AACC-AOCS Joint Meeting

PROGRAM

AMERICAN OIL CHEMISTS' SOCIETY
and
AMERICAN ASSOCIATION OF CEREAL CHEMISTS

March 31-April 4, 1968

WASHINGTON HILTON • WASHINGTON, D.C.

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MONDAY MORNING—APRIL 1
7:30 A.M.—Military Room
SESSION CHAIRMEN BREAKFAST

MONDAY MORNING—APRIL 1
9:00 A.M.—International Ballroom West
AACC OPENING SESSION
Presiding—Kenton L. Harris

9:00 **CALL TO ORDER**
Introduction of overseas visitors
Tribute to deceased members
Results of elections

PRESENTATION OF HONORARY MEMBERSHIPS
Recipients: Ellis D. English, Harold Plimpton, Jr.

PRESENTATION OF GEDDES MEMORIAL AWARD
Recipient: Paul E. Ramstad

ADJOURNMENT

MONDAY MORNING—APRIL 1
9:00 A.M.—International Ballroom East
AOCS OPENING SESSION
Presiding—Raymond Reiser

9:00 **CALL TO ORDER**

GREETINGS & ANNOUNCEMENTS
Edith Christensen, General Chairman
AACC-AOCS Joint Meeting

MINUTES OF THE 58th ANNUAL MEETING
G. C. Cavanagh, Secretary

REPORT OF THE TREASURER
M. T. Joyner

ADDRESS OF THE PRESIDENT
Raymond Reiser

CONSTITUTIONAL REVISIONS

MISCELLANEOUS BUSINESS

REPORT OF THE NOMINATING & ELECTION COMMITTEES AND CERTIFICATION OF OFFICERS
C. W. Hoerr, Chairman

ADJOURNMENT

MONDAY MORNING—APRIL 1
10:30 A.M.—International Ballroom
JOINT OPENING SESSIONS

10:30 **CALL TO ORDER**

ANNOUNCEMENTS

ADDRESS OF THE PRESIDENT OF THE AOCS
Raymond Reiser

ADDRESS OF THE PRESIDENT OF THE AACC
Kenton L. Harris

KEYNOTE SPEAKER
To be announced

ADJOURNMENT AND LATE ANNOUNCEMENTS

MONDAY AFTERNOON—APRIL 1
2:00 P.M.—Lincoln Ballroom West
AFLATOXIN I—METHODOLOGY
Chairman—**W. A. Pons, Jr.**, USDA Southern Regional Research Laboratory, New Orleans, La.

2:00 1. **A RAPID METHOD OF DETECTING AFLATOXINS IN PEANUTS**
Charles E. Holaday

2:20 2. **A RAPID AND PRECISE PHYSICOCHEMICAL ASSAY METHOD FOR AFLATOXIN IN PEANUTS AND PEANUT PRODUCTS**
Arthur E. Waltking, George Bleffert, and Mary Kiernan

2:40 3. **NONINTERFERENCE OF SEED COAT CONSTITUENTS OF COTTONSEED IN A MODIFIED PONS-GOLDBLATT AFLATOXIN PROCEDURE**
T. C. Campbell, P. B. Marsh, and J. H. Snider

3:00 4. **A SIMPLIFIED PROCEDURE FOR THE DETERMINATION OF AFLATOXIN B_1 IN COTTONSEED MEALS**
James Velasco

3:20 5. **IMPROVED OBJECTIVE FLUOROMETRIC DETERMINATION OF AFLATOXINS IN COTTONSEED PRODUCTS**
Walter A. Pons, Jr., Alva F. Cucullu, A. O. Franz, and L. A. Goldblatt

3:40 6. **AN IMPROVED SEPARATION OF AFLATOXINS**
Mabry Wiley and A. C. Waiss

4:00 7. **SEPARATION AND PURIFICATION OF AFLATOXINS AND COMPARISON OF SEMI-SYNTHETIC AFLATOXINS B_2 AND G_2 WITH NATURALLY OCCURRING AFLATOXINS B_2 AND G_2**
J. V. Rodricks

4:20 8. **CONDITIONS AND TECHNIQUES FOR THIN-LAYER CHROMATOGRAPHY OF AFLATOXINS**
Stanley Nesheim

MONDAY AFTERNOON—APRIL 1
1:30 P.M.—Crystal Ballroom
BAKING I
Chairman—**K. H. Tipples**, Board of Grain Commissioners, Winnipeg, Canada

1:30 **INTRODUCTORY REMARKS**

1:35 9. **STARCH DAMAGE AND ALPHA-AMYLASE AS A BASIS FOR MATHEMATICAL MODELS RELATING TO FLOUR WATER ABSORPTION**
E. A. Farrand

1:55 10. **STATISTICAL EVALUATION OF THE RELATIONSHIP OF VARIOUS LEVELS OF NONFAT DRY MILK AND EMULSIFIERS TO CRUMB FIRMNESS**
J. K. Seibel and A. M. Swanson

2:15 11. **TEMPERATURE, MIXER RPM AND SALT EFFECTS ON FARINOGRAPH CHARACTERISTICS**
J. F. Conn and T. P. Kichline

2:35 12. **OXIDATION OF SULFHYDRYL GROUPS OF FLOUR BY BROMATE UNDER VARIOUS CONDITIONS AND DURING THE BREADMAKING PROCESS**
C. C. Tsen

2:55 **BREAK**

3:15 13. **EFFECT OF FLOUR DAMAGED STARCH LEVEL IN VARIOUS BREADMAKING METHODS**
K. H. Tipples and R. H. Kilborn

3:35 14. **USE OF LEVULOSE CONTAINING CORN SYRUP IN YEAST-LEAVENED BAKED GOODS**
S. Redfern, H. Gross, B. R. Strong, and E. K. Wardrip

MONDAY AFTERNOON—APRIL 1
2:00 P.M.—Jefferson Ballroom West
BLOOD LIPIDS I
Chairman—**R. F. Witter**, National Communicable Disease Center, Atlanta, Ga.

2:00 15. **STEROL METABOLISM AND ATHEROSCLEROSIS IN CEBUS MONKEYS**
Hugh B. Lofland, Jr., T. B. Clarkson, R. W. St. Clair, and N. D. M. Lehner

2:20 16. **AUTOMATED TECHNIQUES FOR THE DETERMINATION OF CHOLESTEROL, PHOSPHOLIPIDS, AND TRIGLYCERIDES**
Gerald Kessler

2:40 17. **A STUDY OF TRIGLYCERIDE ANALYSES AS PERFORMED BY 19 LABORATORIES**
Virginia S. Whitner, Rebecca Wylie, and Robert F. Witter

3:00 18. **SERUM TRIGLYCERIDE METHODS FOR FIELD STUDIES**
Gerald Korbel, G. V. A. Pantulu, J. T. Anderson, and A. Keys

3:20 19. **PLASMA TRANSPORT OF MENADIONE**
K. E. Guyer

3:40 20. **VOLATILE FREE FATTY ACIDS OF HUMAN SERUM**
V. Mahadevan and L. Zieve

4:00 21. **QUANTITATIVE DETERMINATION OF PHOSPHOLIPID CLASSES IN HUMAN SERUM BY COMBINED THIN-LAYER CHROMATOGRAPHY**

AND PHOSPHORUS ANALYSIS
J. H. Williams, M. Kuchmak, and R. F. Witter

MONDAY AFTERNOON—APRIL 1

1:30 P.M.—Jefferson Ballroom East

OILSEEDS I

Chairman—**J. D. Mullen**, General Mills, Inc., Minneapolis, Minn.

1:30 **INTRODUCTORY REMARKS**

1:35 22. **NUTRITIONAL AND BIOCHEMICAL EVALUATION OF DEEP-FAT-FRIED SOYBEANS**
L. R. Hackler and W. F. Wilkens

1:55 23. **THE EFFECT OF PROCESSING CONDITIONS ON THE COMPOSITION OF SOY MILK**
W. F. Wilkens and L. R. Hackler

2:15 24. **STEAM PROCESS FOR DEALLERGENIZATION OF CASTOR POMACE**
A. C. Mottola, G. O. Kohler and H. G. Walker, Jr.

2:35 25. **TRYPSIN INHIBITORS IN THE WHOLE WATER-EXTRACTABLE SOYBEAN PROTEINS**
T. Obara, M. K. Kobayashi and T. Kobayashi

2:55 **BREAK**

3:15 26. **PREPARATION OF CHEESE-LIKE SOY PROTEIN FOOD BY ENZYMATIC TREATMENT. I. SELECTION OF PROTEOLYTIC ENZYME**
T. Obara, M. K. Kobayashi and M. Ohata

3:35 27. **EVALUATION OF LIPIDS IN DEFATTED SOY FLAKES AS FLAVOR FACTORS**
D. J. Sessa, D. H. Honig and J. J. Rackis

MONDAY AFTERNOON—APRIL 1

1:30 P.M.—Lincoln Ballroom East

PROCESSING TECHNOLOGY—CEREALS

Chairman—**J. C. Rankin**, USDA Northern Regional Research Lab., Peoria, Ill.

1:30 **INTRODUCTORY REMARKS**

1:35 28. **RICE PROTEIN CONCENTRATES FROM X-M RICE BRAN**
J. W. Hunnell, L. Lynn and J. DeMont

1:55 29. **FERMENTED RICE—A FOOD FROM ECUADOR**
A. G. VanVeen, D. Graham and K. Steinkraus

2:15 30. **WHEAT- AND BARLEY-BASED PROTEIN-FORTIFIED FOOD PRODUCTS FOR FOOD AID PROGRAMS**
R. E. Ferrel, A. P. Mossman, A. D. Shepherd and J. W. Pence

2:35 31. **AIR-CLASSIFICATION OF FLOURS FROM RYE, OATS, AND BARLEY**
A. C. Stringfellow, A. J. Peplinski, L. H. Burbridge and V. F. Pfeifer

2:55 32. **CORN DRY-MILLING: COLD TEMPERING AND DEGERMINATION OF CORN OF VARIOUS INITIAL MOISTURE CONTENTS**
O. L. Brekke and W. F. Kwolek

3:15 **BREAK**

3:35 33. **GELATINIZATION OF CORN GRITS BY ROLL- AND EXTRUSION-COOKING**
R. A. Anderson, H. F. Conway, V. F. Pfeifer, and E. L. Griffin, Jr.

3:55 34. **CHARACTERIZATION OF PREGELATINIZED CEREAL PRODUCTS**
F. Meindl and H. J. Roberts

4:15 35. **IMPROVED PREPARATION OF A QUATERNARY AMMONIUM ALKYL ETHER OF STARCH**
C. L. Mehltretter, T. A. McGuire and C. A. Wilham

4:35 36. **SEPARATION OF WHEAT COMPONENTS IN AN ALKALINE MEDIUM**
B. V. Ettling and M. F. Adams

MONDAY AFTERNOON—APRIL 1

2:00 P.M.—Georgetown Ballroom East

SYMPOSIUM: BROWN ADIPOSE TISSUE I

Chairman—**R. L. Dryer**, Iowa City, Iowa
Co-Chairman—**C. D. Joel**, Harvard Medical School, Cambridge, Mass.

2:00 37. **ONTOGENESIS OF BROWN ADIPOSE TISSUE**
Robert L. Smalley, Judith Jarvis, and Leonore Rowe

2:40 38. **METABOLISM (HEAT PRODUCTION) IN THE SHREW**
R. R. J. Chaffee, M. W. Sorenson, and C. H. Conaway

3:20 39. **METABOLISM OF BROWN ADIPOSE TISSUE**
Cliffe D. Joel

4:00 40. **BIOENERGETICS OF BROWN ADIPOSE TISSUE**
Robert Emrie Smith, Paul A. Herd, and Barbara A. Horwitz

4:40 41. **ATP LEVELS IN CONTROL OF OXIDATION**
Olov Lindberg, Rudolf Eisenhardt, Stanley B. Rusiner, and Barbara Cannon

MONDAY AFTERNOON—APRIL 1

2:00 P.M.—Georgetown Ballroom West

SYMPOSIUM: CHEMICAL SYNTHESIS

Chairman—**D. Swern**, Temple University, Fels Research Institute, Philadelphia, Pa.

2:00 42. **REACTIONS OF ISOPROPENYL STEARATE PROCEEDING THROUGH HEXADECYLKETENE**
Edward S. Rothman

2:30 43. **SYNTHETIC REACTIONS INVOLVING UNSATURATED AND HYDROXY-UNSATURATED FATTY ACIDS**
B. Freedman, M. J. Diamond, T. H. Applewhite, and Glen Fuller

3:00 44. **DIFUNCTIONAL DERIVATIVES OF CYANOETHYLATED HYDROXYSTEARATE**
H. E. Kenney, G. Maerker, and E. T. Donahue

3:30 45. **REACTIONS OF ALDEHYDIC ESTERS**
E. H. Pryde and J. C. Cowan

4:00 46. **CHARACTERIZATION OF NATURALLY OCCURRING α-HYDROXYLINOLENIC ACID**
Cecil R. Smith, Jr.

4:30 47. **PHOTOLYSIS OF METHYL α-IODOSTEARATE**
David R. Howton and Guey-Shuang Wu

MONDAY AFTERNOON—APRIL 1

4:00 P.M.

AACC TECHNICAL COMMITTEE MEETINGS

Individual committees will meet in concurrent sessions from 4:00 P.M. to 6:00 P.M. Room assignments will be posted at the registration desk.

