a single band on the column. Thus, the method presented here will separate a mixture containing only the straight chain saturated fatty acids C_5 to C_{10}.

After the chromatographic separation of the straight-chain fatty acids C_5 to C_{10}, the individual fatty acid in each band can be tentatively identified by its threshold volume (4). The threshold volume for a given compound is an empirical figure which may be defined as the volume of percolate collected under specified conditions while the compound is moving from the top of the column to the point of egress at the bottom. The threshold volumes for the fatty acids C_5 to C_{10} are quite reproducible, and characteristic of the individual acids. Therefore, it is feasible, using pure acids, to standardize the column with respect to the threshold volumes of the acids. The identification of a fatty acid can be confirmed by admixture with an approximately equal amount of an authentic sample of the suspected acid, followed by passage of this admixture through a column of suitable size prepared in the same way. The formation of only one band then indicates that the isolated acid is identical with the authentic speci-

men, or at least is very closely related; *i.e.*, it very probably contains the same number of carbon atoms.

No acids except the branched-chain acids C_5 to C_{10}, and possibly the unsaturated acids C_5 to C_{10}, in the fatty acid series are known or expected to interfere in the method. The behavior of the unsaturated fatty acids C_5 to C_{10} on the column, however, was not investigated. Acids such as oxalic, succinic, malic, citric, and tartaric, if placed on the column, remain practically stationary at the top since they are almost insoluble in isooctane. Homologs in the fatty acid series below valeric and above capric do not interfere. Formic, acetic, propionic and n-butyric acids move much more slowly on the column than valeric; and by the time the valeric acid has moved completely through the column these acids will have separated into three bands, a n-butyric band, a propionic band, and a band containing both acetic and formic acid. The separation of the valeric and n-butyric acids appears to be quite complete. Hendecanoic (undecylic), lauric, and myristic acid, when present to the extent of about 10 mg each, will separate into visible bands, but the bands are so close together that the separations are not complete. However, the separation of capric from undecylic appears to be fairly complete, and the separation of capric from lauric quite complete. Acids higher than myristic do not interfere in the method, but when present in great excess as compared with the acids C_5 to C_{10}, it is necessary to collect the C_5 to C_{10} acids as a single group, and then to refractionate on a fresh column. The indicator appears to become quite insensitive to small amounts of acid after a large amount of acid has passed through the column.

How pure the acid in each eluted band may be is difficult to establish on a few milligrams by the usual criteria of purity. It requires about 2 milligrams of acid (about 2 for capric and somewhat less for valeric) to make a visible band on a column 1 inch in diameter and containing 20 grams of gel; for a 2 gram column in a smaller tube only about 0.2 milligram is required. Thus, on the larger column 2 milligrams of residual acid might remain unnoticed after passage of the visible band. When a single acid is passed through the column, the volume of eluate necessary to recover all of the added acid is a little larger than the volume apparently required to elute the same acid from a mixture. Whether, in a mixture, a following band tends to push the one in front of it through faster (in a smaller volume) than the front band would move, if alone, or whether the following band tends to engulf the tail of the one in front of it, is a difficult question to answer. However, when a mixture of the six straight-chain acids C_5 to C_{10} was separated by partition chromatography and each of the separated acids refractionated on a fresh column, each acid was found to contain about 5% of the acid which immediately preceded it on the column. To test more accurately the completeness of separation of any given acid in the C_5 to C_{10} group from its homologs by two or more

passages through a partition column, chromatography of a mixture of the acid with a synthetic sample of each homolog containing a radioactive atom as a tracer, could be employed.

The working range of the method presented here is 2 to 20 milligrams of each acid in the solution transferred to the column. When only even numbered carbon acids, or only odd numbered carbon acids, are being separated, the capacity of the column is appreciably greater. A column larger than the one used in this analytical method would, of course, have a correspondingly greater capacity. It would be practical to use a column large enough for isolating and purifying several grams of the fatty acids.

Preliminary experiments indicate that the proposed method will be useful in studies on fermentation, decomposition of foods, the composition of certain fats, and the composition of ester-containing flavors, both natural and synthetic; and that the method will also be useful in checking the purity of fatty acids, and possibly in routine control work in connection with the manufacture of the fatty acids.

METHOD

APPARATUS

(1) *Chromatographic tubes.*
- (a) Large size: 27 mm O.D.×200 mm long prepared from Pyrex tubing, or from Pyrex test tubes by sealing into the bottom of each a piece of glass tubing 6 mm O.D.×25 mm long.
- (b) Small size: 18 mm O.D.×250 mm long prepared from 18 mm O.D. Pyrex tubing.
- (c) Any other suitable container.

(2) Suitable pressure source such as compressed air or a cylinder of nitrogen, and a means of keeping the pressure constant such as a column of mercury or a diaphragm type pressure regulator.

REAGENTS

1. *Silicic acid.*[a]
2. *Methanol.*
3. *Isooctane (2, 2, 4-trimethylpentane*[b] *solvent.*—If not free of acid, wash with alkali, dry, and distil. To ca one l. of isooctane add 20 ml of 90% methanol, shake vigorously, allow to separate, and discard the methanol layer.
4. *Bromocresol green solution.*—Dissolve 200 mg of the dye in 25 ml of methanol.
5. *Phenolphthalein soln.*—Dissolve 1 g of phenolphthalein in 100 ml of absolute alcohol and neutralize with sodium ethylate.
6. *Ammonium hydroxide solution,* ca *N.*
7. *Sodium ethylate, standard 0.02 N solution.*—Prepare 0.02 *N* sodium ethylate using aldehyde-free absolute alcohol (5), by the usual procedure (6).
8. *Straight chain fatty acids.*—Valeric, caproic, enanthic, caprylic, pelargonic, capric, and lauric.

PROCEDURE

(1) *Determination of the optimum ratio of methanol to silicic acid:* To 20 g of the sample of silicic acid in a mortar, add 10 ml of methanol and mix well with a pestle.

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[a] Mallinckrodt's Analytical Reagent grade precipitated powder used in this work.
[b] Pure grade product of Phillips Petroleum Company used in this work.

Continue the addition of methanol stepwise in 2 ml portions, mixing well after each addition, until the silicic acid is gummy and sticky; at this point it will not produce a smooth slurry with isooctane. The optimum amount of methanol to be added to the silicic acid usually lies from 2 to 5 ml below this point and must be determined by actually preparing a slurry with the isooctane, transferring the slurry to a chromatographic tube, and observing whether the gel packs down quite firmly under pressure without cracking or drying out.

(2) *Preparation of the chromatographic partition column:* To 1 ml of the bromocresol green soln, add sufficient methanol to give the optimum amount as determined in (1) above and add enough of the ammonium hydroxide soln (1 to 3 drops is usually sufficient) to give the alkaline color of the indicator. Add the methanol soln to 20 g of silicic acid in a mortar and mix thoroughly with a pestle. If the silicic acid is not alkaline enough to be pale bluish-green in color, add more of the ammonium hydroxide solution dropwise, mixing thoroly after each addition, until this color is obtained. Add ca 60 ml of the isooctane and make a slurry. Place a very small cotton plug firmly in the neck of the constriction at the bottom of the large size chromatographic tube, and clamp the tube in an upright position. Pour the slurry into the tube, preferably by transferring from the mortar to a 100 ml beaker and pouring from the beaker into the tube; connect the top to a suitable pressure source through the gas pressure regulator; and apply 2 to 5 pounds of pressure. During the packing down process, tap the tube from time to time to aid in obtaining a smooth, level surface. Release the pressure when the silicic acid has packed down firmly, but before the column begins to dry out, crack, or separate from the walls of the tube. (If the column begins to dry out before the acids are added, remove the gel from the tube, slurry again with the isooctane, retransfer to the tube, and repack.)

(3) *Testing the silicic acid for its suitability and standardization of the chromatographic column:* To neutralize the slight but unavoidable excess of ammonium hydroxide on the column before use, place 2 ml of isooctane containing ca 10 mg of lauric acid on the column, adding carefully from a pipet so that the top surface of the column is not disturbed. Connect to the pressure source and apply pressure. At the instant all the solution has sunk into the gel, release the pressure, disconnect, and add 1 ml of isooctane. Renew the pressure until this wash solvent has sunk into the gel, and repeat the washing again using 1 ml of the isooctane. Pass ca 15 ml of additional isooctane through the column, stopping when all of the solvent has sunk into the gel. The column is now ready for use. Place a graduated cylinder under the column to collect the forerun, including the lauric acid, and pipet onto the column 2 ml of a standard solution in isooctane, of fatty acids, which contains about 10 mg of each acid. Allow the solution to sink into the gel under pressure, and wash with two 1 ml portions of isooctane as above. Fill up the tube with solvent and adjust the pressure so that the rate of percolation is from 3 to 5 ml per minute (rate not critical). (A separatory funnel or other suitable container may be fitted in the chromatographic tube to serve as a reservoir for the solvent.) Change the receiver when the lower edge of a band reaches the constriction of the tube, disregarding the lauric acid band which is caught in the forerun. In general collect the whole band in one fraction, changing receivers when the following band reaches the constriction, and continue until all of the desired bands have passed. In case there is no following band, change receivers when the visible band has passed through the tube, collecting 5 ml fractions, and titrating each. Continue until the titration agrees with the blank value.

Record the volume of percolate for the forerun and for each band. Transfer each of the fractions to an Erlenmeyer flask, add a few drops of the phenolphthalein soln, and titrate with the standard sodium ethylate. Correct the titration for a blank in each case, using approximately the same quantity of isooctane as was used to elute the acid being titrated.