TUESDAY MORNING—APRIL 2

8:00 A.M.—Military Room

AACC PAST PRESIDENTS' BREAKFAST

TUESDAY MORNING—APRIL 2

9:00 A.M.—Lincoln Ballroom West

AFLATOXIN II—BIOCHEMICAL ASPECTS

Chairman—**W. A. Pons, Jr.**, USDA Southern Regional Research Laboratory, New Orleans, La.

9:00 48. **SAMPLE PREPARATION FOR AFLATOXIN ASSAY, THE NATURE OF THE PROBLEM AND APPROACHES TO A SOLUTION**
L. Stoloff, A. D. Campbell, S. Nesheim, and A. C. Beckwith

9:20 49. **SAMPLING STORED CORN FOR AFLATOXIN ASSAY**
Robert M. Johnson, W. T. Greenaway, and C. Golumbic

9:40 50. **SURVEY OF CEREAL GRAINS AND SOYBEANS FOR THE PRESENCE OF AFLATOXINS**
O. L. Shotwell, W. F. Kwolek, H. R. Burmeister, G. M. Shannon, M. L. Goulden, E. Vangegraft, M. S. Milburn, H. H. Hall, and C. W. Hesseltine

10:00 51. **SCREENING COTTONSEED FOR AFLATOXINS**
Marion E. Whitten

10:20 52. **REMOVAL OF AFLATOXINS FROM OILSEED MEALS BY EXTRACTION WITH AQUEOUS ISOPROPANOL**
Eric T. Rayner and Frank G. Dollear

10:40 **53. TEMPERATURE-DOSE RELATIONSHIPS WITH AFLATOXIN ON THE BRINE SHRIMP, ARTEMIA SALINA**
R. F. Brown, J. D. Wildman, and R. M. Eppley

11:00 **54. MYCOTOXIN INHIBITION OF BACILLUS MEGATERIUM**
Nancy L. Clemments

11:20 **55. AFLATOXIN-PRODUCING ABILITY OF FUNGI**
Harry W. Schroeder and R. A. Boller

11:40 **56. THE BIOASSAY OF AFLATOXINS AND RELATED SUBSTANCES WITH BACILLUS MEGATERIUM SPORES AND CHICK EMBRYOS**
A. Jayaraman, E. J. Herbst, and Miyoshi Ikawa

TUESDAY MORNING—APRIL 2
9:00 A.M.—Crystal Ballroom
BLOOD LIPIDS II
Chairman—**R. F. Witter**, National Communicable Disease Center, Atlanta, Ga.

9:00 **57. QUANTITATIVE DETERMINATION OF PHOSPHOLIPIDS AND GLYCOLIPIDS: THIN-LAYER AND COLUMN CHROMATOGRAPHY WITH SPECTROPHOTOMETRIC ASSAY**
George Rouser

9:30 **58. THE DETERMINATION OF THE INDIVIDUAL PHOSPHOLIPIDS IN ERYTHROCYTES AND PLASMA OF VARIOUS MAMMALIAN SPECIES**
Gary J. Nelson

9:50 **59. LIPID COMPOSITION OF HUMAN ERYTHROCYTES**
John D. Turner and George Rouser

10:10 **60. THE EFFECT OF INCUBATION ON THE PLASMA PHOSPHOLIPID PATTERN IN HEALTHY PERSONS, AFTER HEPARIN ADMINISTRATION AND IN MYOCARDIAL INFARCTION**
R. Berlin, C. O. Oldfelt, and O. Vikrot

10:30 **61. THE METABOLISM OF LIPOPROTEIN LIPASE**
James Felts

10:50 **62. CORRELATION BETWEEN POST-HEPARIN LIPASE AND PHOSPHOLIPASE ACTIVITIES IN HUMAN PLASMA**
William C. Vogel and Edwin L. Bierman

11:10 **63. DETERMINATION OF HEPARIN INDUCED LIPOPROTEIN LIPASE IN BLOOD PLASMA**
J. Boberg

11:30 **64. PLASMA LIPIDS OF YOUNG ADULTS ON CONTROLLED EXPERIMENTAL DIETS**
A. Kuksis, L. Marai, and O. Stachnyk

TUESDAY MORNING—APRIL 2
9:00 A.M.—Jefferson Ballroom East
CEREAL CHEMISTRY (GENERAL) I
Chairman—**V. L. Youngs**, North Dakota State University, Fargo, N. Dak.

9:00 **INTRODUCTORY REMARKS**

9:05 **65. COLOR DETERMINATION OF SPAGHETTI BY THE TRESTIMULUS METHOD**
D. E. Walsh, K. A. Gilles, and W. C. Shuey

9:25 **66. A WHEAT-HARDNESS INDEX**
W. T. Greenaway

9:45 **67. ESTIMATION OF LYSINE AND METHIONINE ADDED TO FORTIFIED WHEAT FOOD PRODUCTS**
R. E. Ferrel, Nancy Bellard, A. D. Shepherd, and J. W. Pence

10:05 **68. STABILITY DURING STORAGE OF LYSINE IN LYSINE-INFUSED WHEAT KERNELS**
R. E. Ferrel, A. D. Shepherd, D. G. Guadagni, and J. W. Pence

10:25 **69. NONPOLAR LIPIDS IN HAND-DISSECTED YELLOW CORN FRACTIONS**
C. W. Blessin

10:45 **70. USE OF THE BIURET AND DYE-BINDING TECHNIQUES FOR ESTIMATING PROTEIN IN MILLED AND BROWN RICE**
Lucila Parial, L. W. Rooney, and B. D. Webb

11:05 **71. SCREENING FOR HIGH-PROTEIN RICE VARIETIES FROM THE IRRI WORLD COLLECTION**
B. O. Juliano, C. C. Ignacio, V. M. Panganiban, and C. M. Perez

11:25 **72. HEXANAL AS A MEASURE OF STORAGE DETERIORATION OF READY-TO-EAT CEREALS**
C. W. Fritsch, J. P. Nelson, and J. L. Olson

11:45 **72B. SCREENING METHOD FOR LYSINE ASSAY**
R. Palter, L. White, M. A. Gauger, and G. O. Kohler

TUESDAY MORNING—APRIL 2
9:00 A.M.—Lincoln Ballroom East
CEREAL PROTEINS I
Chairman—**J. E. Bernardin**, University of Oregon, Eugene, Oreg.

9:00 **INTRODUCTORY REMARKS**

9:05 **73. SOME PROPERTIES OF THE PROTEINS OF THE ENDOSPERM, SUB-ALEURONE LAYER, AND ALEURONE LAYER OF WHEAT**
G. A. H. Elton and J. Pace

9:25 **74. PEPTIDES FROM ALPHA-GLIADIN**
J. E. Bernardin, Maureen T. O'Sullivan, and D. K. Mecham

9:45 **75. COMPARATIVE STUDIES ON GLUTENINS FROM DIFFERENT CLASSES OF WHEAT**
F. R. Huebner

10:05 **76. ISOLATION AND CHEMICAL COMPOSITION OF PROTEIN BODIES AND MATRIX PROTEINS FROM CORN ENDOSPERM**
D. D. Christianson, H. Nielsen, U. Khoo, M. J. Wolf, and J. S. Wall

10:25 **77. EXTRACTION AND STRUCTURE STUDIES OF CORN GLUTELIN PROTEINS**
H. C. Nielsen, J. W. Paulis, C. James, and J. S. Wall

10:45 **78. INTERACTIONS BETWEEN PROTEIN AND STARCH**
Itsuo Takeuchi

11:05 **79. EFFECT OF FLOUR FRACTIONS ON HYDROGEN SULFIDE RELEASE FROM DOUGHS MIXED UNDER NITROGEN**
D. K. Mecham and Maura M. Bean

11:25 **80. EFFECTS OF OXIDIZING AND REDUCING AGENTS ON CHANGES OF FLOUR PROTEINS DURING DOUGH MIXING**
C. C. Tsen

TUESDAY MORNING—APRIL 2
8:45 A.M.—Jefferson Ballroom West
SORGHUM GRAIN
Chairman—**L. W. Rooney**, Texas A&M University, College Station, Texas

8:45 **INTRODUCTORY REMARKS**

8:50 **81. SORGHUM GRAIN: ITS WORLDWIDE SIGNIFICANCE AND POTENTIAL**
K. O. Rachie

9:30 **82. COMPOSITION AND STRUCTURE OF SORGHUM GRAIN**
J. S. Wall and C. W. Blessin

9:50 **83. THE DRY MILLING OF GRAIN SORGHUM**
R. R. Hahn

10:10 **84. LABORATORY WET MILLING STUDIES OF SELECTED GRAIN SORGHUM TYPES**
J. E. Freeman and S. A. Watson

10:30 **85. PROTEIN QUALITY STUDIES ON GRAIN SORGHUM**
C. W. Deyoe, P. E. Sanford, L. M. Murphy, and D. H. Waggle

10:50 **86. AMINO ACID COMPOSITION OF MILLED SORGHUM PRODUCTS**
F. K. Shoup, C. W. Deyoe, and JoAnn Campbell

11:10 **87. PIGMENT CHARACTERIZATION IN GRAIN SORGHUMS**
W. K. Nip and E. E. Burns

11:30 **88. THE LIPIDS OF SORGHUM GRAIN. I. CHARACTERIZATION OF FREE AND BOUND LIPIDS FROM SELECTED GRAIN SORGHUM VARIETIES**
L. W. Rooney

TUESDAY MORNING—APRIL 2
9:00 A.M.—Georgetown Ballroom East
SYMPOSIUM: BROWN ADIPOSE TISSUE II
Chairman—**R. L. Dryer**, Iowa City, Iowa
Co-Chairman—**C. D. Joel**, Harvard Medical School Cambridge, Mass.

9:00 **89. METABOLISM OF FREE BROWN ADIPOSE TISSUE CELLS**
John N. Fain and Nora Reed

9:40 **90. DEHYDROGENASE KINETICS IN BROWN ADIPOSE TISSUE**

R. L. Dryer and J. R. Paulsrud

10:20 91. **FATTY ACID METABOLISM IN BROWN ADIPOSE TISSUE**
Eric G. Ball

11:00 92. **CONTROL MECHANISMS OF THERMOGENESIS**
J. R. Williamson, S. Rusiner, and B. Chance
GENERAL DISCUSSION

TUESDAY MORNING—APRIL 2
8:30 A.M.—Thoroughbred Room
SYMPOSIUM: DETERGENTS I
Chairman—**I. Schmolka**, Wyandotte Chemicals Corp.

8:30 93. **IDENTIFICATION OF POLYOXYALKYLENE-TYPE NONIONIC SURFACTANTS BY PAPER CHROMATOGRAPHY**
G. L. Selden and J. H. Benedict

8:50 94. **THE DEVELOPMENT OF A RADIOACTIVE PARTICULATE SOIL FOR DETERGENCY STUDIES**
B. E. Gordon and E. L. Bastin

9:10 95. **THE IMPORTANCE OF LIQUID-TO-CLOTH RATIO IN DETERGENCY**
W. H. Smith and A. R. Martin

9:30 96. **COMPUTERIZATION IN FABRIC DETERGENCY TESTING**
J. C. Illman, G. M. Hartwig, and J. W. Roddewig

9:50 97. **CHLORINE-STABLE MACHINE DISHWASHING PRODUCTS**
I. R. Schmolka, T. M. Kanekd, and
E. A. Weipert

10:10 98. **HEXYLENE GLYCOL AS A COSOLVENT IN DRY-CLEANING**
M. Wentz and A. R. Martin

TUESDAY MORNING—APRIL 2
10:30 A.M.—Thoroughbred Room
SYMPOSIUM: DETERGENTS II
THE NEXT PHASE: TEXTILES AND DETERGENTS OF THE '70'S
Chairman—**E. Jungermann**, Armour & Co., Chicago, Ill.

10:30 99. **SYNTHETIC FATTY ALCOHOLS AND ACIDS: NEW ADDITIONS TO THE RAW MATERIAL POOL FOR SOAPS AND DETERGENTS**
J. G. Moffett and W. de Acetis

10:50 100. **THE IMPACT OF NEW FIBRES AND FABRICS AND NEW LEVELS OF CONSUMER PERFORMMANCE REQUIREMENTS**
Fred Fortess

11:10 101. **THE EFFECTS OF THE CHANGING TEXTILE TECHNOLOGY ON DETERGENT FORMULATIONS**
R. T. Hunter

11:30 102. **THE NEW TOILET SOAPS**
Eric Jungermann and A. B. Herrick

11:50 103. **CHANGES IN HARDWARE**
D. T. Donavan

TUESDAY MORNING—APRIL 2
12:00—International Ballroom West
AOCS INAUGURATION LUNCHEON

TUESDAY AFTERNOON—APRIL 2
2:00 P.M.—Crystal Ballroom
BLOOD LIPIDS III—SERUM LIPOPROTEINS, METHODS, AND STRUCTURE
Chairman—**F. T. Lindgren**, University of California, Berkeley, Calif.

2:00 104. **SCREENING FOR SERUM LIPOPROTEIN ABNORMALITIES—COMPARISON OF ULTRACENTRIFUGAL, PAPER, AND THIN-LAYER STARCH-GEL ELECTROPHORETIC TECHNIQUES**
Lena A. Lewis

2:20 105. **ASSAY OF LIPOPROTEINS BY RADIAL IMMUNODIFFUSION**
Robert S. Lees

2:40 106. **COMPARISON OF LIPOPROTEIN ANALYSIS BY AGAROSE GEL ELECTROPHORESIS AND ULTRACENTRIFUGATION**
R. P. Noble, F. T. Hatch, J. A. Mazrimas,
F. T. Lindgren, L. C. Jensen, and
G. L. Adamson

3:00 107. **CHARACTERIZATION OF HUMAN LOW-DENSITY SERUM LIPOPROTEINS**
F. T. Lindgren, L. C. Jensen,
R. D. Wills, and N. K. Freeman

3:20 **DISCUSSION**

3:35 108. **EFFECTS OF HYDROGEN PEROXIDE ON LIPOPROTEINS AND ASSOCIATED LIPIDS**
Dale A. Clark, Emmett L. Foulds, and
Frederick H. Wilson, Jr.

3:55 109. **ULTRAVIOLET AND FAR-ULTRAVIOLET CIRCULAR DICHROISM SPECTRA OF HUMAN SERUM HIGH-DENSITY LIPOPROTEIN (HDL)**
A. Scanu and R. Hirz

4:15 110. **PREPARATION AND PROPERTIES OF AN APOPROTEIN DERIVATIVE OF HUMAN SERUM β-LIPOPROTEIN**
A. M. Gotto, R. I. Levy, and
D. S. Fredrickson

4:35 111. **PHYSICAL AND CHEMICAL CHARACTERIZATION OF LOW-DENSITY LIPOPROTEIN SUBFRACTIONS FROM HUMAN PLASMA**
Diana M. Lee, Petar Alaupovic, and
Robert H. Furman

4:55 **DISCUSSION**

TUESDAY AFTERNOON—APRIL 2
1:30 P.M.—Jefferson Ballroom East
CEREAL CHEMISTRY (GENERAL) II
Chairman—**V. L. Youngs**, North Dakota State University, Fargo, N. Dak.

1:30 **INTRODUCTORY REMARKS**

1:35 112. **PROTEIN QUANTITY AND QUALITY AS FACTORS IN THE EVALUATION OF BREAD WHEATS**
W. Bushuk

1:55 113. **THE PRECISION AND ACCURACY OF LABORATORY TESTS**
J. P. Woolcott

2:15 114. **A MODIFIED MICRO SEDIMENTATION TEST FOR SCREENING EARLY-GENERATION WHEAT SELECTIONS**
J. S. Kitterman and M. A. Barmore

2:35 115. **A COMPARISON OF STARCH GELATINIZATION AND OTHER PHYSICAL PROPERTIES OF 24 WHEAT FLOURS**
Ruth H. Matthews

2:55 116. **STUDIES ON PIGMENT DESTRUCTION DURING PROCESSING OF SPAGHETTI**
R. R. Matsuo, J. W. Bradley, and
G. N. Irvine

3:15 **BREAK**

3:35 117. **EFFECT OF GLUTEN QUALITY ON THE COOKING QUALITY OF SPAGHETTI**
R. R. Matsuo and G. N. Irvine

3:55 118. **EXPERIMENTAL PRODUCTION OF ATTA**
M. Shafiq Chaudhry, M. M. MacMasters,
E. P. Farrell, and W. J. Hoover

4:15 119. **AMINO ACID ANALYSES OF RICE AND RICE BYPRODUCTS**
D. F. Houston, M. E. Allis, and G. O. Kohler

TUESDAY AFTERNOON—APRIL 2
2:00 P.M.—Lincoln Ballroom West
FATS AND OILS I
Chairman—**M. Sahasrabudhe**, Canadian Department of National Health and Welfare, Ottawa, Canada

2:00 120. **GAS-LIQUID RADIOCHROMATOGRAPHY OF INTACT NATURAL TRIGLYCERIDES**
W. C. Breckenridge and A. Kuksis

2:20 121. **STUDIES ON THE COURSE OF CIS-TRANS ISOMERIZATION OF POLYUNSATURATED FATTY ACIDS**
E. C. Nickell and O. S. Privett

2:40 122. **IMPROVED PROCEDURES FOR THE PREPARATION AND PURIFICATION OF POLYUNSATURATED FATTY ACIDS**
O. S. Privett, J. D. Nadenicek, F. J. Pusch,
R. J. Chapman, and K. K. Beutel

3:00 123. **RADIOLYSIS OF LIPIDS. I. MODE OF CLEAVAGE IN TRIGLYCERIDE FATTY ACIDS**
M. D. Dubravcic and W. W. Nawar

3:20 124. **PREPARATION OF SOME LONG-CHAIN 2-OXAZOLIDONES**
M. E. Dyen and D. Swern

3:40 125. **POSITIONAL DISTRIBUTION OF DOCOSAHEXAENOIC, DOCOSAPENTAENOIC, AND EICOSAPENTAENOIC ACIDS IN AQUATIC ANIMAL TRIGLYCERIDES**
C. Litchfield

4:00 126. **LOCATION OF DOUBLE BONDS IN MIXTURES**

OF UNSATURATED FATTY ACIDS BY OZONOLYSIS AND THIN-LAYER CHROMATOGRAPHY
James B. Saddler, Hugo Krueger, Ian Tinsley, and Robert Lowry

4:20 127. **LIPIDS OF MATURING GRAIN OF CORN (ZEA MAYS)**
Evelyn J. Weber

4:40 128. **REMOVAL OF CHLORINATED PESTICIDES FROM CRUDE VEGETABLE OILS BY SIMULATED COMMERCIAL PROCESSING PROCEDURES**
K. J. Smith, P. B. Polen, D. Devries, and F. Coon

TUESDAY AFTERNOON—APRIL 2
2:00 P.M.—International Ballroom East

MARKETING AND DEVELOPMENT
Chairman—**R. E. Martin**, Swift & Co., Chicago, Ill.

2:00 **INTRODUCTORY REMARKS**

2:05 129. **DO MARKETING AND R & D HAVE A COMMON GROUND?**
W. Fenton Guinnee

2:35 130. **THE BRIDGE AND THE GAP BETWEEN MARKETING AND R & D**
A. L. Powell

3:05 131. **ASPECTS OF TECHNICAL SELLING**
Dale W. Johnson

3:35 132. **THE "SNAP-CRACKLE-POP" OF OBTAINING A U. S. PATENT**
Wilbur C. Davis

4:05 133. **THE ROLE OF TECHNICAL SERVICE IN THE MARKETING OF FOOD INGREDIENTS**
Joseph Rakosky, Jr.

4:35 134. **A SALES-MINDED RESEARCH DIRECTOR'S VIEWS ON MARKETING**
Justin J. Alikonis

TUESDAY AFTERNOON—APRIL 2
1:30 P.M.—Thoroughbred Room

OILSEEDS II
Chairman—**J. S. Mullen**, General Mills, Inc. Minneapolis, Minn.

1:30 **INTRODUCTORY REMARKS**

1:35 135. **HEAT DENATURATION OF SOYBEAN 11S PROTEIN**
W. J. Wolf and T. Tamura

1:55 136. **POLYACRYLAMIDE GEL ELECTROPHORESIS OF REDUCED AND ALKYLATED SOYBEAN TRYPSIN INHIBITORS**
A. C. Eldridge and W. J. Wolf

2:15 137. **DENATURATION OF SOYBEAN PROTEINS WITH ORGANIC SOLVENTS AND ENZYMATIC HYDROLYSIS OF DENATURED PROTEINS**
Danji Fukushima

2:35 138. **BIOLOGICAL EVALUATION OF CRAMBE SEED MEALS AND DERIVED PRODUCTS BY RAT FEEDING**
C. H. Van Etten, M. E. Daxenbichler, T. A. Wolff, and A. N. Booth

2:55 **BREAK**

3:20 139. **USE OF RAPE SEED MEAL IN PRODUCTION OF BROILERS**
R. Costabal, N. Gonzalez, T. McAullife, and J. McGinnis

3:40 140. **SOY PROTEIN NOMENCLATURE**
W. J. Wolf, M. P. Tombs, N. Catsimpoolas, I. Koshiyama, K. Shibasaki, and P. Melnychyn

OILSEEDS DIVISION BUSINESS MEETING IMMEDIATELY AFTER SESSION

TUESDAY AFTERNOON—APRIL 2
2:00 P.M.—Georgetown Ballroom West

SYMPOSIUM: PRACTICAL APPLICATIONS OF CHROMATOGRAPHY IN LIPID ANALYSES I
Chairman—**G. L. Feldman**, Baylor University College of Medicine, Waco, Texas

2:00 141. **GAS CHROMATOGRAPHIC DETERMINATION OF STRUCTURE OF LIPIDS BY HYDROGENATION, HYDROGENOLYSIS, AND OZONOLYSIS**
Morton Beroza

3:00 142. **QUANTITATIVE ANALYSIS OF URINARY STEROID HORMONES BY GAS-LIQUID CHROMATOGRAPHY**
Billy G. Creech

4:00 143. **AUTOMATED THIN-LAYER CHROMATOGRAPHY**
Fred Snyder

TUESDAY AFTERNOON—APRIL 2
2:00 P.M.—Lincoln Ballroom East

PROCESSING TECHNOLOGY—OILSEEDS
Chairman—**D. Arndtsen**, The DeLaval Separator Company, Chicago, Ill.

2:00 144. **X-M RICE OIL DEWAXING PROCESS**
D. Norman Kinsey, John W. Hunnell, and Lawrence Lynn

2:20 145. **NEW RICE OILS**
Lawrence Lynn, R. M. Anderson, and Gary Steen

2:40 146. **CATALYTIC INVESTIGATION FOR THE HYDROGENATION OF COTTONSEED OIL**
S. Stefanovic and L. Albright

3:00 147. **OXIDATIVE DETERIORATION OF PARTIALLY PROCESSED SOYBEAN OIL**
L. H. Going

3:20 148. **PROBLEMS ASSOCIATED WITH THE DEVELOPMENT OF A HIGH-PROTEIN SOY BEVERAGE IN ALLEVIATING PROTEIN MALNUTRITION**
L. Ross Hackler

WEDNESDAY MORNING—APRIL 3
9:00 A.M.—Jefferson Ballroom West

CHEMICALS AND FATTY ACIDS
Chairman—**V. Babayan**, Stokely Van Camp, Inc., Indianapolis, Ind.

9:00 149. **ETHYLENE ADDUCT OF CONJUGATED OCTADECADIENOIC ACIDS: OXIDATIVE DERIVATIVES**
E. J. Dufek, J. P. Friedrich, and L. E. Gast

9:20 150. **HYDROFORMYLATION OF UNSATURATED FATTY ESTERS**
E. N. Frankel, S. Meltin, W. K. Rohwedder, and I. Wender

9:40 151. **HOMOGENEOUS CATALYTIC HYDROGENATION OF UNSATURATED FATS: GROUP VIB—METAL CARBONYL COMPLEXES**
E. N. Frankel and F. L. Little

10:00 152. **SOME RADIOCHEMICAL EXPERIMENTS ON MINOR CONSTITUENTS IN SOYBEAN OIL**
T. L. Mounts, C. D. Evans, H. J. Dutton, and J. C. Cowan

10:20 153. **REACTIONS OF PEROXIDES. II. REACTION OF PEROXIDES AND IODINE WITH CARBOXYLIC ACIDS. A NEW TYPE OF HUNSDIECKER REACTION**
Leonard S. Silbert

10:40 154. **REACTION OF CYCLOPROPENE ESTERS WITH HYDROGENATION CATALYSTS**
Zigrida M. Zarins, R. K. Willich, and R. O. Feuge

11:00 155. **THE GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF CYCLOPROPENOID FATTY ACIDS**
E. L. Schneider, S. P. Loke, and D. T. Hopkins

11:20 156. **VOLATILES FROM SOYBEAN OIL: ISOLATION BY VACUUM DISTILLATION AND IDENTIFICATION BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY**
E. Selke, Helen A. Moser, and W. K. Rohwedder

TUESDAY AFTERNOON—APRIL 2
4:00 P.M.

AACC TECHNICAL COMMITTEE MEETINGS
Individual committees will meet in concurrent sessions from 4:00 P.M. to 6:00 P.M. Room assignments will be posted at the registration desk.

TUESDAY AFTERNOON—APRIL 2
3:00 P.M.—Military Room

AACC ADVISORY COUNCIL MEETING

TUESDAY EVENING—APRIL 2
6:30 P.M.—Sirloin and Saddle Restaurant
(Adjacent to Washington Hilton)

AACC OILSEEDS DIVISION DINNER

WEDNESDAY MORNING—APRIL 3

9:00 A.M.—Lincoln Ballroom West

FATS AND OILS II

Chairman—**M. Sahasrabudhe,** Canadian Department of National Health and Welfare, Ottawa, Canada

9:00 157. **GLYCERYL ETHERS IN INSECTS: IDENTIFICATION OF THE ALK-1-ENYL AND ALKYL ALKENYL ETHERS IN THE PHOSPHOLIPID FRACTION OF THREE INSECT SPECIES**
Edward N. Lambremont and Randall Wood

9:20 158. **OXYGENATED FATTY ACIDS OF SUNFLOWER SEED OIL**
K. L. Mikolajczak, R. M. Freidinger, C. R. Smith, Jr., and I. A. Wolff

9:40 159. **DIHYDROSTERCULIC, A MAJOR FATTY ACID COMPONENT OF DIMOCARPUS LONGANS SEED OIL**
R. Kleiman, F. R. Earle, and I. A. Wolff

10:00 160. **DECREASE, BY MILD OXIDATION, OF THE TOXICITY OF FRESH VEGETABLE OILS**
H. Kaunitz and Ruth E. Johnson

10:20 161. **DETERMINATION OF 15-16 DOUBLE BOND IN HYDROGENATED SOYBEAN OIL**
L. T. Black and R. E. Beal

10:40 162. **REACTION OF THIN-LAYER CHROMATOGRAPHY IN THE ANALYSIS OF PLASMALOGENS**
C. V. Viswanathan, F. Phillips, and W. O. Lundberg

11:00 163. **GAS-LIQUID AND THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF FATTY AMINO ACIDS**
Bernard Freedman

11:20 164. **AUTOMATED GC ANALYSIS OF LIQUID SAMPLES**
F. G. McCarty

WEDNESDAY MORNING—APRIL 3

9:00 A.M.—Crystal Ballroom

FLAVORS

Chairman—**Wm. J. Downey,** Fritzsche Bros., Inc. New York, N. Y.