The first band of the mixture to elute is capric acid; the second, pelargonic; the third, caprylic, etc. The threshold volume for capric acid is the volume of the forerun (includes lauric acid band). The threshold volume for pelargonic acid is the forerun volume plus the volume of solvent required to elute the capric acid. Obtain threshold volumes of the remaining acids in a similar manner. These threshold volumes constitute the standardization of the column for 10 mg of each of the fatty acids under the specific conditions actually employed.

Consider the silicic acid suitable if, upon following the above procedure, the acid bands are clearly visible, well separated as determined by visual observation, and if the recoveries of the added acids are in the range 90–110%.

(4) *Determination and identification:* The preparation of the sample will vary from product to product. The first step in any event, however, will be the separation and concentration of the acids either by steam distillation or solvent extraction, or a combination of the two processes. Neutralize the distilled or extracted acids, concentrate to a small volume, and transfer to a test tube. Remove the water in a current of air at steam bath temperature, add 2 to 4 drops of $H_2SO_4(1+1)$ (enough to turn congo red paper to its acid color) and a few glass beads. Wet the solid salts by rotating the glass beads in the tube, using a long thin-bladed spatula to break up the crust or lumps if necessary. Extract the liberated acids with 2, 1, and 1 ml portions of the isooctane; and transfer each portion to the column with an eyedropper pipet, allowing the solvent to sink into the gel each time before the next portion is added.

Collect the fractions, determine the threshold volume of each acid band, and titrate as in (3) above.* Compare the threshold volume of the unknown acid in each case with approximately the same quantity of authentic acid (see (3) above), and thus make a tentative identification of the unknown acids.

To confirm this tentative identification add ca 5 ml of water to the neutralized isooctane solution of each unknown acid, evaporate the isooctane on the steam bath, transfer to a small test tube and continue evaporation to dryness. However, when the volume of isooctane solution is 50 ml or greater, extract the sodium salt from the solvent by shaking with three small successive portions of water in a separatory funnel and drawing off each portion into a small beaker. Concentrate the aqueous solution to ca 5 ml and transfer to a test tube and evaporate as above. Convert the sodium salt of each acid to a solution of the free acid in isooctane as described above, and pipet an aliquot of ca 1 ml, containing ca 2ml of 0.02 N acid (4 to 7 mg of the acid), onto a freshly prepared column. Pipet an approximately equal amount of the authentic acid, in not more than 1 ml, onto the column; gently swirl to mix; and proceed to develop the chromatogram. If only one band is obtained, the tentative identification is confirmed. Report the results in mg of acid found.

Table 1 shows the recoveries of the fatty acids when present in admixture in the range 2 to 20 mg each, 5 different mixtures being analyzed, 2 of them in duplicate. As can be seen, recoveries are generally within ± 5% except mixture "E," where the recoveries on 2 mg quantities are high. No corrections have been made for homologs known to be present as impurities in the acids used, but in no case in this table would the results be appreciably affected by such slight impurities.

Table 1 also shows the threshold volumes obtained in each experiment for

* Where results of the highest degree of accuracy are required, each of the separated acids should be steam distilled and the distillates titrated with standard aqueous alkali (CO_2 free), using phenolphthalein as the indicator. Results reported in this paper, however, were obtained by omitting the steam distillation step.

EXPERIMENTAL RESULTS AND DISCUSSION

TABLE 1.—*Recoveries of the acids C_5 to C_{10} when present in admixture.*

ACID	ADDED	FOUND	RECOVERY	THRESHOLD VOLUME
Name	*mg*	*mg*	*per cent*	*ml*
		Mixture A		
Capric	2.03	1.96	97	47
Pelargonic	2.04	1.86	91	59
Caprylic	2.03	2.04	101	73
Enanthic	2.02	2.16	107	97
Caproic	2.00	1.88	94	130
Valeric	2.04	1.90	93	174
		Mixture B		
Capric	5.08	5.0	98	44
		5.2	102	43
Pelargonic	5.11	4.7	92	55
		5.2	102	55
Caprylic	5.08	5.1	100	70
		5.1	100	69
Enanthic	5.06	5.1	101	90
		5.1	101	90
Caproic	4.99	4.9	98	123
		5.3	106	122
Valeric	5.11	4.8	94	172
		5.0	98	173
		Mixture C		
Capric	10.17	9.5	93	42
		10.1	99	43
Pelargonic	10.22	10.0	98	53
		10.1	99	53
Caprylic	10.15	10.7	105	66
		10.8	106	66
Enanthic	10.12	9.3	92	88
		9.7	96	88
Caproic	9.98	9.5	95	177
		9.6	96	119
Valeric	10.22	9.7	95	167
		9.9	97	159
		Mixture D		
Capric	20.33	19.3	95	41
Pelargonic	20.43	20.2	99	51
Caprylic	20.30	20.4	100	63
Enanthic	20.23	20.6	102	83
Caproic	19.96	18.7	94	112
Valeric	20.43	20.6	101	152
		Mixture E		
Capric	9.91	9.5	96	47
Pelargonic	2.02	2.1	104	60
Caprylic	10.16	9.8	96	73
Enanthic	2.03	2.4	118	100
Caproic	10.05	9.5	95	127
Valeric	2.02	2.3	114	199

each acid. It will be noted that the threshold volume of an acid is definitely affected by the amount of such acid placed on the column; the larger the amount of acid the smaller the threshold volume, *i.e.*, large amounts of an acid travel faster on the column than small amounts of the same acid. In actual practice, then, the quantity of an acid must be taken into consideration in making the tentative indentification by this means.

The threshold volume of an acid is not appreciably affected by large amounts of the homologs of the acid. In carefully controlled experiments using the same amount of a given acid, the threshold volume varied as much as 10% ($\pm$ 4 ml for capric acid; $\pm$ 10 ml for valeric). The cause of these small variations was not definitely determined. However, it was found that the rate of percolation within rather wide limits, 2 to 6 ml per min., did not affect the threshold volume, and that the quantity of methanol within the range 10 to 20 ml per 20 g of silicic acid (using silicic acid from the same lot each time) was also without effect.

The color of the band formed by a definite quantity of capric acid, say 0.02 millimol, is not as strong as that formed by the same quantity of a lower acid. In fact the strength of the color contrast increases noticeably from capric to valeric acid. The fact that only the acid dissolved in the methanol can affect the color of the indicator, and that the distribution of the lower acids favors the methanol solvent, is probably the explanation of this behavior.

Completeness of Separation on the Column:

Since the acid bands widen and "tail out" as the chromatogram is developed, and since, when all six of the acids are present in the mixture being chromatographed, the bands which first elute are fairly close together, it appears that there is a possibility of contaminating a separated acid with its next higher homolog. However, the probability that a separated acid is also contaminated by its next lower homolog is not as great, since it has been found that the front of a visible band is sharp, permitting accurate fraction cuts to be made; and since, if the acid is pure, no acid elutes in front of this visible band.

An experiment was performed to determine the degree of contamination of acids separated by the proposed method, by their next higher homologs. A mixture consisting of ca 10 mg each of the six fatty acids C_5 to C_{10} was separated on a column. The separated acids, except capric and valeric, were refractionated individually on a fresh column, the percolate being collected in small fractions in the zone where the next higher acid, if present as a contaminant, would be found. It should be noted that even if the contamination of one acid with another in this case were as much as 10 per cent, the impurity would not show as a band, because 1 mg of an acid is not enough to produce a clearly visible band. Therefore, it is necessary to collect eluate fractions ahead of the main band in order to detect the presence of the next higher homolog. Results of this

experiment are tabulated below, in Table 2. In this experiment the identity of the homolog impurity was not confirmed by fractionation of an admixture with an authentic sample because the amount of impurity was quite small. However, the reliability of the procedure was tested by adding 1 mg of capric acid to 10 mg of pelargonic acid in one experiment and by adding 1 mg of caprylic acid to 10 mg of enanthic acid in another experiment. The recovery of the homolog impurity in both cases by the above procedure was ca 90 per cent.

TABLE 2.—*Degree of contamination of an acid separated by partition chromatography with its next higher homolog*

NAME OF ACID REFRACTIONATED	AMT. OF ACID	FRACTION COLLECTED	FRACTION NORMALLY CONTAINING NEXT HIGHER HOMOLOG*	HOMOLOG FOUND		CONTAMINANT
				NAME		
	ml 0.02 *N*	*ml*			*ml* 0.02 *N*	*per cent*
Pelargonic	2.90	51– 58	47– 59	Capric	0.15	5
		58– 60			nil	
Caprylic	3.55	63– 72	59– 73	Pelargonic	0.15	4
		72– 73			nil	
Enanthic	3.70	80– 92	73– 97	Caprylic	0.14	4
		92– 96			nil	
Caproic	4.09	105–125	97–130	Enanthic	0.19	5
		125–132			nil	

* From mixture A of Table 1.

Based on the recoveries in Table 1 it also appears that the contamination of one acid by another is generally not over 5 per cent, and may be less. Of course, when only the even numbered carbon acids, or only the odd numbered carbon acids, are separated at one time, the bands are farther apart, and the degree of contamination of one acid by another may be then of the order of 1 per cent. However, as pointed out above, the purity of an eluted fatty acid in the amounts used is extremely difficult to determine.