9:00 **INTRODUCTORY REMARKS**

9:05 165. **THE EFFECT OF LIPOXIDASE ON THE LIPID COMPOSITION AND FLAVOR OF SOY MILK**
W. F. Wilkens

9:25 166. **THE FORMATION OF 1-OCTEN-3-OL IN SOYBEANS DURING SOAKING**
A. F. Badenhop and W. F. Wilkens

9:45 167. **CITRUS FLAVOR TRANSFORMATIONS**
J. A. Rogers

10:05 168. **THE ISOLATION AND IDENTIFICATION OF VOLATILE FLAVOR COMPOUNDS PRODUCED DURING DEEP FAT FRYING**
S. S. Chang, Tsukasa Kawada, R. G. Krishnamurthy, B. R. Reddy, J. A. Thompson, and Kosaku Yasuda

10:25 169. **VOLATILE COMPONENTS OF DEEP-FAT-FRIED SOYBEANS**
W. F. Wilkens, A. F. Badenhop, and L. R. Hackler

10:45 170. **FLAVORING READY-TO-EAT CEREALS**
J. P. Littlejohn

11:05 171. **FLAVORING SYNTHETIC FOODS**
J. Broderick

11:25 172. **TEXTURE AND FLAVOR RELATIONSHIPS RELATED TO SIMULATED MEAT PRODUCTS**
W. E. Hartman

WEDNESDAY MORNING—APRIL 3

9:00 A.M.—Georgetown Ballroom West

SYMPOSIUM: PRACTICAL APPLICATIONS OF CHROMATOGRAPHY IN LIPID ANALYSES II

Chairman—**G. L. Feldman,** Baylor University College of Medicine, Waco, Texas

9:00 173. **CHROMATOGRAPHIC ANALYSIS OF INDIVIDUAL MOLECULAR SPECIES OF PHOSPHATIDES AND GLYCOLIPIDS**
Ossi Renkonen

10:00 174. **ISOLATION AND CHARACTERIZATION OF SPHINGOLIPIDS**
Gerald L. Feldman, A. Stewart Windeler, and Larry L. Pitt

11:00 175. **ANALYSIS OF GLYCERYL-ALKYL AND ALK-1-ENYL ETHERS BY GAS-LIQUID CHROMATOGRAPHY AND THIN-LAYER CHROMATOGRAPHY**
Randall Wood and Fred Snyder

WEDNESDAY MORNING—APRIL 3

9:00 A.M.—Lincoln Ballroom East

CEREAL PROTEINS II—ENZYMES

Chairman—**W. Bushuk,** University of Manitoba, Winnipeg, Canada

9:00 **INTRODUCTORY REMARKS**

9:05 176. **ROLE OF GLUTEN PROTEIN GROUPS IN BREAD-BAKING**
R. C. Hoseney, K. F. Finney, M. D. Shogren, and Y. Pomeranz

9:25 177. **THE ROLE OF FLOUR WATER SOLUBLES IN BREAD-BAKING**
R. C. Hoseney, K. F. Finney, M. D. Shogren, and Y. Pomeranz

9:45 178. **DETECTION OF PROTEOLYTIC ACTIVITIES IN WHEAT PROTEINS BY STARCH GEL ELECTROPHORESIS**
E. Kaminski and W. Bushuk

10:05 179. **WHEAT ALPHA-AMYLASES—ISOLATION AND PROPERTIES**
J. H. Kruger and R. Tkachuk

10:25 180. **WATER-SOLUBLE PROTEINS OF DURUM SEMOLINA—THEIR INFLUENCE ON MACARONI QUALITY**
D. E. Walsh, K. A. Gilles, and C. E. McDonald

10:45 181. **CORRELATION OF ENZYMIC ACTIVITIES WITH PROCESSING CONDITIONS OF CORN PRODUCTS FOR CSM**
H. W. Gardner and G. E. Inglett

11:05 182. **COMPUTER USE IN OPTIMIZING COMPOSITIONS OF CEREAL-BASED FOODS FOR DEVELOPING COUNTRIES**
G. E. Inglett, J. F. Cavins, W. F. Kwolek, and J. S. Wall

11:25 183. **VARIABILITY IN THE LYSINE CONTENT OF WHEAT, RYE, AND TRITICALE**
E. Villegas, C. E. McDonald, and K. A. Gilles

WEDNESDAY MORNING—APRIL 3

9:00 A.M.—International Ballroom East

PROTEIN-ENRICHED CEREAL FOODS FOR WORLD NEEDS I

Chairman—**M. Milner,** UNICEF, United Nations, New York, N. Y.

9:00 **CHAIRMAN'S REMARKS**

9:05 **INTRODUCTORY STATEMENT**
Mrs. Dorothy Jacobson, Asst. Secretary for International Affairs, USDA, Washington, D.C.

9:15 184. **INFANT AND CHILD MALNUTRITION IN DEVELOPING COUNTRIES AND THE NEED FOR PROTEIN-RICH CEREAL FOODS**
W. H. Sebrell, Jr.

9:55 185. **NUTRITIONAL VALUE OF CEREAL PROTEINS**
D. M. Hegsted

10:35 186. **NEW OR UNCONVENTIONAL PROTEINS FOR CEREAL FOOD SUPPLEMENTATION**
M. Milner

11:15 187. **FORMULATION AND TESTING OF WEANING AND SUPPLEMENTARY FOODS CONTAINING OILSEED PROTEINS**
R. Bressani

WEDNESDAY MORNING—APRIL 3

9:00 A.M.—Jefferson Ballroom East

SYMPOSIUM: SPECTROSCOPY AND X-RAY DIFFRACTION I

Chairman—**R. T. O'Connor,** USDA Southern Regional Research Laboratory, New Orleans, La.

9:00 188. **THE GREAT VARIETY OF SPECTROSCOPIC TECHNIQUES AVAILABLE FOR THE ANALYSIS OF FATTY ACIDS AND LIPIDS**
Robert T. O'Connor

9:40 189. **NEUTRON ACTIVATION ANALYSIS AND ITS APPLICATION TO THE ANALYSIS OF FOOD PRODUCTS**
Vincent P. Guinn

10:20 190. **APPLICATION OF WIDE-LINE N-M-R TO ANALYSIS OF CEREAL PRODUCTS AND FATS AND OILS**
W. D. Pohle and R. L. Gregory

11:00 191. **HIGH RESOLUTION N-M-R SPECTROSCOPY**
C. Y. Hopkins

11:40 192. GAS-LIQUID CHROMATOGRAPHY OF LIPIDS, CARBOHYDRATES, AND AMINO ACIDS
S. F. Herb

WEDNESDAY AFTERNOON—APRIL 3
1:30 P.M.—Crystal Ballroom
BAKING II
Chairman—K. H. Tipples, Board of Grain Commissioners, Winnipeg, Canada

1:30 INTRODUCTORY REMARKS

1:35 193. A RAPID, STABLE BREW CONCENTRATE FOR WHEY/CYSTEINE BREAD METHODS
R. G. Henika and Marlene R. Henselman

1:55 194. RESEARCH WITH A PILOT SCALE CONTINUOUS BREADMAKING UNIT. V. EFFECT OF BREW FERMENTATION TIME AND MAKEUP ON FLAVOR OF CONTINUOUS PROCESS BREAD
S. Redfern, H. Gross, R. L. Bell, and F. Fischer

2:15 195. RESEARCH WITH A PILOT SCALE CONTINUOUS BREADMAKING UNIT. VI. BUFFERING EFFECTS OF CALCIUM PROPIONATE IN NO-FLOUR BREWS
H. Gross, S. Redfern, R. L. Bell, and F. Fischer

2:35 196. FARINOGRAPH STUDIES ON THE EFFECT OF VARIOUS OXIDIZING AGENTS IN THE SPONGE-AND-DOUGH SYSTEM
K. Ikezoe and K. H. Tipples

2:55 BREAK

3:20 197. WHITE LAYER CAKE STUDIES INVOLVING USE OF VARIOUS SHORTENINGS
D. D. Disch, A. M. Swanson, and C. H. Amundson

3:40 198. EFFECT OF PROTEOLYTIC ACTIVITY OF FLOURS ON RHEOLOGY OF CONTINUOUS MIX DOUGHS
A. M. Swanson and R. D. Marshall

WEDNESDAY AFTERNOON—APRIL 3
1:30 P.M.—Jefferson Ballroom West
CARBOHYDRATES
Chairman—W. J. Hoover, Kansas State University, Manhattan, Kans.

1:30 INTRODUCTORY REMARKS

1:35 199. PROPERTIES OF WAXY AND ISOGENIC NON-WAXY RICES DIFFERING IN STARCH GELATINIZATION TEMPERATURE
B. O. Juliano, M. B. Nazareno, and N. B. Ramos

1:55 200. NEW STARCHES: PROPERTIES OF FIVE VARIETIES OF COW PEA (VIGNA SINENSIS) STARCH
E. Tolmasquim, A. M. N. Corrêa, and S. T. Tolmasquim

2:15 201. BARLEY STARCH. III. A STUDY OF THE STARCH PROPERTIES OF 30 BARLEY GENOTYPES
K. J. Goering, E. Eslick, C. A. Watson, and Juin Keng

2:35 202. NEW STARCHES. IV. THE PROPERTIES OF THE STARCH FROM TYPHA LATIFOLIA
K. J. Goering and Yolanda A. Rigault

2:55 203. STARCH-COMPLEXED LIPIDS: QUALITATIVE DEPENDENCE UPON TWO SOLVENT SYSTEMS
S. Rogols, J. E. Green, and R. L. High

3:15 BREAK

3:35 204. GLYCOLIPIDS—THEIR DISTRIBUTION, CHEMISTRY, AND ROLE IN BREADMAKING
Y. Pomeranz and K. F. Finney

4:15 205. VISCOSITY DEVELOPMENT IN STARCH: A METHOD FOR EVALUATING EFFECTS OF STARCH TYPE, COOKING TEMPERATURE, SLURRY CONCENTRATION, AND PH
J. E. Freeman and W. J. Verr

4:35 206. THE SUGARS OF WHEAT BRAN
Robin M. Saunders and H. G. Walker, Jr.

CARBOHYDRATES DIVISION BUSINESS MEETING IMMEDIATELY AFTER SESSION

WEDNESDAY AFTERNOON—APRIL 3
1:30 P.M.—Lincoln Ballroom West
LIPOLYTIC ENZYMES
Chairman—R. G. Jensen, University of Connecticut, Storrs, Conn.

1:30 207. CHARACTERIZATION OF MILK LIPASE
W. J. Harper and T. J. Gaffney

1:50 208. EFFECT OF EPINEPHRINE ON CARDIAC LIPOPROTEIN LIPASE ACTIVITY
S. Mallov and Adawia Alousi

2:10 209. STUDIES WITH CLEARING FACTOR. VI. FRACTIONATION OF LIVER LIPASES ON SEPHADEX G-200
A. N. Payza, H. B. Eiber, and S. Walters

2:30 210. ERRORS IN DETERMINING THE STRUCTURE OF TRIGLYCERIDES BY ENZYMATIC HYDROLYSIS
F. H. Mattson and R. A. Volpenhein

3:10 211. SOME FACTORS INFLUENCING ADIPOSE TISSUE LIPOLYSIS
J. N. Pereira

3:30 212. ACID LIPASE OF THE CASTOR BEAN
R. L. Ory

3:50 213. ON THE STRUCTURE OF SOME BACTERIAL POLYGLYCERO-PHOSPHATIDES-PHOSPHOLIPASE A HYDROLYSIS EXPERIMENTS
Robert W. Walker and Gordon L. Howard

4:10 214. SUITABILITY OF GEOTRICHUM CANDIDUM LIPASE FOR THE STEREOSPECIFIC ANALYSIS OF SOME TRIGLYCERIDES
J. Sampugna and R. G. Jensen

4:30 215. ISOLATION AND STUDY OF BOVINE PANCREATIC LIPASE
I. M. Khan and K. M. Shahani

4:50 216. THE PHYLOGENY OF LIPASE SPECIFICITY
D. L. Berner and E. G. Hammond

WEDNESDAY AFTERNOON—APRIL 3
1:30 P.M.—Georgetown Ballroom West
SYMPOSIUM: PRACTICAL APPLICATIONS OF CHROMATOGRAPHY IN LIPID ANALYSES III
Chairman—G. Y. Feldman, Baylor University College of Medicine, Waco, Texas

1:30 217. THE ROHRSCHNEIDER METHOD OF COLUMN CLASSIFICATION
Walter Supina

2:30 218. CONTAMINANTS IN EDIBLE FATS AND OILS
David Firestone

3:30 219. IDENTIFICATION OF METHYL ESTERS BY RETENTION DATA
Robert Ackman

4:30 220. THIN-LAYER AND GAS CHROMATOGRAPHIC ANALYSIS OF THE NATURAL LIPIDS THAT ARE FOUND BETWEEN THE TRIGLYCERIDES AND HYDROCARBON SPOTS IN TLC PLATES
Nicholas Pelick, L. Newton, and M. S. Shamma

WEDNESDAY AFTERNOON—APRIL 3
1:30 P.M.—International Ballroom East
PROTEIN-ENRICHED CEREAL FOODS FOR WORLD NEEDS II
Chairman—M. Milner, UNICEF, United Nations, New York, N. Y.