Table 3 shows the sensitivity of the method in detecting the homologs of a fatty acid added to such acid as impurities. In general, by one or more refractionations 0.5 to 1 per cent of an acid as a homolog impurity can be detected. It should be noted that although capric acid must contain about 20 per cent of pelargonic acid before it can be detected in a single passage through the column, suitable refractionation will detect as little as 1 per cent pelargonic in capric acid. The procedure used here is similar to that described below in detail with regard to the application of the method to some commercial products.

experiment are tabulated below, in Table 2. In this experiment the identity of the homolog impurity was not confirmed by fractionation of an admixture with an authentic sample because the amount of impurity was quite small. However, the reliability of the procedure was tested by adding 1 mg of capric acid to 10 mg of pelargonic acid in one experiment and by adding 1 mg of caprylic acid to 10 mg of enanthic acid in another experiment. The recovery of the homolog impurity in both cases by the above procedure was ca 90 per cent.

TABLE 2.—*Degree of contamination of an acid separated by partition chromatography with its next higher homolog*

NAME OF ACID REFRACTIONATED	AMT. OF ACID	FRACTION COLLECTED	FRACTION NORMALLY CONTAINING NEXT HIGHER HOMOLOG*	HOMOLOG FOUND		CONTAMINANT
				NAME		
	ml 0.02 *N*	*ml*			*ml* 0.02 *N*	*per cent*
Pelargonic	2.90	51– 58	47– 59	Capric	0.15	5
		58– 60			nil	
Caprylic	3.55	63– 72	59– 73	Pelargonic	0.15	4
		72– 73			nil	
Enanthic	3.70	80– 92	73– 97	Caprylic	0.14	4
		92– 96			nil	
Caproic	4.09	105–125	97–130	Enanthic	0.19	5
		125–132			nil	

* From mixture A of Table 1.

Based on the recoveries in Table 1 it also appears that the contamination of one acid by another is generally not over 5 per cent, and may be less. Of course, when only the even numbered carbon acids, or only the odd numbered carbon acids, are separated at one time, the bands are farther apart, and the degree of contamination of one acid by another may be then of the order of 1 per cent. However, as pointed out above, the purity of an eluted fatty acid in the amounts used is extremely difficult to determine.

Table 3 shows the sensitivity of the method in detecting the homologs of a fatty acid added to such acid as impurities. In general, by one or more refractionations 0.5 to 1 per cent of an acid as a homolog impurity can be detected. It should be noted that although capric acid must contain about 20 per cent of pelargonic acid before it can be detected in a single passage through the column, suitable refractionation will detect as little as 1 per cent pelargonic in capric acid. The procedure used here is similar to that described below in detail with regard to the application of the method to some commercial products.

mixtures were fractionated again and then identified. Fraction 3 containing the tailings of the acid in the main band plus its lower homologs, if any, was refractionated on a fresh column and the fractions measured and identified in a manner similar to that for fraction 1.

Silicic Acid:

The most critical requirement of this chromatographic method is a suitable silicic acid. Different lots of silicic acid from the same source vary in their chromatographic properties, and not all lots have been found entirely satisfactory. Silicic acid prepared in the laboratory from different lots of commercial waterglass by a definite procedure (3) also has not been entirely satisfactory. In Table 5 is a comparison of different samples of silicic acid. Samples 1 to 4 are different lots from the same source, number 1 being the batch of silicic acid used in this work unless otherwise noted. Samples 5 and 6 are the laboratory prepared products. It should be noted that silicic acid 2, which held the smallest quantity of methanol, gave the smallest threshold volume and would not separate the C_9 and C_{10} acids; on the other hand, silicic acid 6, which had the largest capacity for methanol, gave the largest threshold volume and caused the bands to move farthest apart.

TABLE 5.—*A comparison of different samples of silicic acid*

20 G SAMPLE OF SILICIC ACID	THRESHOLD VOLUME FOR 10 MG OF CAPRYLIC ACID	NOTES
	ml	
1	70	Silicic acid found to be most suitable.
2	32	Would not separate pelargonic from capric acid. Held only 12 ml of methanol instead of 20 ml on 20 g.
3	72	Usable but the acid bands were not as clear as 1.
4	74	Usable but the acid bands were not as clear as 1.
5	98	Acid bands not as clear as 1 but moved farther apart in development. Had greater capacity for methanol; used 30 ml.
6	116	Similar to 5. Had largest capacity for methanol; used 35 ml.

The laboratory-prepared gels were of a finer mesh, and the percolation rate was slower than that of the commercial gels under the same pressure. The percolation rate for a given gel is affected somewhat by the quantity of methanol added to the gel; the greater the amount added, the greater the percolation rate. However, as previously indicated, it was found that a range of methanol all the way from 10 to 20 ml per 20 g powder, in the

case of silicic acid number 1, did not affect the threshold volume of caprylic acid.

In brief then, experiments indicate that, in order to be suitable a silicic acid should give, under the conditions outlined in the method, a threshold volume of 60 to 80 ml for 10 mg of caprylic acid; should show clearly visible acid bands when 2 mg of each acid are used; and should be capable of taking up about 100 per cent of its weight of methanol without drying out of the column at the top or bottom during use. However, it appears that the only way to find out whether a silicic acid is suitable is to try it out using the procedure outlined above.

The requirement of a suitable silicic acid is not peculiar to this particular method. A silicic acid prepared according to one procedure (7) was not suitable for the separation of the organic acids of fruit, because this silicic acid was too highly "adsorptive." (8) The preparation of a rather weakly adsorptive silicic acid suitable for separating the fruit acids has been described. (8) However, attempts to duplicate this preparation in another laboratory were unsuccessful. (9)

SUMMARY

A method is presented, based on partition chromatography, for the separation and determination of the saturated straight-chain fatty acids C_6 to C_{10}.

The fatty acids are separated on a column of silicic acid, using methanol as the immobile solvent, 2, 2, 4-trimethylpentane as the mobile solvent, and bromocresol green as the indicator. The separated acids are titrated with standard sodium ethylate and tentatively identified by their threshold volumes; and the identification in each case is confirmed by adding an approximately equal amount of an authentic sample of the suspected acid and testing the chromatographic homogeneity of the mixture on a fresh column.

It appears that the method may be suitable for routine use in the study of fermentation, food decomposition, the composition of certain fats, and the composition of natural and synthetic flavors and esters.

BIBLIOGRAPHY

(1) Hilditch, T. P., "The Chemical Constitution of Natural Fats," p. 369, (1940).
(2) Ramsey, L. L., and Patterson, W. I., *This Journal*, **28**, 644 (1945).
(3) Elsden, S. R., *Biochem. J.*, **40**, 252 (1946).
(4) Weiss, J., *J. Chem. Soc.*, **1943**, 297.
(5) *Methods of Analysis, A.O.A.C.*, **31.24**, p. 497, sixth ed. (1945).
(6) *Ibid.*, **23.29(b)**, p. 354.
(7) Gordon, A. H., Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, **37**, 79 (1943).
(8) Isherwood, F. A., *Ibid.*, **40**, 688 (1946).
(9) Tristram, G. R., *Ibid.*, **40**, 721 (1946).

THE DETERMINATION OF FREE TRYPTOPHANE IN MILK, CREAM, AND BUTTER

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Milk contains abundant amounts of the three major components of food-proteins, fats, and carbohydrates; it also contains growth factors needed by many micro-organisms. Since milk does not contain any natural inhibitor of bacterial growth, microbiological activity begins immediately after milking.

Many different types of micro-organisms are commonly found in raw milk or cream. Some of these organisms produce lactic acid almost exclusively, which results in clean sour milk or cream when these micro-organisms are the predominant types present. This so-called "clean sour" milk or cream is preferred for certain uses. Other micro-organisms elaborate proteolytic enzymes which decompose the milk proteins. Still other micro-organisms produce lipolytic enzymes which attack the milk fat and cause rancidity. The inter-relationship of these types of organisms is dependent upon a number of highly variable factors, but under certain conditions they may develop concurrently in milk and cream. In the uncontrolled microbial decomposition of milk and other foods there is, of course, no sharp boundary where a desirable type of chemical action stops and another, and undesirable, one begins.

The chemical reactions involved in the microbial degradation of proteins to amino acids and the resulting changes in these acids are known in a general way (1, 2). The proteins are hydrolyzed by enzymatic activity into simpler substances: proteoses, peptones, amino acids, etc. Although many characteristics of the more complex products of protein hydrolysis, *e.g.*, peptones and polypeptides, are known, the chemical characteristics of the amino acids are more clearly defined and better known.

Certain amino acids can be chemically measured in much smaller quantities than others; tryptophane, in particular, has been the subject of numerous investigations, especially in the field of nutrition, and methods capable of measuring microgram amounts of tryptophane are available. However most of these workers (3, 4, 5 6, 7) have been concerned with the total quantity of tryptophane present, and very little attention has been given to the isolation of small quantities of free tryptophane from natural products.

Tryptophane has been suspected of being connected with decomposition in foods in earlier investigations on indole (8, 9, 10, 11). These investigators inferred that the indole was formed from tryptophane. It seems reasonable to suppose that, in the decomposition of foods, tryptophane is formed first by hydrolysis of proteins and then converted in part

to indole. Thus there is a good chance that tryptophane might be present in much larger amounts than the resultant indole. Even though this amino acid is not a final end product in the process of protein degradation, it is logical to reason that the products of the earlier enzymatic breakdown might be found more frequently and abundantly in decomposed food than an end product produced by fewer species of microorganisms. As with any useful chemical criterion, the stage of decomposition at which it appears, and the rate of its formation, and of its disappearance, are very important. This report describes a method for the isolation and determination of free tryptophane in milk, cream, and butter, and presents experiments showing the effect of time and temperature on the formation of free tryptophane in milk and cream.