1:30 INTRODUCTORY REMARKS

1:35 221. NUTRITIONAL EVALUATION IN INFANTS OF NEW PROTEIN FOODS
G. Graham

2:15 222. FORMULATED CEREAL FOODS IN THE FOOD FOR PEACE PROGRAM
F. R. Senti

2:55 223. LOW-COST PROTEIN FOODS: FORTIFIED CEREALS AND NEW PROTEIN BEVERAGES
A. M. Altschul

3:35 224. PROBLEMS IN ACCEPTANCE AND MARKETING
S. M. Cantor

WEDNESDAY AFTERNOON—APRIL 3
1:30 P.M.—Thoroughbred Room
SALMONELLA
Chairman: T. J. Potts, Ralston Purina Co., St. Louis, Mo.

1:30 225. SALMONELLOSIS
James H. Steele

1:50 226. SALMONELLA ECOLOGY
V. D. Foltz

2:10 227. THE SALMONELLA PROBLEM FROM AN ENFORCEMENT STANDPOINT
Kenneth R. Lennington

2:30 228. **PROFILE OF A SALMONELLA TESTING METHOD**
James Rutledge

2:50 229. **GLASSWARE APPARATUS FOR SALMONELLA TESTING**
George J. Banwart

3:10 230. **SALMONELLA RESERVOIRS IN ANIMALS AND FEEDS**
E. M. Ellis

3:30 231. **HUMANS AND PETS AS SOURCES OF SALMONELLA**
Mildred M. Galton

3:50 232. **BACTERIOLOGICAL PROBLEMS IN THE MANUFACTURE OF OILSEED PROTEINS**
R. Paul Elliott

4:10 233. **A METHOD OF DESTROYING SALMONELLA**
O. H. M. Wilder

4:30 234. **CONTROL OF SALMONELLA IN EGG PROCESSING**
D. H. Berquist and Richard Forsythe

4:50 235. **FACTORS AFFECTING THE HEAT RESISTANCE OF SALMONELLA**
Henry Ng

5:10 236. **SALMONELLA IN RELATION TO AGRICULTURE**
E. E. Saulmon

WEDNESDAY AFTERNOON—APRIL 3
2:00 P.M.—Jefferson Ballroom East

SYMPOSIUM: SPECTROSCOPY AND X-RAY DIFFRACTION II

Chairman—**R. T. O'Connor**, USDA Southern Regional Research Laboratory, New Orleans, La.

2:00 237. **ATOMIC ABSORPTION SPECTROSCOPY**
Biagio Piccolo and Robert O'Connor

2:40 238. **THE ROLE OF CRYSTALLOGRAPHY IN THE STUDY OF TRIGLYCERIDES**
C. W. Hoerr and F. R. Paulicka

3:20 239. **APPLICATIONS OF INFRARED ABSORPTION SPECTROSCOPY IN THE ANALYSIS OF LIPIDS**
N. K. Freeman

4:00 240. **SOME APPLICATIONS OF MASS SPECTROMETRY TO THE ELUCIDATION OF STRUCTURE**
Ralph T. Holman and Kwok Sun

4:40 241. **A REVIEW OF COMPUTER-AIDED SPECTROSCOPY**
R. O. Crisler

WEDNESDAY EVENING—APRIL 3
6:30 P.M.—Terrace Level

ALLIED TRADES PARTY

7:30 P.M.—International Ballroom

BANQUET AND DANCE

THURSDAY MORNING—APRIL 4
9:00 A.M.—Georgetown Ballroom East

ANTIOXIDANTS

Chairman—**D. Firestone**, Division of Food Chemistry, Food and Drug Administration, Washington, D. C.

9:00 242. **TOCOPHEROL DESTRUCTION AND PEROXIDE FORMATION IN ETHYL OLEATE, LINOLEATE, LINOLENATE, AND ARACHIDONATE**
L. A. Witting

9:20 243. **VITAMIN E IN FOODS: DETERMINATION OF TOCOLS AND TOCOTRIENOLS**
H. T. Slover, J. Lehmann, and R. J. Valis

9:40 244. **ANTIOXIDANTS IN BIOLOGICAL SYSTEMS**
H. H. Draper

THURSDAY MORNING—APRIL 4
9:00 A.M.—Lincoln Ballroom West

BIOLOGY AND NUTRITION

Chairman—**F. Quackenbush**, Purdue University, Lafayette, Ind.

9:00 245. **PROGRESSIVE CHANGES IN THE COMPOSITION OF THE LIPIDS OF THE MAJOR ORGANS OF IMMATURE RATS AFTER HYPOPHYSECTOMY**
B. Jensen and O. S. Privett

9:20 246. **FATTY ACID COMPOSITION OF ADULT SCHISTOSOMA MANSONI**
Thomas M. Smith, H. B. White, Jr, and T. J. Brooks, Jr.

9:40 247. **DETOXIFICATION OF CASTOR MEAL AND ITS UTILIZATION IN LIVESTOCK RATIONS**
Jim Ridlehuber and Charles Hay

10:00 248. **THE EFFECT OF TEMPERATURE ON THE ARTIFACTUAL FORMATION OF METHYL ESTERS IN MOUSE LIVER**
Jon J. Kabara and Etienne Mueller, Sr.

10:20 249. **THE KETOGLUTARATE SHUNT AND LIPID SYNTHESIS IN THE BRAIN OF THE NEONATAL RAT**
A. F. D'Adamo, J. C. Smith, and G. Frigyesi

10:40 250. **LIPID DEPLETION AND DNA CONTENT OF FAT CELLS FROM EPIDIDYMAL FAT PADS IN COLD-EXPOSED RATS**
Donald Therriault and Roger Hubbard

11:00 251. **GLYCEROKINASE ACTIVITY OF THE ISOLATED EPIDIDYMAL FAT CELL OF THE RAT**
Roger Hubbard, Paul Voorheis, and Donald Therriault

THURSDAY MORNING—APRIL 4
9:00 A.M.—Jefferson Ballroom East

COMPOSITION OF WHEAT AND WHEAT PRODUCTS

Chairman—**W. A. Gortner**, Human Nutrition Research Division, ARS, USDA, Beltsville, Md.

9:00 **INTRODUCTORY REMARKS**

9:05 252. **DESCRIPTION OF SAMPLES OF WHEATS AND WHEAT PRODUCTS FOR MULTINUTRIENT ANALYSES**
F. N. Hepburn and J. H. Tulloss

9:25 253. **EXTRACTABLE AND "BOUND" FATTY ACIDS IN WHEAT AND WHEAT PRODUCTS**
F. W. Quackenbush and J. A. Inkpen

9:45 254. **B-VITAMINS IN WHEATS AND WHEAT PRODUCTS**
Marilyn M. Polansky, E. W. Toepfer, and F. N. Hepburn

10:05 255. **DISTRIBUTION OF MANGANESE, COPPER, NICKEL, ZINC, AND MAGNESIUM IN WHEATS AND WHEAT FOOD PRODUCTS**
Elizabeth G. Zook and Florida E. Greene

10:25 256. **TOCOPHEROLS IN WHEATS AND WHEAT PRODUCTS**
H. T. Slover, Joanna Lehmann, and R. J. Valis

10:45 257. **ANALYSIS OF FREE STEROLS AND SATURATED STEROL ESTERS ISOLATED FROM DIFFERENT FLOUR MILL STREAMS**
C. P. Berry, V. L. Youngs, and K. A. Gilles

THURSDAY MORNING—APRIL 4
9:00 A.M.—Lincoln Ballroom West

DRYING OILS AND PAINT

Chairman—**R. Austin**, North Dakota State University, Fargo, N. Dak.

9:00 258. **REACTION OF ALDEHYDE OILS WITH A RESINOUS POLYOL STYRENE ALLYL ALCOHOL COPOLYMER**
A. E. Rheineck and P. R. Lakshmanan

9:20 259. **URETHANE COATINGS BASED ON ALDEHYDE OILS**
A. E. Rheineck and P. R. Lakshmanan

9:40 260. **PRODUCTION OF VINYLIC UNSATURATION BY PARTIAL DEHYDROBROMINATION OF VICINAL DIBROMO FATTY DERIVATIVES**
A. E. Rheineck and B. Sreenivasan

10:00 261. **RESIN-CATALYZED EPOXIDATION OF METHYL UNDECYLENATE**
A. E. Rheineck, B. Sreenivasan, and Joel Bender

10:20 262. **GLYCEROLYSIS OF LINSEED OIL: A COMPOSITIONAL STUDY**
A. E. Rheineck, B. Sreenivasan, and Richard Bergseth

THURSDAY MORNING—APRIL 4
9:00 A.M.—Crystal Ballroom

SURFACTANTS IN FOODS

Chairman—**I. A. MacDonald**, Ashland Chemical Co., Minneapolis, Minn.

9:00 **INTRODUCTORY REMARKS**

9:05 263. **EFFECT OF CIS-TRANS ISOMERS AND RELATED PHYSICAL PROPERTIES OF MONOUN-**

SATURATED LIPIDS ON SHORTENING POWER
Joyce Ostrander and Ada Marie Campbell

9:25 264. EXPERIENCE WITH SOME MIXTURE DESIGNS —OPTIMIZATION OF EMULSIFIER BLENDS IN BAKERS' SHORTENING
R. P. Basson and D. T. Rusch

9:45 265. THE FUNCTIONS OF SODIUM STEAROYL-2-LACTYLATE IN YEAST-LEAVENED AND CHEMICALLY LEAVENED BAKED PRODUCTS
R. J. Tenney and D. M. Schmidt

10:05 266. THE EFFECTS OF SODIUM STEAROYL-2-LACTYLATE ON PASTE VISCOSITY OF FOOD STARCHES
R. J. Tenney, M. W. Ward, and R. N. Van Vactor

10:25 267. CHROMATOGRAPHIC ANALYSIS OF SORBITAN FATTY ACID ESTERS
M. Sahasrabudhe

10:45 268. THE EFFECT OF VARIOUS MESOMORPHIC PHASES OF MONOGLYCERIDE:WATER SYSTEMS IN STARCH PRODUCTS
N. Krog

THURSDAY MORNING—APRIL 4

9:00 A.M.—Georgetown Ballroom West

SYMPOSIUM: PRACTICAL APPLICATIONS OF CHROMATOGRAPHY IN LIPID ANALYSES IV

Chairman—**G. L. Feldman**, Baylor University College of Medicine, Waco, Texas

9:00 269. RAPID GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF METHYL ESTERS
Milton D. Jellum

10:00 270. FRACTIONATION AND ANALYSIS OF GLYCERIDES AND RELATED LIPIDS BY REVERSED-PHASE PARTITION CHROMATOGRAPHY
Orville S. Privett, Lowell J. Nutter, E. C. Nickell, and Roland Gross

THURSDAY MORNING—APRIL 4

11:30 A.M.—Jefferson East

ANNUAL AACC BUSINESS MEETING

CALL TO ORDER

MINUTES OF THE 1967 MEETING ......Donald K. Dubois
Board of Directors ....Kenton L. Harris
Treasurer ....Edwin J. Bass
Executive Vice President ....Raymond J. Tarleton
Scientific Editor ....Welker G. Bechtel
Director of Publications ....Merrill J. Busch
Technical Policy Committee ....Wilbur C. Schaefer
(Covering reports of the various technical committees)

Administrative Committees
Published Reports (April 1968 CST)..Donald K. Dubois
Unpublished Reports ....Committee Chairmen

NEW BUSINESS

ADJOURNMENT

ABSTRACTS OF PAPERS

1

A RAPID METHOD OF DETECTING AFLATOXINS IN PEANUTS. Charles E. Holaday, ARS, USDA, Albany, Georgia.

The thin-layer chromatographic procedure for detecting and quantifying aflatoxin in peanuts is too time-consuming, costly, and difficult to be practical for use by untrained personnel. Colorimetric and fluorometric methods, also, were found to be impractical and to lack sensitivity. A method based on milli-column chromatography was tested and found to be both rapid and simple. The milli-column is developed in a chloroform-methanol-acetone extract of a peanut sample. If afflatoxin is present, a blue fluorescent band at the lower end of the column is observed when the column is exposed to long-wave radiation. Sensitivity is in the order of 10-20 ppb, and an assay can be completed in 10-15 min. The columns are prepared by filling a length of 4-mm. glass tubing with silica gel to a depth of about 4.5 cm. Columns are plugged at both ends with glass fiber filter paper to hold the silica gel in place. Before using, the columns are equilibrated in an atmosphere of 80% relative humidity. Some degree of quantification is possible by comparison with columns developed in extracts with known aflatoxin contents.

2

A RAPID AND PRECISE PHYSICOCHEMICAL ASSAY METHOD FOR AFLATOXIN IN PEANUTS AND PEANUT PRODUCTS. Arthur E. Waltking, George Bleffert, and Mary Kiernan, Corn Products Company, Bayonne, New Jersey.