METHOD FOR FREE TRYPTOPHANE IN MILK, CREAM, AND BUTTER

REAGENTS

Color Reagent.—Dissolve 0.4 g. p-dimethylaminobenzaldehyde in 5 ml acetic acid and mix with 92 ml phosphoric acid and 3 ml conc. HCl. Purify the commercial reagent according to Organic Syntheses Collective Vol. I, p. 214–7, Gilman & Blatt, J. Wiley and Sons, N. Y. (1941).

Hydrochloric Acid.—C.P.—Conc.

Acetic Acid.—C.P.—Glacial. This may be conveniently dispensed from a large separatory funnel on a ringstand.

Lactic Acid Soln.—2.5% W/V.

Dilute Hydrogen Peroxide Soln.—Dilute 1 ml 3% H_2O_2 to 5 ml with H_2O. Prepare fresh daily. (Add 1 drop of this soln to 20 ml of acetic acid immediately before use).

Sodium Hydroxide Soln.—10% W/V.

Acetone—90%.—Redistill before dilution, discarding approximately 5% at the end of the distillation. Neutralize to phenolphthalein with 0.1 *N* NaOH just before using. Dilute 90 ml acetone with 10 ml water.

Alumina Cream.—*Methods of Analysis, A.O.A.C.*, 6th Ed., **34.19(b)**, p. 562. The washing operation may be speeded up by centrifuging. This reagent should have a smooth gelatinous appearance. Solns having the appearance of a suspended powder do not filter satisfactorily.

Standard Tryptophane Soln.—Accurately weigh 20 mg tryptophane and dissolve in ca 100 ml water. Dilute to exactly 200 ml. Dispense from a 10 ml microburette. This solution may be diluted with water to a more convenient working strength. Keep stock soln under refrigeration, and do not use after 3 days. (Tryptophane is difficult to wet and therefore dissolves only slowly. Frequent shaking will hasten solution.)

PREPARATION OF SAMPLE

Cream and Milk.—Weigh 25 g of well mixed sample into a 250 ml centrifuge bottle. If the cream is plastic or very thick, dilute with 5 ml hot water or heat on steam bath until the cream is free flowing.

Butter.—Weigh 50 g of well mixed butter into a 250 ml centrifuge bottle and melt at a temperature not above 100°C. Centrifuge at ca 2000 r.p.m. for several minutes and remove and discard the fat layer with a cream pipette. (Or dissolve the 50 g portion in 50 ml ethyl ether by vigorous shaking, add 50 ml pet. ether, shake, and centrifuge at ca 2000 r.p.m. for several minutes. Remove ether-fat layer by means of a siphon or pipette.) Care must be exercised in order that none of the aqueous phase is removed with the fat.

EXTRACTION AND DETERMINATION OF TRYPTOPHANE

Using 3–4 drops of 1% alcoholic phenolphthalein as an indicator, neutralize the sample by the dropwise addition of 10% NaOH soln. Mix the solution after the addition of each drop of alkali. Discharge the pink color by the dropwise addition of 2.5% lactic acid soln and add 1.0 ml in excess. If the sample is warm, cool to room temperature. Add 100 ml of 90% acetone, shake vigorously for at least two minutes, and centrifuge for several minutes at ca 2000 r.p.m. Carefully inspect the sample after centrifuging; if the precipitated protein layer appears to be fluid, or if there is a liquid layer (except fat) in the bottom of the bottle, add 10 ml water, shake vigorously for two min. and centrifuge again. This treatment must be repeated yet again if the precipitate is still fluid. (Tryptophane may be lost at this point if the acetone extract is decanted from a fluid or liquid layer.) Filter, thru a rapid folded filter paper which will retain the fat, into a 250 ml beaker. (Several ml of butter fat usually separates during the centrifuging and is left on the filter paper and discarded.) Evaporate the filtrate to a volume of 5–7 ml on a hot plate, after addition of glass beads to prevent bumping. If the soln develops a basic reaction during evaporation of the acetone, neutralize and add lactic acid in excess as before. If solns have a cloudy appearance after evaporation to 5–7 ml, repeat the neutralization and heat for several minutes. Allow to cool to room temperature, add 2 ml alumina cream, and filter into a 25 ml glass stoppered graduate (C.S.S. 589 White Ribbon filter paper is satisfactory.) Wash the beaker with 2–3 ml water and pour thru the filter. Continue the washing until the volume of the filtrate is 10 ml. (Alternately, the soln may be filtered thru a cotton pledget, to remove fat, into a graduate containing 2 ml alumina cream, made to 10 ml. vol. by washing, and then filtered or centrifuged. Less time is required by this method.) Shake well, pipet 2.0 ml of the sample soln into a 50 ml beaker, and evaporate to ½ ml or less on a hot plate. (Caution: Do not take to dryness.) Allow to cool to room temperature. Add 1 ml hydrochloric acid and 1 ml of the color reagent, mix by swirling, and allow to stand for 15±1 min. Pour the mixture into a dry, 25 ml glass stoppered graduate and wash the beaker with ca 20 ml acetic acid to which has been added 1 drop of the diluted H_2O_2. Make to 25 ml with acetic acid and mix well by inverting the graduate several times. Allow to stand 10±1 min., pour into a suitable photometer cell, and measure the color at 560 mμ.

(While NaCl is often precipitated upon the addition of the strong acids, it generally settles out, leaving a clear supernatant liquid; however, the color solution may be filtered during the final standing if the NaCl does not appear to be settling out.)

(Cream and milk solutions which contain large amounts of tryptophane may require longer than 10 minutes for color development. This condition is easily recognized since a constant reading cannot be obtained. In such instances repeat the determination, using a smaller aliquot.)

In each series of determinations on milk, cream, or butter, determine the blank on an aliquot of each different sample soln in the same manner, omitting only the addition of the color reagent.

Since the fat contents of milk, cream, and butter are so different, calculate all results to the fat-free basis for ease of comparison.

The following formula is convenient for calculating the results:

$$\text{Tryptophane—fat-free basis p.p.m.} = \frac{A \times (B/C)}{g.(100 - F)}$$

Where A = net micrograms tryptophane in aliquot
B = total ml of sample solution
C = ml of aliquot used
G = grams of sample used
F = per cent fat in sample

DISCUSSION OF METHOD

The tryptophane combined in the casein molecule will react with the color reagent and form blue or purplish blue solutions. It is therefore necessary to isolate the free tryptophane resulting from enzymatic or bacterial action in such a manner as will not liberate tryptophane from the casein molecule.

Acetone was selected both as a combined solvent for free tryptophane and a denaturant for interfering milk proteins, after testing a number of common denaturants, *e. g.*, trichloracetic acid, sodium tungstate, sodium chloride, alcohol, etc. The acetone is readily removed by evaporation. One 100 ml portion of the acetone solution is sufficient to extract the free tryptophane completely enough to be within the experimental error of the method.

The varying protein content of milks and creams (and to a lesser extent, of butters), as well as the varying salt content of butters, makes it practically impossible to prescribe an acetone-water ratio which will perform equally well on all samples of milk, cream, and butter. An acetone solution which contains too much water will not satisfactorily coagulate the protein; if, on the other hand, the acetone concentration is too high, the salt solution from the butter will not be dissolved, and significant quantities of tryptophane might then be lost at this step in the procedure.

Unsuccessful attempts were made to dissolve tryptophane in butter fat; further, no tryptophane was detectable in butter fat from cream which contained large amounts of free tryptophane.

Tryptophane is destroyed by heating in acid solution. This effect is more pronounced when the strong mineral acids are used, instead of lactic acid, to neutralize the samples. However, the acetone extraction of the prepared samples must be made from acid solutions, since cloudy concentrates which are almost impossible to filter are obtained if the extraction is made from basic solutions. The acidity recommended approximates the titratable acidity of fresh cream.

A number of reactions have been proposed for the determination of tryptophane in protein hydrolyzates, but one of the most widely used determinations, as well as one of the most sensitive, measures the blue color formed when tryptophane reacts with p-dimethylaminobenzaldehyde in the presence of strong acids. A trace of an oxidizing agent. *e.g.*, H_2O_2 or $NaNO_2$, speeds up the reaction. The blue color can be measured photometrically at 560 mμ.

Sample solutions obtained from cream and milk have a yellow color, probably due to the presence of the water soluble milk pigment, riboflavin. Sample solutions from butter have only a tinge of this yellow color, but such solutions exhibit a fluorescence in ultraviolet light which is typical of riboflavin. The blank due to this yellow color in cream or milk sample solutions, in the absence of the color reagent, usually amounts

to not more than 1–2 mm, using the 100 mm cell and the neutral wedge photometer (12). A 2 ml aliquot of butter sample solution has no detectable blank. In view of the small magnitude of the error thus introduced into the determination, only two (unsuccessful) attempts were made to eliminate this color.

The selection of a 2.0 ml aliquot of the sample solution in the actual determination has been governed by a number of considerations: (a) The interference due to the natural pigment is slightly more pronounced, and brownish off-colors are often obtained, when aliquots larger than 2.0 ml are used. (b) In sample solutions obtained from salted butter, the salt precipitates upon evaporation of the aliquot and is not redissolved by the strong acids used in developing the color. This interference is not serious in aliquots up to 2.0 ml; however, if larger aliquots are used, the salt must be removed from the color solution by filtration or some other means. (c) The 10 ml sample solution contains a total of 25 mg lactic acid, and when larger (3–5 ml) aliquots are used, the acid must be neutralized before evaporation.