A practical, short-cut, sensitive method for rapidly determining aflatoxin in peanuts and peanut butter has been developed. This was in response to the need to reduce the time required for analyses of peanut products in process. Through reductions in solvent volumes, utilization of pressure filtration for clarification, and substitution of liquid/liquid extraction for a lengthy column cleanup, equivalent results are possible in ⅓ the time required for the current official procedure. Sensitivity, reproducibility, and accuracy are comparable to the current method for raw nuts and peanut butter. It is now possible to analyze a given ground sample of peanuts within a period of less than 90 min. Hence, the manufacturer can now do a better policing job than previously possible in assuring his peanut butter production to be free of aflatoxin.

3

NONINTERFERENCE OF SEED COAT CONSTITUENTS OF COTTON SEEDS IN A MODIFIED PONS-GOLDBLATT AFLATOXIN PROCEDURE. T. C. Campbell and J. H. Snider, Virginia Polytechnic Institute, Blacksburg, Virginia, and P. B. Marsh, ARS, USDA, Beltsville, Maryland.

The normal constituents of seed coats and fibers of fuzzy cotton seeds were not found to interfere with aflatoxin analysis of embryos (meats). Several modifications of the Pons and Goldblatt (*J. Am. Oil Chem. Soc.* *42*:471, 1965) procedure for aflatoxin analyses in cottonseed are presented which aid not only in making better defined spots, but also improve TLC resolution. The so-called blue spot of Ashworth *et al.* (*J. Am. Oil Chem. Soc.* *44*:394, 1967) was not found to either partially or totally obscure

aflatoxin B_1 on the plates, as claimed by these workers. An improved method of aflatoxin estimation on TLC plates is presented.

4

A SIMPLIFIED PROCEDURE FOR THE DETERMINATION OF AFLATOXIN B_1 IN COTTONSEED MEALS. JAMES VELASCO, ARS, USDA.

A simplified procedure for the detection of aflatoxin B_1 and B_2 in cottonseed meals has been developed. The procedure substantially reduces the time and cost of aflatoxin analysis. A single chromatographic column of celite is used to concentrate and purify the aflatoxin fractions. The number of samples which can be screened for aflatoxin contamination on 1 TCL plate is doubled by reuse of the plate. The use of technical reagents and stainless-steel beakers also helps to reduce the time and cost of analysis. The procedure is sensitive to about 5 μg of B_1 per Kg. of meal.

5

IMPROVED OBJECTIVE FLUOROMETRIC DETERMINATION OF AFLATOXINS IN COTTONSEED PRODUCTS. WALTER A. PONS, JR., ALVA F. CUCULLU, A. O. FRANZ, and L. A. GOLDBLATT, Southern Utilization Research and Development Division, ARS, USDA, New Orleans, Louisiana.

The aqueous acetone-lead acetate-silica gel method for aflatoxins in cottonseed products (*J. Am. Oil Chem. Soc. 49*:554, 1966) has been modified to give essentially complete recovery of added B and G aflatoxins, and to incorporate a more accurate and precise densitometric evaluation of aflatoxins on TLC plates. Extraction with 85% acetone containing acetic acid improved recovery of added aflatoxins B_1, B_2, G_1 and G_2, and the lead acetate purification step was shortened to eliminate use of centrifugation and the washing of lead precipitates. Use of a new type of silica gel and a chloroform:acetone elution solvent in the column cleanup step improved recovery of aflatoxins. TLC separations were improved by use of a new silica gel, and TLC development with chloroform: acetone:2-propanol. Each phase of the procedure was evaluated by fluorescence densitometry on TLC plates.

6

AN IMPROVED SEPARATION OF AFLATOXINS. MABRY WILEY and A. C. WAISS, Western Regional Research Laboratory, ARS, USDA, Albany, California.

After precipitation with hexane of crude aflatoxin from a chloroform extract of *Aspergillus flavus* cultures, the crude mixture was chromatographed on 100-200 mesh silica gel columns, using ethyl acetate as eluant. On this column, there was no separation of aflatoxins from each other, but most of the brown, oily material is removed. The next step in the purification was chromatography on 100-200 mesh silica gel columns with chloroform and 2% methanol in chloroform as eluants. A large part of the B_1 was purified, but B_2, G_1, and G_2 did not separate, and M_1 had a brown oil that prevented crystallization. The M_1 was cleaned up by chromatography on Sephadex LH-20 with chloroform; the brown material was retained while the M passed through. The separation of aflatoxin B_2, G_1, and G_2 was achieved by column chromatography on very fine silica gel—Silica Gel H for TLC—with chloroform as eluant. The purity and identity of the compounds was established by 100 MC NMR.

7

SEPARATION AND PURIFICATION OF AFLATOXINS AND COMPARISON OF SEMI-SYNTHETIC AFLATOXINS B_2 AND G_2 WITH NATURALLY OCCURRING AFLATOXINS B_2 AND G_2. J. V. RODRICKS, Food and Drug Administration, Washington, D. C.

Crude extracts of *Aspergillus flavus* cultures containing aflatoxins B_1, B_2, G_1, and G_2 were passed rapidly through an acid alumina column using benzene and benzene/chloroform, I/I, V/V, as the eluting solvents. Two major fractions were obtained; 1 contained aflatoxins B_1, B_2, and G_1, and a trace of aflatoxin G_2; the second contained aflatoxin G_2 and a number of low R_f materials. This last fraction has not been successfully resolved. Slow evaporation to a small volume of the benzene solution containing the 4 aflatoxins afforded a colorless, crystalline material which was a mixture of all 4 aflatoxins. Silica gel column chromatography of the crystalline mixture (400 g. silica gel for 1 g. aflatoxin) using benzene, benzene/chloroform gradient, chloroform, and chloroform/methanol gradient as the elution series gave fractions containing aflatoxins B_2, aflatoxins B_1 and B_2, aflatoxins B_2 and G_1, aflatoxin G_1, and aflatoxin G_2. A solvent flow rate of c. 0.5 ml./hr./g. silica gel was maintained for effective separation. Fractions which contained aflatoxin B_1, when taken to dryness, yielded a colorless residue which could be easily crystallized from chloroform; the same procedure was effective for aflatoxins G_1 and G_2. Rechromatography of the fractions which contained aflatoxins B_1 and B_2 and aflatoxins B_2 and G_1, using the same column conditions, afforded separation of these mixtures. Subsequent purification of aflatoxin B_2 was carried out as with the other 3 toxins. The factors governing the separation, along with a discussion of the amounts of toxins obtained by this procedure, will be included. There will also be a presentation of criteria of purity for the 4 aflatoxins. Partial hydrogenation of aflatoxins B_1 and G_1 afforded semi-synthetic aflatoxins B_2 and G_2, respectively. The semi-synthetic toxins proved to be physically indistinguishable from the naturally occurring aflatoxins B_2 and G_2, and toxicologically indistinguishable in the chicken embryo.

8

CONDITIONS AND TECHNIQUES FOR THIN-LAYER CHROMATOGRAPHY OF AFLATOXINS. STANLEY NESHEIM, Food and Drug Administration, Washington, D. C.

Possible causes of poor resolution were investigated, and optimum conditions for the TLC were sought. Poor resolution and spot tailing in chromatograms were most frequently attributable to unknown quality factors in the commercial silica gel-$CaSO_4$ adsorbent preparations. Variation in quality even from container to container within the same lot was observed. Other variables found to affect the TLC to a lesser degree were: adsorbent particle size, concentration and nature of the $CaSO_4$ binder, silica gel layer thickness, moisture content of the layer at the time of development, vapor phase composition in developing chamber, temperature during development, and trace water in some developers. Variables which appeared to have little or no effect were: heating of the silica gel-$CaSO_4$ powder prior to slurry preparation, speed of spreading the slurry, type of spreader, aging of wet plates before drying, "leveling" of wet plates by agitation on a mechanical shaker, ethanol concentrations of 1-2% in the $CHCl_3$ used for "spotting," evaporation aids for "spoting," spotting under N, rate of "spotting" sample solution, size of developing chamber (0.40-20 L), development distance (10-15 cm.), and reuse of the developing solvent. The commonly used developing solvents, 10/90, acetone/chloroform; 7/93, methanol/chloroform; and upper phase of 46/35/19, benzene/ethanol/water, give good to excellent separations. Other solvents which also were found to give good to excellent separations were: 100/10/10, benzene/methanol/acetic acid; 80/9/10, benzene/acetone/acetic acid; 10/90, acetic acid/benzene; 90/25/4, benzene/dioxane/acetic acid; and 10/90, acetone/chloroform/ether. In this last combination, the chloroform/ether ratio can be varied from 30/10 to 10/80.

9

STARCH DAMAGE AND ALPHA-AMYLASE AS A BASIS FOR MATHEMATICAL MODELS RELATING TO FLOUR WATER ABSORPTION. E. A. FARRAND, Ranks Hovis McDougall Ltd., London, England.

The relative amounts of water absorbed by the main components of flour are postulated as major factors contributing to the rheological properties of dough and to flour quality attributes in terms of baking characteristics. Dough formation is considered a problem of hydrating protein to form gluten, disaggregation of flour particles, and spreading the gluten over the starch to form a continuous matrix. Damaged starch is introduced in terms of arbitrarily measured absorption characteristics and consequent changes in surface area to be covered by the gluten. The relationship is derived expressing the optimum level of starch damage as a function of protein content. The absorption is finally expressed in terms of protein, moisture, and starch damage, the calibrating constants being obtained from a statistical evaluation of the Brabender Farinograph absorption for reference flours at optimum starch damage. The mathematical model estimates flour-water absorption with similar precision to routine Brabender Farinograph measurements, but has the advantage of giving information concerning the nature of the absorption. An ancillary equation is also described, relating optimum levels of alpha-amylase to the level of starch damage, thereby ensuring doughs with adequate gassing power and minimum rheological instability.

10

STATISTICAL EVALUATION OF THE RELATIONSHIP OF VARIOUS LEVELS OF NONFAT DRY MILK AND EMULSIFIERS TO CRUMB FIRMNESS. J. K. SEIBEL and A. M. SWANSON, University of Wisconsin, Madison, Wisconsin.

A series of experiments were planned and completed to evaluate the effects of nonfat dry milk (NFDM) and emulsifiers on staling and toasting properties of bread made by both sponge-and-dough and liquid-ferment methods. Four levels of NFDM, 0, 2, 4, and 6%; three levels of emulsifiers (mono- and diglycerides), 0, 0.6, and 1.2%; and two methods of breadmaking, sponge-and-dough and liquid-ferment, by laboratory and pilot procedures were used. All possible combinations of NFDM and emulsifier were run in a random manner for each of the breadmaking procedures. At the 0.0% emulsifier level, 3.0% fat (lard) was used. As emulsifier was added, the fat-emulsifier level remained at 3%. The previously established formulas and procedures for making bread by laboratory and pilot methods for sponge-and-dough and liquid ferment bread were used. The bread was allowed to cool on the racks for 1 hr. after baking; then they were placed in polyethylene bags and held in the bake shop for the various storage times. The temperature of the air-conditioned shop was 72° F. Evaluations were made on the freshly sliced loaves after holding for 18, 42, and 72 hr. for the pilot (10 loaf doughs) procedure, and 18 and 42 hr. for the laboratory (4 loaf doughs) procedure. Compressibility and toasting properties evaluations were made. The data were subjected to statistical analysis by factorial analysis and analysis of variance. The liquid-ferment procedure appears to have real value in producing softer bread. The level of milk had a very highly significant effect, and at the 6% level, NFDM was highly desirable. Emulsifier addition was found to be the only significant factor in reducing the compressibility of sponge-and-dough bread. The data for milk in sponge-and-dough bread were found not to be significant.

11

TEMPERATURE, MIXER RPM, AND SALT EFFECTS ON FARINOGRAPH CHARACTERISTICS. J. F. CONN and T. P. KICHLINE, Monsanto Company, St. Louis, Missouri.

Operating temperature of the Farinograph was increased from 30 to 40°C. to correspond with continuous mix dough temperatures. The increased temperature reduced curve height. However, normal curve height without reduction in amount of water used was achieved by increasing mixer rpm. Two curve peaks were observed in all flour tests made at 40°C. over a wide range of mixer speeds. The first peak consistently occurred early in mixing and may be related to hydration of the flour. The second peak occurred considerably later in mixing and appeared to be related to gluten development. Farinograph mixing time was greatly increased by addition of salt, and the increase was directly related to the quantity of salt added.

12

OXIDATION OF SULFHYDRYL GROUPS OF FLOUR BY BROMATE UNDER VARIOUS CONDITIONS AND DURING THE BREADMAKING PROCESS. C. C. TSEN, American Institute of Baking, Chicago, Illinois.

Bromate is a slow oxidizing agent for cysteine and glutathione, particularly for the sulfhydryl group of flour. The oxidation of sulfhydryl groups in flour-water suspension by bromate can be increased slightly by lowering the suspension's pH, but markedly by raising the suspension's temperature. This temperature effect is confirmed with the determination of sulfhydryl groups oxidized by bromate in nonfermented and fermented doughs heated at various temperatures. It is also supported by the results of baking tests with bromated and non-bromated doughs, whether fermented or not. The effect of dough fermentation on the oxidation by bromate is insignificant. The present findings indicate the major bromate effect on oxidation of sulfhydryl groups occurs when dough is heated during the early stage of baking. The synergistic action through the use of a combination of flour improvers is discussed.

13

EFFECT OF FLOUR DAMAGED STARCH LEVEL IN VARIOUS BREADMAKING METHODS. K. H. TIPPLES and R. H. KILBORN, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, Canada.

An Alpine pin mill was used to increase the levels of damaged starch in several flours from Canadian hard red spring wheat. Preliminary experiments were carried out to determine the pin-mill speeds required to give the required increments of increased starch damage. The range of starch damage level studied was 19-34% by the Farrand method. Four baking methods were used: the GRL Remix method, a sponge-and-dough method, and two batch mechanical development methods, one for making English Chorleywood-type bread and the other for making bread of high specific volume. For each method the flour samples were baked with and without malt syrup. Changes in optimum baking absorption, as judged by handling properties of doughs at panning, were noted, and each sample tested was baked at several levels of absorption. In the Remix method, involving a vigorous 2¾-hr.

fermentation time with 1% salt and 3% yeast, no increase in baking absorption was obtained by increasing damaged starch. In the presence of malt, which is necessary for achievement of maximum volume potential by this method, optimum baking absorption tended to decrease at higher levels of damaged starch. In the sponge-and-dough method, baking absorption increased with increasing damaged starch in the absence of malt, whereas in the presence of malt, absorption was in some cases decreased. By the mechanical development processes involving no period of bulk fermentation, baking absorption increased with increasing damaged starch. Malt was not necessary for achieving maximum volume potential, and higher levels of absorption were possible in the absence of malt. In many cases, high levels of damaged starch had a marked effect on the mixing curves; the significance of these changes is discussed.

14

USE OF LEVULOSE-CONTAINING CORN SYRUP IN YEAST-LEAVENED BAKED GOODS. S. REDFERN and H. GROSS, The Fleischmann Laboratories, Standard Brands, Inc., Stamford, Connecticut, and B. R. STRONG and E. K. WARDRIP, Clinton Corn Processing Co., Standard Brands Inc.

By the use of a newly developed glucose isomerase enzyme, it is possible to produce a series of corn syrups which contain varying levels of the sugar levulose. The first commercially available syrup of this family is now available for use in baking applications. The characteristics and performance of this new syrup in yeast-leavened goods will be compared with other sweeteners.

15

STEROL METABOLISM AND ATHEROSCLEROSIS IN *CEBUS* MONKEYS. HUGH B. LOFLAND, JR., T. B. CLARKSON, R. W. ST. CLAIR, and N. D. M. LEHNER, Bowman Gray School of Medicine, Winston-Salem, North Carolina.

Seventy-two *Cebus albifrons* monkeys have been maintained on either an atherogenic diet (0.5% cholesterol, 25% lard) or a basal diet, identical except for the absence of cholesterol. Four age and sex groups were represented: young (prepuberal) males and females, and adult, sexually mature males and females. The duration of the experiment was 2 years. Among control monkeys of either age or sex, no differences were observed in serum cholesterol levels. On the other hand, among animals fed cholesterol, over the entire experimental period old male animals had significantly higher levels of serum cholesterol than did the other groups (mean value of 574 mg./100 ml. for old males). Young males had the lowest serum cholesterol values (mean value of 327 mg./100 ml.). Females of either age had cholesterol values that were intermediate between old and young males. Sterol turnover and excretion were studied in these monkeys by following the kinetics of the disappearance of cholesterol-4-^{14}C administered intravenously as labeled lipoprotein. Subgroups of each age and sex were placed in metabolism cages for collection of urine and feces, on which determinations of the excretion rates of labeled bile acids and neutral sterols were made. Results of these studies have indicated that young male monkeys compensate for dietary cholesterol by virtue of an enhanced turnover and more rapid excretion of the products of sterol degradation. Aortic atherosclerosis was exacerbated in the monkeys fed cholesterol, but only to a modest extent. On the other hand, atherosclerosis in the coronary and carotid arteries was strikingly more severe in the animals consuming cholesterol-containing diets. These findings suggest that this species may provide an excellent primate model for the study of coronary and carotid artery atherosclerosis. (Aided by grants from the U.S.P.H.S. H-09357 and H-4371).

16

AUTOMATED TECHNIQUES FOR THE DETERMINATION OF CHOLESTEROL, PHOSPHOLIPIDS, AND TRIGLYCERIDES. GERALD KESSLER, Jewish Hospital of St. Louis, St. Louis, Missouri.

Recent emphasis on atherosclerotic heart disease research has aroused increased interest in measuring various serum lipid fractions. This report describes procedures utilizing an automated system of analysis (Autoanalyzer) for determining cholesterol, phospholipids, and triglycerides on the same isopropanol extract. Serum lipid extracts (1:20) are manually prepared using isopropanol as the extraction solvent. For determining phospholipids, an aliquot of the lipid extract is subjected to hot-acid digestion with sulfuric acid and hydrogen peroxide. Cholesterol can also be measured in the extracts at this point. The serum isopropanolic extracts are then treated with a Zeolite-Lloyd-$CuSO_4$-$Ca(OH)_2$ mixture. Zeolite and Lloyds reagents completely remove phospholipids, while interference from glucose is eliminated by the copper-lime treatment. The treated extracts are now usable for determining triglycerides, and if desired, cholesterol can also be measured. Cholesterol is analyzed using an automated modification of the Zlatkis technique, employing ferric ion, acetic, and sulfuric acids. The ferric chloride-acetic acid reagent is preheated and then the isopropanol extract is added. This is immediately followed by the addition of concentrated sulfuric acid. After mixing, the resulting purple color is measured at 550 mμ, using a 15-mm. flow cell. For the measurement of phospholipids, untreated aliquots of the isopropanol extract undergo hot-acid digestion for destruction of organic material, and conversion of phospholipids to phosphate. The phosphate is then determined by an automated stannous chloride-molybdic acid technique. The colored product is measured at 660 mμ using a 15-mm. flow cell. Triglycerides are quantitated using the Hantzsch condensation reaction between an amine, beta diketone, and an aldehyde. The automated system carries out the saponification of triglycerides to glycerol, oxidation to formaldehyde, followed by condensation with diacetylacetone and ammonia to give a fluorescent product. Fluorescence is measured in a 6-mm. cuvette using a 405-mμ interference filter as the primary and a Corning 3-71 as the secondary filter. Blank determinations are performed by eliminating the saponification step.

17

A STUDY OF TRIGLYCERIDE ANALYSES AS PERFORMED BY 19 LABORATORIES. VIRGINIA S. WHITNER, REBECCA WYLIE, and ROBERT F. WITTER, National Communicable Disease Center, Atlanta, Georgia.

Nineteen laboratories joined a program designed to study the precision and accuracy in the determination of serum triglycerides. The methods employed are all based on the determination of glycerol. One hundred fifty frozen serum samples were to be analyzed by each laboratory during a period of 18 months. These samples were drawn from several serum pools differing in triglyceride concentration. The program is divided into 3 parts. All laboratories have completed the 1st part and 12, the 2nd part (120 analyses during 12 months). This presentation will summarize results to date regarding (1) laboratory precision when samples were analyzed on a given day, on different days, and over a period of several months, and (2) the agreement of triglyceride levels among laboratories.

18

SERUM TRIGLYCERIDE METHODS FOR FIELD STUDIES. GERALD KOBBEL, G. V. A. PANTULU, J. T. ANDERSON, and A. KEYS, University of Minnesota, Minneapolis, Minnesota.

An analytical procedure, Method STG-5, has been developed for determination of triglycerides in blood serum. Florisil 60-80 mesh, adjusted to 7% H_2O is packed into a 4 x 100-mm. column in the stem of a funnel, and washed with 8 ml. of 2:1 chloroform: methanol (CM). Serum, 0.1 ml., is pipetted onto 22 cm.^{2} of ashless filter paper in a 16 x 150-mm. tube. Four 4-ml. portions of CM are shaken with the sample on the paper and passed through the column to remove phospholipids. The eluate is evaporated to dryness saponified with 1 ml. 0.1*N* ethanolic KOH at 60°C. for 30 min. and acidified with 1 ml. of 0.2*N* H_2SO_4. Cholesterol and fatty acids are removed by extraction with 4 ml. of petroleum ether (PE). After 10 min. at 80°C. to remove excess PE, 0.1 ml. of 0.05*M* $NaIO_4$ is added and mixed in. Ten min. later, 0.2 ml. of 0.5*M* Na_3AsO_3 is added. After mixing and 30 min. later, 10 ml. of a mixture of 15 parts of 0.7% aqueous solution of chromotropic acid (disodium salt) and 85 parts of 24*N* H_2SO_4 are added. After 30 min. in a boiling water bath and cooling, the absorbance is read at 570 mμ. Tripalmitin is used as standard. The analyses have been compared with those from the other laboratories participating in the Triglyceride Standardization Program of the National Communicable Disease Center. For use in field studies in distant locations, a method utilizing pipetted serum samples dried on filter paper is used. When fasting serum cannot be obtained, casual samples are taken; the material floated by centrifuging at 20,000 x g. for 1 hr. at 4°C. is discarded, and the subnatant fraction is analyzed.

19

PLASMA TRANSPORT OF MENADIONE. K. E. GUYER, Medical College of Virginia, Richmond, Virginia.

The administration of menadione (vitamin K_3) to animals results in such low circulating levels of the vitamin that transport studies are extremely difficult even with isotopically labeled material. Other investigators have shown that after addition of menadione to a protein solution, not all the menadione may be recovered by extraction. In addition, it has been found that menadione will react with sulfhydryl groups. In the present study a slurry of celite (coated with menadione) in a saline solution was allowed to react with plasma or a plasma protein solution during agitation at 4°C. After removal of particulate menadione and celite by filtration and centrifugation, the solubilized menadione was studied by colorimetric determination and scintillation counting. In order to show the importance of sulfhydryl groups we have investigated the interaction of plasma (or purified proteins) with menadione in the presence of silver ions or *N*-ethyl maleimide. Our results suggest that the major portion of menadione solubilized by plasma is that solubilized by albumin. The presence of sulfhydryl reagents has a negligible effect on the total menadione solubilized under the described conditions, but the irreversibly bound menadione is decreased markedly.

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VOLATILE FREE FATTY ACIDS OF HUMAN SERUM. V. MAHADEVAN and L. ZIEVE, Minneapolis Veterans Hospital, Minneapolis, Minnesota.

Methods for the determination of circulating volatile free fatty acids (VFA) of human blood by gas-liquid chromatography are described. Blood from normal healthy subjects was collected in the fasting state in heparinized tubes and separated into plasma and red cells. The red cells were washed thrice with saline. Aliquots of plasma and red cells were acidified with 0.2 vol. of 1.0*N* H_2SO_4, then homogenized with 20 vol. of chloroform-methanol (2:1, v/v) and filtered. Mixing with 0.2 vol. of water resulted in the formation of a 2-phase system. The upper layer was neutralized with dil. KOH, evaporated to dryness and acidified with phosphoric acid to pH 3.0, and steam-distilled in the presence of $MgSO_4$. Alkalinized distillates were evaporated to dryness, re-acidified with aqueous phosphoric acid, and the aqueous solutions of the VFA were analyzed by gas-liquid chromatography using columns packed with porapak Q. The overall accuracy of the extraction and separation of the acids was tested by using known standard solutions of mixed acids. The mg./100 ml. and the standard deviations of the circulating VFA of the initial 6 normal human plasma and red cells, respectively analyzed were as follows: acetate, 1.64 ± 0.37, and 1.403 ± 0.26; propionate, 0.16 ± 0.02, and 0.19 ± 0.02; isobutyrate, 0.14 ± 0.03, and 0.16 ± 0.003; butyrate, 0.03 ± 0.003, and 0.02 ± 0.003; isovalerate, 0.15 ± 0.08 and nil.

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QUANTITATIVE DETERMINATION OF PHOSPHOLIPID CLASSES IN HUMAN SERUM BY COMBINED THIN-LAYER CHROMATOGRAPHY AND PHOSPHORUS ANALYSIS. J. H. WILLIAMS, M. KUCHMAK, and R. F. WITTER, National Communicable Disease Center, Atlanta, Georgia.

An improved procedure for the quantitative determination of phospholipids in human serum will be presented. The 4 main phospholipid classes—lecithin, sphingomyelin, lysolecithin, and phosphatidyl ethanolamine—are separated on thin-layer chromatoplates with chloroform-methanol-water/acetic acid, 65/45/8/1, v/v/v. The zones of silica gel containing the cleanly separated phospholipids are transferred to small chromatographic tubes equipped with sintered glass filters. The phospholipids are eluted with methanol-chloroform-water, 80/10/10, v/v/v, and phosphorus is determined in the effluent. The average recoveries of phosphorus were, for a reference phospholipid mixture, 96.7%, and for serum extracts, 95.1%.

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NUTRITIONAL AND BIOCHEMICAL EVALUATION OF DEEP-FAT-FRIED SOYBEANS. L. R. HACKLER and W. F. WILKENS, Cornell University, Geneva, New York.

Much research has been conducted on soybean products suitable for human consumption in the last few years. Soy milk and synthetic meat products have received considerable attention. Recently, we have investigated the possibility of soybean "nuts" as a food supplement. This investigation was concerned with the utilization of deep-fat-fried soybeans and changes in amino acids as a result of processing in oil at an initial temperature of 190°C. for 0-5 min. Soybean "nuts" are more nutritious than peanuts and might be an additional source of high-quality, low-cost protein in the developing countries. The results obtained for soybean cotyledons deep-fat fried for 0, 1, 2, 3, 4, and 5 min., fed to young weanling rats to determine protein efficiency ratio, will be discussed, along with changes in amino acids as determined by ion-exchange chromatography.