The term "free tryptophane" as used in this paper, means tryptophane reacting substances in the sample solutions. Preliminary experiments with the method of Horn and Jones (6) indicate that the "free tryptophane" determined in milk or cream by this method is actually tryptophane. Although primarily of academic interest, further study of the point is being undertaken.

The method used was systematically investigated with respect to time of reaction, concentration and character of the acid-aldehyde reagent, diluent, presence of water and concentration of the hydrogen peroxide. The following general conclusions are drawn from theses experiments:

1. Fifteen minutes is a generous allotment of time for the initial reaction. After addition of the acetic acid-peroxide, however, the time limits should be observed closely since the color slowly fades.

2. The phosphoric acid-aldehyde color reagent has a slightly greater sensitivity than a solution of the aldehyde in hydrochloric acid.

3. An oxidizing agent has been found necessary to develop the maximum color. The optimum concentration of the peroxide solution is a 1+4 dilution of 3% U.S.P. hydrogen peroxide. Stronger solutions promote rapid fading. Solutions of KNO_2 and KNO_3 are satisfactory for the development of the color. The color developed by these oxidants is more stable than that developed by H_2O_2 but may require 15–60 minutes for maximum development of color.

4. Acetic acid was selected as the diluent, since no color is developed when the solutions are diluted to the necessary volume with water. Probably the other diluents which have been used in this reaction would be equally satisfactory.

5. The final sample solution is concentrated in order to remove the excess water, which causes fading and, when more than 1 ml is present, results in the development of less color. This fading is not diminished by the use of larger amounts of color reagent and hydrochloric acid.

6. The concentration of p-dimethylaminobenzaldehyde in the color reagent is not particularly critical.

7. The quantities of hydrochloric acid and color reagent used in developing the color are not critical. The amounts indicated have been found to be adequate for as much as 300 mmg tryptophane.

8. It is preferable to finish the determinations on the same day that they are begun; however, the solutions may be stored overnight under refrigeration.

9. When the quantity of tryptophane measured is greater than 10 mmg, a straight line is obtained by plotting photometer readings against micrograms tryptophane.

TABLE 1.—*Recovery of tryptophane added to cream and butter*

SAMPLE	TRYPTOPHANE P.P.M.			
	Present	*Added*[1]	*Total*	*Recovery*
Cream	0	1.0	1.0	1.1
Cream	0	2.0	2.0	2.4
Cream	0	3.0	3.0	3.0
Cream	0	4.0	4.0	4.1
Cream	0	4.0	4.0	4.1
Cream	0	6.0	6.0	5.6
Cream	0	20.0	20.0	17.6
Cream	0	1.0	1.0	1.0
Butter	1.0	1.0	2.0	1.8
Butter	1.0	1.0	2.0	1.9
Butter	1.5	1.0	2.5	2.2
Butter	1.1	1.5	2.6	1.3
Butter	1.3	3.0	4.3	3.8
Butter	1.4	1.0	2.4	2.2

[1] Multiply by 25 to obtain micrograms of tryptophane added to cream sample, and by 50 for butter sample.

RECOVERY EXPERIMENTS

Table 1 shows the recovery of tryptophane added to cream and butter, using the above procedure. The data indicate that the method is sufficiently accurate to detect small differences in the tryptophane content of cream and butter.

EXPERIMENTAL

The following experiments were undertaken to show that free tryptophane actually is formed in the microbial degradation of milk proteins:

Two lots of milk of known history were used in obtaining the data presented in Table 2, and in Fig. 1.

Milk and cream samples A, B, and C were all from one lot of milk sampled approximately four hours after milking at a receiving plant in Crosby, Mississippi. Cream samples B and C contained 38 per cent and

56 per cent fat, respectively. Both were separated from the same milk represented by sample A.

Sub-samples of the milk and of the two cream samples, consisting of 3 sets of 12 half-pint portions and 3 sets of half gallon portions, were placed in sterile containers. One set of each sub-sample was stored at 37°C (98.6°F); the second was held at room temperature which ranged from 18–25°C (64.5–77°F); and the third was placed in a refrigerator at a temperature of 0–4°C (32–39°F). Tryptophane and titratable acidity (13) determinations were made at the time intervals indicated in Table 2. The one-half gallon samples were stirred thoroughly before each analysis. The one-half pint samples were well mixed immediately before analysis, and a different bottle was used for each set of tryptophane and acidity determinations. Thus, the one-half pint samples were aged without intermittent mixing, and the one-half gallon samples were mixed prior to each determination.

Efforts were made to obtain a homogenous mixture throughout these experiments. However, the cream layer on the milk was not completely miscible when the acidity reached ca 0.3 per cent. No difficulty was encountered in obtaining visual homogeneity in the cream samples until the acidity reached 0.4 per cent. Separation was noted both in the milk and in the 38 per cent fat cream samples after standing undisturbed for 12 hours; and it was increasingly difficult to reincorporate the upper layer as the acidity increased. It is possible that some of the inconsistences in the results may be attributed to the difficulty of obtaining perfect homogeneity.

Milk sample D was from a milking observed by the writer at a New Orleans, Louisiana, dairy. Cream sample E was from the same dairy, separated 12 hours earlier and placed in the cooler immediately after separation. Triplicate sub-samples of D and E were placed in sterile one-half pint jars; one set of each was aged as before at 0–4°C; another at room temperature, 23–29°C. (73–84°F); and the third at 37°C.

Inspection of these tables shows that the tryptophane content, and, of course, the titrable acidity of both milk and cream increases with age. The rate of formation of tryptophane and acid increases with rise in temperature. Only the cream data have been used in preparing Fig. I, because it was possible to follow the cream decomposition for longer periods than the milk. This graph clearly illustrates the effect of time and temperature upon the formation of tryptophane in cream. The data given in Table 2 show that milk behaves in the same manner.

From a comparison of the tryptophane content of milk sample A, and cream samples B and C held at 37°C. for periods up to and including 36 hours, it can be seen that the formation of tryptophane in independent of the size of the sample, and is not significantly influenced by the fat content of the sample.

Table 2.—*Effect of time and temperature on development of tryptophane and titratable acidity*

AGE (HRS.)	0–4° C.		18–25° C.		37° C.	
	TITRATABLE ACIDITY (% LACTIC)	FREE TRYPTOPHANE P.P.M.	TITRATABLE ACIDITY (% LACTIC)	FREE TRYPTOPHANE P.P.M.	TITRATABLE ACIDITY (% LACTIC)	FREE TRYPTOPHANE P.P.M.
SAMPLE A, MILK—5% FAT						
4	(See text)...	....	0.17	0.9......	....	....
12	0.19 (0.18)	2.1 (1.0)[1]	0.19 (0.21)	1.8 (1.6)	0.23 (0.22)	1.7 (1.7)
24	0.17 (0.18)	1.2 (1.8)	0.50 (0.36)	3.6 (1.8)	0.72 (0.77)	5.3 (4.8)
36	0.17 (0.17)	0.5 (1.1)	0.80 (0.74)	3.1 (2.7)	0.88	6.3
48	0.18 (0.16)	1.0 (1.2)	0.85	4.2	0.91	7.8
96	0.14 (0.17)	1.9 (1.4)				
144	0.17 (0.17)	1.2 (1.2)				
192	0.16 (0.17)	1.7 (1.3)				
240	0.18 (0.17)	1.6 (1.3)				
288	0.18 (0.19)	2.8 (2.0)				
336	0.20 (0.18)	5.8 (3.0)				
408	0.32 (0.22)	8.4 (3.9)				
480	0.42 (0.38)	7.8 (9.3)				
SAMPLE B, CREAM—38% FAT						
4	(See text)...	....	0.08	0........	....	....
12	0.11 (0.13)	1.2 (0)	0.12 (0.13)	1.0 (1.0)	0.15 (0.16)	1.5 (1.1)
24	0.12 (0.13)	0.8 (0)	0.22 (0.33)	1.9 (1.3)	0.55 (0.56)	3.1 (3.5)
36	0.11 (0.12)	0 (0)	0.54 (0.56)	1.0 (2.8)	0.61 (0.61)	6.3 (5.8)
48	0.12 (0.13)	0 (1.1)	0.60 (0.61)	2.4 (3.1)	0.63 (0.63)	7.7 (7.3)
96	0.13 (0.13)	1.0 (1.0)	0.48 (0.67)	5.8 (5.9)	0.81 (1.02)	16.2 (18.4)
144	0.13 (0.13)	1.6 (1.0)	0.74 (0.73)	11.0 (9.4)	1.08 (1.31)	32.3 (34.8)
192	0.12 (0.12)	1.3 (1.0)	0.80 (0.81)	15.9 (11.6)	1.09	55.9
240	0.13 (0.12)	1.1 (1.6)	0.81	19.2	1.29	82.6
288	0.13 (0.11)	1.4 (1.1)	0.88	23.6	1.42	139.4
336	0.15 (0.13)	1.0 (1.6)	0.95	27.0	1.42	135.5
408	0.29 (0.15)	5.5 (1.6)				
480	0.51 (0.25)	8.5 (2.4)				
SAMPLE C, CREAM—56% FAT						
4	(See text)...	....	0.08	0........	....	....
12	0.07 (0.10)	1.0 (0)	0.09 (0.11)	0 (0)	0.11 (0.10)	0 (1.4)
24	0.09 (0.09)	1.0 (0)	0.20 (0.25)	1.7 (1.1)	0.38 (0.43)	4.7 (3.7)
36	0.09 (0.09)	0 (0)	0.35 (0.37)	1.7 (2.2)	0.40 (0.33)	6.9 (7.0)
48	0.08 (0.10)	0 (0)	0.41 (0.40)	3.0 (4.1)	0.42 (0.40)	11.9 (10.1)
96	0.11 (0.10)	1.0 (1.4)	0.46 (0.41)	6.8 (6.2)	0.64 (0.52)	35.5 (21.7)
144	0.11 (0.10)	1.1 (0)	0.50 (0.48)	12.0 (9.6)	0.92 (0.62)	56.8 (30.0)
192	0.09 (0.09)	1.1 (0.8)	0.50	13.8	0.97	74.6
240	0.09 (0.10)	1.5 (1.4)	0.64	18.3	1.09	102.7
288	0.09 (0.10)	1.5 (1.2)	0.72	26.4	1.15	145.5
336	0.15 (0.15)	2.6 (1.4)	0.83	31.9	1.63	136.4
408	0.22 (0.23)	4.0 (2.6)				
480	0.26 (0.39)	8.5 (3.7)				
SAMPLE D,[2] MILK—3.5% FAT						
0	(See text)...	....	0.15	1.2......	....	....
6	0.17	1.1	0.17	1.3	0.18	1.7
12	0.17	1.1	0.17	0.9	0.17	1.5
18	0.17	1.0	0.16	1.3	0.21	3.2
24	0.16	0.9	0.17	2.2	0.36	3.9
30	0.17	0.9	0.19	3.2	0.59	4.5
36	0.17	0.9	0.21	4.2	0.77	7.8
48	0.16	1.9	0.75	6.8	0.81	11.6
72	0.17	1.4	0.91	7.0	0.85	12.2
96	0.16	1.3				
120	0.18	1.2				
168	0.17	1.4				
SAMPLE E,[2] CREAM—21.3% FAT						
0	(See text)...	....	0.13	0.9......	....	....
6	0.16	0.9	0.16	0.8	0.16	1.1
12	0.16	0.6	0.16	0.9	0.21	1.7
18	0.16	0.8	0.19	1.1	0.64	4.8
24	0.16	0.8	0.55	2.4	0.70	8.0
30	0.16	0.8	0.69	3.9	0.74	8.9
36	0.16	0.8	0.70	4.0	0.74	11.7
48	0.15	1.2	0.76	6.3	0.77	14.4
72	0.17	0.8	0.79	8.0	0.79	21.9
96	0.15	1.2				
120	0.17	1.2				
168	0.15	0.9				

Note: Tryptophane values calculated to fat-free basis.
[1] Values enclosed in parentheses were obtained from ½-gallon sub-samples.
[2] 23–29°C. (Room temperature in these experiments.)

The samples held under refrigeration remained fresh; the tryptophane content and titratable acidity remained reasonably constant up to 240 hours. Thereafter a slow increase in both tryptophane and acidity is noticeable. There are greater variations between the ½ gallon and the ½

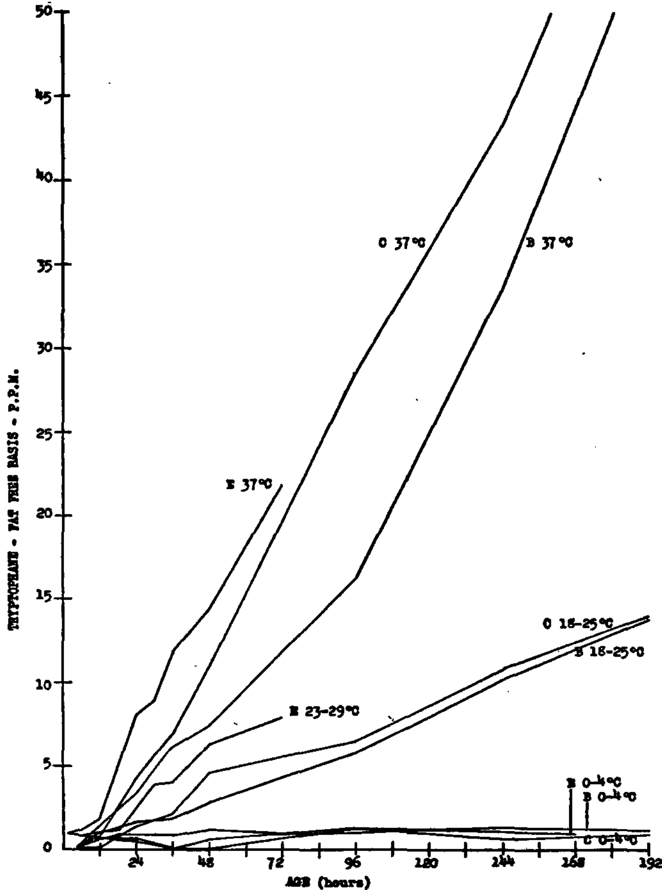

Fig. 1—Effect of temperature on rate of development of tryptophane in cream.

pint subsamples after 336 hours of storage and, with the exception of sample A at 480 hrs., higher tryptophane values were obtained on the ½ pint jars which had been held without mixing. In some cases there was also a noticeable difference in the acidity. No logical explanation, except the erratic growth habits of micro-organisms at low temperatures, is offered for this behavior.

The samples held at room temperature also show an increase in tryptophane content with age. The rate of formation of tryptophane and titratable acidity is noticeably greater than in the samples held at refrigerator termperatures. However, the milk was clabbered at the end of 36 hours, thus making sampling difficult at an early stage of this experiment. The ½ gallon of 56 per cent cream was granular in appearance, and therefore difficult to sample at the end of 48 hours storage. However, measurable quantities of tryptophane were found in the milk before the clabbering point was reached.

The rate of formation of tryptophane and acid was noticeably hastened by incubation at 37°C. The milk samples were clabbered at the end of 24 hours, and, judging from the acidity, had been clabbered for some time before.

TABLE 3.—*Free tryptophane content of butter made from cream of known age*

SAMPLE	AGE (DAYS)	CREAM		BUTTER
		TITRATABLE ACIDITY (% LACTIC)	FREE TRYPTOPHANE FAT-FREE BASIS P.P.M.	FREE TRYPTOPHANE FAT-FREE BASIS P.P.M.
I	0	0.09	0	0
	2	0.48	7.5	7.5
	4	0.57	9.9	9.8
	7	0.76	19.6	13.1
	10	0.95	28.0	23.8
	46	1.34	123.3	—
II	0	0.09	0	0
	3	0.90	10.3	9.2
	6	1.49	28.7	23.8
	14	1.86	77.5	56.0

Table 3 shows the results of similar experiments where cream was stored at room temperature and churned by hand into butter at intervals up to 46 days. These data show that there is a rather close relationship between the free tryptophane content of butter and that of the cream from which the butter was made.

TRYPTOPHANE CONTENT OF COMMERCIAL MILK AND CREAM

In order to gain some information on the normal content of free tryptophane in sweet milk and cream, 18 samples of milk and 22 samples of cream were analyzed. These samples were collected from retail stores in New Orleans, Louisiana, and represent the output of as many of the dairies in this area as could be readily sampled. Each sample represents either a different dairy or a different day's output. These results are presented in Table 4. The three samples of sour cream are representative of the product prepared by inoculating pasteurized sweet cream with a starter. Re-

TABLE 4.—*Free tryptophane content of market milk and cream*

PRODUCT	SAMPLE NO.	TITRATABLE ACIDITY, % LACTIC	FREE TRYPTOPHANE FAT-FREE BASIS P.P.M.
Milk	1	0.11	0.6
	2	0.12	1.0
	3	0.12	0.8
	4	0.10	1.0
	5	0.10	0
	6	0.11	1.0
	7	0.12	0.8
	8	0.10	0.9
	9	0.12	0.7
	10	0.08	1.2
	11	0.10	1.5
	12	0.10	0.9
	13	0.10	1.7
	14	0.10	1.0
	15	0.10	1.0
	16	0.11	1.6
	17	0.13	1.0
	18	0.14	0.9
Breakfast Cream	1	0.11	0.7
	2	0.08	0.8
	3	0.10	2.7
	4	0.09	0
	5	0.10	0
	6	0.10	0
	7	0.10	0.8
	8	0.10	0.8
	9	0.11	2.0
	10	0.12	0.7
	11	0.06	1.5
	12	0.12	2.1
	13	0.09	0.8
	14	0.21	2.4
	15	0.16	1.2
	16	0.09	2.0
	17	0.06	0.7
Whipping Cream	18	0.14	0
	19	0.10	1.0
Sour Cream	20	0.94	4.4
	21	1.05	2.2
	22	0.70	1.3

turned milk and cream are used in the preparation of this product. The free tryptophane in these samples is negligible, compared to the values obtained on raw milk or cream allowed to reach corresponding acidities.

SUMMARY AND CONCLUSIONS

An investigation has been made of the occurrence of free tryptophane in milk, cream, and butter. A method for the extraction and measurement of the free tryptophane in these products has been described.

The following conclusions have been reached:

(1) Negligible quantities of free tryptophane are present in normal sweet milk and cream.

(2) The amount of free tryptophane in milk and cream increases with age if the products are held under conditions conducive to bacterial and enzymatic activity.

(3) The amount of free tryptophane in butter depends upon the free tryptophane content of the original cream.

ACKNOWLEDGMENTS

The author is indebted to J. O. Clarke, Chief, Central District, for his invaluable suggestions throughout the investigation. He also wishes to express his appreciation for the indispensable aid of M. E. Warren and W. A. Bell of New Orleans Station.

REFERENCES

(1) PORTER, J. R., "Bacterial Chemistry & Physiology," pp. 849–889, J. Wiley & Sons, New York (1946).
(2) HARROW and SHERWIN, "Test book of Biochemistry," p. 343, W. B. Saunders Co., Philadelphia (1935).
(3) BATES, R. W., Proc. Am. Soc. Biol. Chem., *J. Biol. Chem.*, **119**, p. vii (1937).
(4) SULLIVAN, M., MILENE, H. S., and EVERITT, E. L., *Ibid.*, **125**, 471 (1937).
(5) SHAW, J. L. D., and MCFARLANE, W. D., *Ibid.*, **132**, 387 (1940).
(6) HORN, M. J., and JONES, D. B., *Ibid.*, **157**, 153 (1945).
(7) GRAHAM, C. E., *et al.*, *Ibid.*, **168**, 711 (1945).
(8) CLARKE, J. O., *et al.*, *This Journal*, **20**, 475 (1937).
(9) KING, W. H., FLYNN, F. F., GOWANLOCH, J. N., *Ibid.*, **28**, 385 (1945).
(10) BEACHAM, L. M., *Ibid.*, **29**, 89 (1946).
(11) DUGGAN, R. E., and STRASBURGER, L. W., *Ibid.*, **29**, 177 (1946).
(12) CLIFFORD, P. A., and BRICE, B. A., *Ind. Eng. Chem.* (*Anal. Ed.*), **12**, 218 (1940).
(13) *Methods of Analysis, A.O.A.C.*, 6th Ed., **22.4**, p. 303 (1945).

NOTES

Note on the Microscopic Identification of Caffeine

By George L. Keenan*

The microchemical identification of caffeine with mercuric chloride reagent already has been described (1) and the crystalline precipitate has been photomicrographed by Stephenson (2). The precipitate appears as needles, as also does the crystalline precipitate formed with gold chloride reagent.

The purpose of this note is to call attention to a much more distinctive test suggested by Arreguine (3) but considerably simplified as to technic. The method of procedure is as follows: A drop of Lugol's Reagent (iodine-in-potassium iodide) is

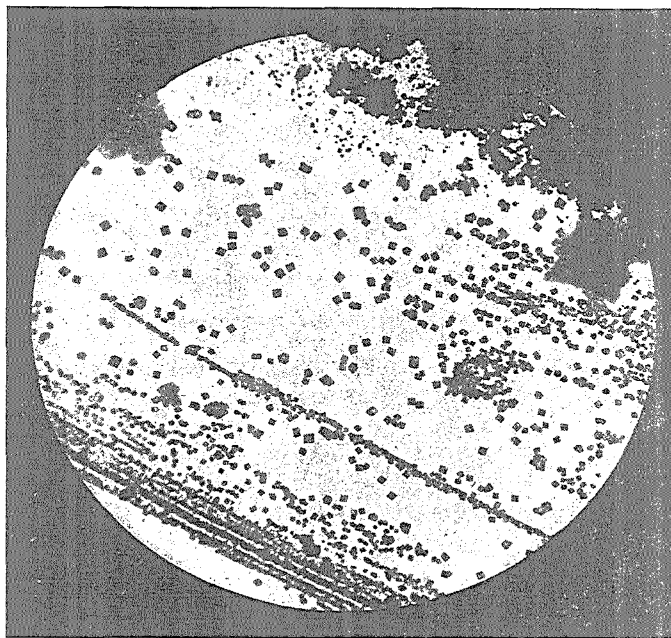

Fig. 1.—Caffeine with Lugol's reagent and ammonium sulfate.

added to a small amount of the test material on an object slide and stirred in. Then a few fragments of ammonium sulfate are dropped into the preparation. As the drop evaporates at the periphery, numerous dark red to brown to almost black (depending on their thickness) quadrilateral plates will appear, many of these so oriented as to show a box-like habit (Figure 1). They resemble rhombohedra in form and exhibit strong birefringence with crossed nicols. This microchemical test is more characteristic and striking than that obtained with either mercuric chloride or gold chloride reagents.

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REFERENCES CITED

(1) *This Journal*, **13**: 46 (1930).
(2) STEPHENSON, CHARLES H.: "Some Microchemical Tests for Alkaloids," J. B. Lippincott Co., 1921, pp. 30–31 (Plate VIII, Fig. 1.)
(3) ARREGUINE, V., *Rev. Univ. nacl.* (Cordoba, Arg.) **29**, 1732 (1942); *Chem. Abs.*, **37**: 6819, Nov. 20, 1943.

Separation of n-Butyric and Isobutyric Acids by Partition Chromatography

L. L. RAMSEY (Food Division,* Food and Drug Administration, Federal Security Agency, Washington, D. C.)

In applying the method of partition chromatography for separating the straight-chain saturated fatty acids C_5–C_{10} (1) to an unknown mixture of acids derived from a commercial flavoring product, an extraneous acid band was observed between n-butyric acid and n-valeric acid. This extraneous acid was identified as isobutyric acid by the preparation of the characteristic crystalline mercurous salt (2). Previously it was found that a mixture of n-butyric and isobutyric acids does not separate on a chromatographic partition column of silicic acid, using water as the immobile solvent and chloroform containing a small percentage of n-butanol as the mobile solvent (2).

The table below shows the recovery of n-butyric and isobutyric acids when present in admixture, using the same procedure as described for the separation of the straight-chain saturated fatty acids C_5–C_{10} (1). The threshold volumes are also shown. As can readily be seen from the table, the separation of the two acids is not complete. On the column the bands are very close together, in fact, visibly overlapping. A single passage through the column will detect a minimum of about 10% isobutyric acid added to n-butyric acid as an impurity, and about 30% n-butyric acid added to isobutyric acid. Refractionation of appropriate fractions, however, as outlined in the paper on the separation of the acids C_5–C_{10} (1), improves the sensitivity of the method.

The method presented here may be useful in cases where only a small amount of acid is available, where it is desirable to recover the acid after identification, and/or where an approximate determination is satisfactory.

TABLE 1.—*Recovery of n-butyric and isobutyric acids when present in admixture*

NAME OF ACID	ADDED	FOUND	THRESHOLD VOLUME
	mg	*mg*	*ml*
Mixture A			
Isobutyric	10.3	8.2	222
Butyric	10.0	12.3	254
Mixture B			
Isobutyric	5.15	3.7	219
Butyric	5.0	6.4	250

REFERENCES

(1) RAMSEY, L. L. and PATTERSON, W. I., p. 139, *This Journal.*
(2) *Ibid.*, **28**, 644 (1945).

* W. B. White, Chief.

BOOK REVIEWS

Commercial Fertilizers, Their Sources and Use. By Gilbeart H. Collings. Published by the Blakiston Company, Philadelphia-Toronto, 1947. Fourth Edition, 6½×9¼ inches, 522 pages, 160 illustrations, 139 tables. Price $4.50.

The author, Professor of Soils, Clemson Agricultural College, Clemson, South Carolina, and Fertilizer Consultant, Collings and Associates, has had long experience as experiment station agronomist and teacher of soil, fertilizer, and crop courses. Written to satisfy a definite need in the classroom, this book was first published in 1934 with succeeding revised editions appearing in 1938 and 1941. The present new edition, containing eighteen chapters, has been revised to include reference to many changes which occurred in the sources and use of fertilizer materials during World War II. There is an entire new chapter entitled "The Manufacture and Use of Ammonium Nitrate." There are five other chapters on nitrogen, three on phosphate, one on potash, three on other elements in fertilizers including the secondary and minor elements; one chapter each on history, soil reactions and crop growth, purchase of fertilizers, principles of the use of fertilizers; and the final chapter discusses the application of fertilizers and their influence on germination and seedling growth.

In the preface the author states, "Because production and consumption figures for recent years for some countries and states have not been published or are not yet reliable, and because I felt that many conditions as they existed during the war years were not normal, I have purposely used as illustrative material in many chapters the statistics, situations, or practices that prevailed in the normal years just prior to the outbreak of World War II." This treatment of the subject matter is desirable in view of the intended use of the book by students being introduced to general practices relating to a major industry. There is an excellent bibliography of some six hundred references, many of which call attention to original research data. This provides the reader with easy access to a more detailed study on many of the subjects presented in this book. In this way, a vast number of subjects pertinent to the source and use of fertilizers, and which would ordinarily require several volumes, are presented in this single volume. Detailed, technical description of the manufacture and use of fertilizers would be out of place in a book of this nature. The author has done an admirable job in avoiding such detail and the result is an outstanding work on the general subject of commercial fertilizers, their sources and their use.

John O. Hardesty

Fatty Acids, Their Chemistry and Physical Properties. By Klare S. Markley. Interscience Publishers, Inc., New York, 1947. X+668 pp. Price $10.00.

The purpose of this work, states the author, is "to bring together in an organized and readily accessible form as much as possible of the present accumulation of facts and data pertaining to the chemical reactions and physical properties of the fatty acids . . . " and thus "obviate many hours of searching the literature by the large and growing body of chemists, physicists, engineers, and technologists who are interested in the fatty acids and their numerous products and by-products."

The scope and emphasis of the book may be indicated to a certain degree by noting the number of pages devoted to the major divisions of the book, together with the names of the chapters under each division. The book is separated into six major divisions:

A. The Nature and History of Fats and Waxes, 9 pp. in one chapter.

B. Classification and Structure of the Fatty Acids, 64 pp., includes two chapters; Classification and Nomenclature, and Isomerism.

C. Physical Properties of the Fatty Acids, 166 pp., includes five chapters; Crystal Properties, Spectral Properties, Thermal Properties, Solubility of Fatty Acids and Solution Properties; and Properties of the Fatty Acids in the Liquid State.

D. Chemical Reactions of the Fatty Acids, 278 pp., includes eleven chapters; Salts of Fatty Acids, Esterification and Interesterification, Alkylation and Alkoxylation, Pyrolysis, Halogenation, Hydrogenation and Hydrogenolysis, Oxidation and Hydroxylation, Oxidation by Atmospheric Oxygen (Autooxidation), Biological Oxidation, Nitrogen Derivatives of Aliphatic Acids, and Sulfur Derivatives of the Fatty Acids.

E. Synthesis of Fatty Acids, 42 pp., includes two chapters; In Vitro Synthesis of Fatty Acids, and Biosynthesis of Fatty Acids.

F. Isolation and Identification of Fatty Acids, 57 pp., includes two chapters; Separation of Fatty Acids, and Identification of Individual Fatty Acids.

A cursory examination of the book discloses that experimental data such as physical constants, and that concepts such as those of crystal structure are well up-to-date, having been taken in many instances from the literature of the past ten years. It is to be regretted, though, that no reference was made to the work of A. W. Weitkamp on the acidic constituents of degras. Weitkamp claims to have isolated and identified thirty-two of the acidic constituents of degras, many of which had not previously been reported as occurring in nature (JACS, **67**, 447 (1945)).

The history of the development of the chemistry of the fatty acids is very closely interwoven with the textual material throughout the book. The work is very well documented, a vast number of pertinent references to the original literature being given.

The text is profusely illustrated by a large number of tables, graphs, and diagrams, together with a few very helpful photographs.

The physical appearance and typographical set-up of the book are good; it is well printed on good paper, and durably bound. Except for an occasional word obviously misspelled, the book appears to be quite free from errors. At the back of the book is a useful author and subject index.

This book is a comprehensive survey of our present knowledge of the fatty acids and it will, no doubt, fill a long felt need in this field. As a reference book it will be found highly useful to chemists and other scientists working on fatty acids, or in related fields.

L. L. RAMSEY

Standard Methods for the Examination of Water and Sewage. Prepared, Approved, and Published Jointly by the American Public Health Association and the American Water Works Association. Publication Office American Public Health Association, New York 19, N.Y. Ninth Edition, 1946, XVII+286 pages. Illustrations. Cloth. Price $4.00.

This edition of one of the best known and most widely distributed laboratory manuals used by water and sewage control technicians, reflects the fine editorial work of Dr. Symons, as well as the activities of the nine cooperating and coordinating committees led by Maxcy, Pirnie, Buswell, Hitchens, Mallmann, Calvert, Black, Hatfield, and Gilcreas; and of the chairman of the joint editorial committee, Norton.

The reading matter on each page has been split in two columns which makes the directions much easier to follow and has been rearranged under fewer captions. It now consists of four parts instead of seven, as follows: Examination of water—physical and chemical; Sewage, Sewage Effluents, Industrial Wastes, Polluted Waters, Sludges and Muds; Microscopical Examination of Water, Sewage Sludge, and Bottom Sediments; and Bacteriological Examination of Water. The two appendices deal with Non-Standard Methods, and with Chemicals and Reagents.

It would be impracticable to refer even briefly to the many deletions, additions, and revisions represented by this edition. One example must suffice. Under "Iron," the two introductory paragraphs have been omitted as such, and "Gravimetric," "Colorimetric-Bypyridine," and "Colorimetric-Phenanthroline" methods have been added. The potassium ferricyanide method for ferrous iron has been deleted. The bibliography for this subject has been increased from two to eight references.

The manual has been immensely improved, and the laboratory worker has a tool in this edition which will greatly increase his knowledge and efficiency. Access to the book is an actual necessity for laboratory workers concerned with the analysis of water and sewage.

J. W. SALE

The Water Soluble Gums. By C. L. Mantell, Ph.D., Reinhold Publishing Company, New York. 1947, 6×9 inches, V+258 pages of text, and a 7-page table "Native Designation of Gums." Price $6.00.

The author's aim in this book, as stated in the preface "is an attempt to coordinate information relating to the water-dispersible products derived largely from a wide variety of plants and known as gums." This aim is only partly fulfilled.

Chapter 1, Classification and Chemistry of the Gums, exemplifies the author's tendency toward brevity. The entire chapter comprises 18 pages; 4 pages, plus a flow sheet, is devoted to classification, leaving 13 pages for the discussion of general chemistry of gums. Physical chemistry of gums, an important subject, is disposed of in six pages. Then follows six chapters on individual gum families, such as acacia, India gums, Astralgus, and seaweed gums. These include much interesting detail on gathering and purifying these products. Considerable space is given in these chapters to their physical and chemical properties. The chapter on acacia gums includes an interesting discussion, the work of deJong and co-workers on "coazervation," or the effects produced by mixing two hydrophilic colloids of opposite electric charge. The chapter on seaweed colloids is particularly informative, due in part to the fact that the producers of agar agar and algin employ modern methods of manufacture and plant control. Excellent flow-sheets of the manufacturing processes are given.

Chapter 8, discussing the modified celluloses, industrial competitors of natural gums, is inadequate, considering the many uses for which these synthetic "gums" are recommended. Only four literature references are given, three dated before 1937. The American literature is entirely neglected. The two chapters on modified starches (dextrins), and on hydrophilic proteins (gelatins, etc.), are similarly brief, the author stating they are adequately treated in existing monographs. Pectin is not discussed.

Industrial uses of gums are disposed of in four chapters, totalling 41 pages. Here again the author seems to have neglected modern research, 100 of the 136 references listed being before 1940. 73 citations refer to patents. As an example, none of the work of Sommer, at Wisconsin, or Mack at Amherst, on stabilizers in ice cream is mentioned, although the statement is made "one of the largest uses of algin in the United States is as a stabilizer for ice cream."

Chapter 15 discusses "Specifications, Identification and Testing." This is too brief to be of practical value to a working chemist. Much important work is omitted. Work by Racicot and Ferguson on detection of gums in foods, and many A.O.A.C. referee reports on the same subject, are not given. The tests copied from *Methods of Analysis, A.O.A.C.* Fifth Ed., and the U. S. Pharmacopoeia XI, are both from obsolete editions. In fact, the XIII edition of the Pharmacopoeia is current. This is especially unfortunate since the Sixth Edition of the *Methods of Analysis* listed many tests and methods not included by the author. The same comment applies to methods published in *The Journal, A.O.A.C.* There is only one reference to articles from this journal dated after 1939. Many of the tests included in this chapter are ab-

stracted too briefly to be of practical value as given. As an example, a method is given (p. 254), for the detection of agar in canned chicken. The paragraph ends "and the precipitate is treated like the like precipitate in the detection of agar in mayonnaise." The method for mayonnaise is given only by literature reference, and that indirectly in the discussion of gums in foods. Again (p. 256) is the statement "The methods for gums in cheese are applicable to vanilla ice cream." Nowhere in the book is a reference to a method for detection of gums in cheese.

One of the most valuable sections of the book is the last chapter. This is a table listing 179 native or trade names of gums, each with its corresponding botanical source, geographical origin and present day classification.

In general, the book is characterized by incomplete literature research. The only other book dealing specifically with gums known to this reviewer, "The Biochemistry of Cellulose, Polyuronides, Lignins, etc." by A. G. Norman (1937) is not mentioned. The many papers by Tseng, of Scripps Institute of Oceanography, University of California, on algin and agar are dismissed with two brief references. Other examples have been given. Mantell's book impresses this reviewer as being too technical for the industrialist or scientist. The author could well have added 100 pages or more without introducing any new subjects. The book is printed on a good quality paper, with easily-read type, and contains informative flow-sheets, graphs, and illustrations. The bibliography, while not complete, is representative, and contains the more important potent citations.

F. LESLIE HART

Cereal Laboratory Methods, Fifth Edition, 1947, XIV, pp. 341, illustrations. Cereal Chemistry Office, University Farm, St. Pail 1, Minn. $4.50.

This is essentially a "methods of analysis" book for particular use in cereal testing laboratories. The new edition has been revised and considerably expanded through the addition of new chapters on experimental malting, vitamin assay methods, and sanitary methods. A number of new methods have also been added in previously existing chapters: specifically the xylene partition and thiobarbituric acid methods as a replacement for the old phloroglucin method for pentosans, the determination of urea and ammoniacal nitrogen, the determination of egg solids in macaroni products, and the fat stability test. Likewise, a number of methods have been deleted: glutenin, proteins soluble in 70% ethyl alcohol, proteins soluble in 5% K_2SO_4 solution, proteins peptized by neutral normal salt solution, albuminoid nitrogen, buffer value of flour, water soluble protein precipitable by 40% alcohol, lipoids, lipoid phosphoric acid, detection of sesame oil, and the accelerated rancidity test. Many of the methods described are the methods adopted by the Association of Official Agricultural Chemists, and by the American Oil Chemists' Society.

A criticism is justified of the provision for sampling feed by the same procedure recommended for sampling flour, rather than by the feed sampling method of the *Methods of Analysis, A.O.A.C.*, and the Official Publication of the Association of American Feed Control Officials, Inc. The (identical) methods of the latter two organizations are generally used in feed enforcement activities.

The increased scope of the subject matter makes this a more valuable book than the previous edition, and it will be a useful addition to any laboratory working in the field of cereal chemistry.

V. E. MUNSEY