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THE EFFECT OF PROCESSING CONDITIONS ON THE COMPOSITION OF SOY MILK. W. F. WILKENS and L. R. HACKLER, Cornell University, Geneva, New York.

Soy milk was prepared from whole and dehulled soybeans, both soaked and unsoaked, by a water extraction process at temperatures from 25 to 100°C. Soak treatments consisted of 1, 3, and 5 hr. at 25-65°C. The major effect of soaking on composition was to reduce the carbohydrate and increase lipid content. Protein was relatively unaffected. The longer soaks at higher temperatures reduced solids yield, especially with whole beans. Extraction of unsoaked beans at 25-100°C. (water temperatures) resulted in maximum solids yield at a 60°C. extraction. Nutritional and flavor aspects of the processing conditions will be discussed.

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STEAM PROCESS FOR DEALLERGENIZATION OF CASTOR POMACE. A. C. MOTTOLA, G. O. KOHLER, and H. G. WALKER, JR., Western Regional Research Laboratory, ARS, USDA, Albany, California.

A process to deactivate castor allergens using steam has been developed. Based on biological measurements of residual allergen activity, we find that pomace must be steamed at 175°C. (120 psig) for 1 hr. to reduce allergens to a low level. Lower temperatures require correspondingly longer times. Some changes in the amino acid composition of treated pomace have been found.

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TRYPSIN INHIBITORS IN THE WHOLE WATER-EXTRACTABLE SOYBEAN PROTEINS. T. OBARA, M. K. KOBAYASHI, and T. KOBAYASHI, Tokyo University of Education, Tokyo, Japan.

The authors have reported that the whole water-extractable soybean proteins can be separated into 4 fractions by gel filtration with Sephadex G-200; only the 2S fraction, which was ultracentrifugally homogeneous, showed trypsin inhibition activity. The trypsin inhibition active 2S fraction was further fractionated into 6 fractions by means of DEAE-cellulose column chromatography with gradient elution. Fractions were eluted with 0, 0.003, 0.07, 0.11, 0.14, and 0.21*M* NaCl and consisted of proteins which have different ratios of trypsin inhibition to chymotrypsin inhibition. Compared with the 3 other fractions separated by Sephadex G-200 gel filtration, the trypsin inhibition active 2S fraction is different in amino acid composition, especially in the higher content of sulfur-containing amino acids. Amino acid composition is also different among the fractions obtained by DEAE-cellulose column chromatography; the difference is greatest in sulfur-containing amino acids. Polyacrylamide gel electrophoresis was used to compare the trypsin inhibition active 2S fraction to the fractions separated on DEAE-cellulose columns.

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PREPARATION OF CHEESE-LIKE SOY PROTEIN FOOD BY ENZYMATIC TREATMENT. I. SELECTION OF PROTEOLYTIC ENZYME. T. OBARA, M. K. KOBAYASHI, and M. OHATA, Tokyo University of Education, Tokyo, Japan.

Experiments have been made to produce an easily digestible, cheese-like food, with good flavor, texture-body, and superior keeping quality, from soy milk. The manufacturing process of the cheese-like food, different from normal cheesemaking process, has the principles of: (1) use of salt agglutinants instead of rennet to make curd, (2) ripening mainly by a proteolytic enzyme, (3) addition of a lactic starter to help ripening and to improve flavor. The most important point in this process is the selection of the enzyme to be used. The following 2 experiments were, therefore, made to select the enzyme.

EXPERIMENT 1: Five popular proteolytic enzymes (trypsin, molsin, pronase, bioprase, and papain) were tested for enzymatic activity by the modified Anson-Hagiwara's method. Then the applicability of these enzymes was examined in the following manner: Each enzyme (same enzyme units) was acted upon soy proteins at pH 6 (optimum for making the cheese-like food) and the degree of proteolysis was determined. Considering the optimum pH of 6 and price, papain is likely to be the most suitable enzyme for this purpose, though the enzymatic activity is in order of bioprase, pronase, and papain.

EXPERIMENT 2: Five cheese-like foods were manufactured using the enzymes described in exp. 1. The optimum manufacturing process was developed by experimentation. Cheese flavor and texture-body were carefully evaluated throughout the ripening period. Better results were obtained with pronase, bioprase, and papain compared with the others. From the results of these experiments, and considering the price and ease of obtaining, papain is the most suitable enzyme to be used in our future study. We are also planning to make further studies on pronase and bioprase because they showed fairly good results.

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EVALUATION OF LIPIDS IN DEFATTED SOY FLAKES AS FLAVOR FACTORS. D. J. SESSA, D. H. HONIG, and J. J. RACKIS, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Oxidative deterioration of unsaturated lipids, during either storage or processing, is a common cause of objectionable flavors in foods. Hexane-ethanol azeotrope (79:21) extracted 3% crude lipids from hexane-defatted soy flakes containing 0.16% residual oil. These are the lipids probably contributing to the flavor of soy flour. The flakes and flavor extracts were analyzed for evidence of oxidative changes. Malonaldehyde was measured by its reaction with 2-thiobarbituric acid (TBA). Low TBA values were obtained with raw and toasted, full-fat and solvent-extracted flakes. The homogenates of full-fat flakes were prepared in the presence of acid to inactivate the lipid-oxidizing enzymes. TBA values for full-fat flakes increased 7-fold when the homogenates were prepared in the absence of acid. Vacuum techniques stripped the volatile carbonyls from defatted soybean flakes. Hexanal at 3 ppm and smaller amounts of ethanol and 2-propanone were the major volatile carbonyls present. Trace amounts of free fatty acid and hydroperoxide occurred in the crude lipid extract, which had an objectionable flavor. Column chromatography was used to separate the neutral lipids and phospholipids into several subfractions. The original distasteful flavor appeared in only one of the phospholipid subfractions. Extracts from toasted defatted flakes contained similar crude lipids and possessed the same objectionable flavor. Our evidence indicates that the lipids themselves when isolated from soy flour possess no unpleasant flavors. Data also show that very little oxidative degradation of lipids occurs during laboratory preparation of the flakes.

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RICE PROTEIN CONCENTRATES FROM X-M RICE BRAN. J. W. HUNNELL, L. LYNN, and J. DEMONT, Riviana Foods Inc., Houston, Texas.

Rice bran has often been suggested as a supplemental food because it is rich in protein. General literature values state 11-14% as typical. However, it is bitter, unstable, and high in bacterial count. The rice bran stream, Protex (trademark, Riviana Foods Inc.), produced in the X-M solvent extractive milling process, is an edible, stable, free-flowing powder and contains 19 ± 2% protein, depending on variety and degree of milling. From 85 to 90% of this protein is a glutelin extractable with dilute alkali. Variable levels, from 0 to a few per cent, of albumins, prolamines, and globulins constitute the remainder. Up to 90% of the glutelin fraction was rapidly solubilized at pH 11.5. Solubilization rapidly increases from pH 10 to a maximum at pH 11.5. Above pH 12, protein degradation and undesirable flavor effects became apparent. After residual solids removal, the crude protein was recovered by precipitation at pH 5.8 to a 70-75% protein, off-white, bland paste. Other protein concentrates demonstrating direct and desirable edible potential were prepared utilizing such pretreatments as precooking the Protex and/or using liquefying amylolytic enzymes. Further color and flavor improvements have been recorded after treatment with activated charcoal. Analyses of amino acid composition, sulfhydryl and disulfide content, available energy, and other biologically active materials as well as palatability evaluation indicate possible acceptable and nutritious creative food technology.

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FERMENTED RICE — A FOOD FROM ECUADOR. A. G. VANVEEN, D. GRAHAM, and K. STEINKRAUS, Cornell University, Ithaca, New York.

"Fermented rice" (arroz fermentado or requemado) is a food consumed in the Ecuadorean Andes about which little information is available. One paper published 10 years ago describes its manufacture, distribution, consumption, and economical aspects. The unhusked rice is fermented by the microorganisms with which it is contaminated. During the fermentation the grain acquires a brownish color. After drying and milling, the resulting product is still colored; after cooking, it develops a faint, specific flavor. We isolated from samples of the fermented product a mixture of fungi and bacilli, in which *Aspergillus flavus* (2 strains), *A. candidus*, and *Bacillus subtilis* were preponderant. A considerable part of the protein is broken down by the microorganisms, and as a result the cooking properties (water absorption) of the rice have been changed. In the rat experiment the nutritive value of the protein had not changed; the riboflavin content increased considerably. The fermented rice samples did not contain aflatoxin, and the 2 *A. flavus* strains were not able to form aflatoxin on the usual wheat medium.

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WHEAT- AND BARLEY-BASED PROTEIN-FORTIFIED FOOD PRODUCTS FOR FOOD AID PROGRAMS. R. E. FERREL, A. P. MOSSMAN, A. D. SHEPHERD, and J. W. PENCE, Western Regional Research Laboratory, ARS, USDA, Albany, California.

Methods were developed for preparing high yields of farina-like products from wheat and barley by inexpensive combinations of moistening, steaming, drying, and simple grinding. The products are well suited as base materials for protein-fortified blended food mixtures for food aid purposes. Pearling procedures effectively reduce crude fiber of the grains to 1.0% or below, with a weight loss of approximating 20% for barley and 10% for wheat. Adjustment of moisture content to between 15 and 40% is followed by steaming for as little as 15 min. to destroy enzymes and to cook the grain partly. After drying, the grain may be coarsely ground in hammer or burr mills to meet hot and cold consistency requirements specified for blended food mixtures. Minerals, vitamins, and other protein sources may then be blended in at desired levels. The steamed barley before drying may be rolled into an attractive flaked product with potential for domestic marketing.

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AIR CLASSIFICATION OF FLOURS FROM RYE, OATS, AND BARLEY. A. C. STRINGFELLOW, A. J. PEPLINSKI, L. H. BURBRIDGE, and V. F. PFEIFER, Northern Regional Research Laboratory ARS, USDA, Peoria, Illinois.

Whole grain samples of rye, oats, and barley were dehulled, milled into flour, reground, and air-classified into 8 fractions. Fractionation responses were good and compared favorably with those previously obtained for hard and soft wheats. Initial protein values (in parentheses) and subsequent ranges produced were: rye (8.2%), 3.4-18.6%; oats (9.8%), 2.3-14.6%; and barley (7.7%), 3.1-23.9%. Protein shift values for rye, 40-50%; defatted, short patent oats, 50%; and barley, 70%.

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CORN DRY-MILLING: COLD TEMPERING AND DEGERMINATION OF CORN OF VARIOUS INITIAL MOISTURE CONTENTS. O. L. BREKKE and W. F. KWOLEK, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Corn initially containing 21, 17, and 12½% moisture was cold-tempered and degerminated on a pilot-plant scale to determine the variation in dry-milling response. Low, moderate, and high addition levels of the first temper water and temper times of ½ to 4 hr. were used. Upon degermination of tempered corn from each of the three lots, the 21% moisture corn gave highest overall yield of flaking grits of lowest oil content and more recoverable oil; it was most consistent in its milling response. The 17% moisture corn gave slightly poorer results and the 12½% corn, appreciably poorer results.

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GELATINIZATION OF CORN GRITS BY ROLL- AND EXTRUSION-COOKING. R. A. ANDERSON, H. F. CONWAY, V. F. PFEIFER, and E. L. GRIFFIN, JR., Northern Regional Research Laboratory, ARS. USDA, Peoria, Illinois.

Recent programs for worldwide feeding involve the production of a food blend called CSM, which contains partially gelatinized corn meal in admixture with soy flour, nonfat dry milk, vitamins, and minerals. Partially gelatinized cornmeal for use in CSM can be produced on heated rolls or by extrusion-cooking. In addition, the wide range of operating conditions available in either form of cooking gives rise to a variety of products from cornmeal, grits, or flour having applications in the food and industrial fields. Studies are described on the processing of corn grits by roll- and extrusion-cooking and on investigating such variables as moisture, temperature, and compression ratios, among others. Products are evaluated by their water absorption and solubility, consistency, and viscosity patterns.

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CHARACTERIZATION OF PREGELATINIZED CEREAL PRODUCTS. FRANK MEINDL and H. J. ROBERTS, Krause Milling Company, Milwaukee, Wisconsin.

A pregelatinized cereal product is one that has been subjected to the combination of sufficient heat and moisture to at least partially gelatinize the starch that is present, yet which still retains its dry appearance. Examples are pregelatinized food starches, foundry core binders, quick-cooking oatmeal, some oil-well drilling mud additives, wheat paste for wallpaper, and certain wet-end additives for paper. Although obviously not cereal products, instant mashed potatoes and pregelatinized potato and tapioca starches are closely related. Two major variables in the manufacture of such products are the degree

to which the starch is gelatinized, and the amount of retrogradation that occurs after gelatinization. This paper reviews methods for determining degree of gelatinization under the general classifications of microscopy, water absorption, solubility, viscosity, and enzymatic susceptibility. The effects of retrogradation on the results of such tests are also discussed. For any specific pregelatinized cereal product, there is probably one test method that will serve as an adequate control of degree of gelatinization. But no single method appears to be capable of characterizing all pregelatinized cereal products. Workers in this field should consider the adoption of a battery of tests that would provide a product profile. Physical characteristics inherent in the profile could accurately reflect the amount and the kind of processing that any cereal product had received.

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IMPROVED PREPARATION OF A QUATERNARY AMMONIUM ALKYL ETHER OF STARCH. C. L. Mehltretter, T. A. McGuire, and C. A. Wilham, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

An improved procedure for preparing a mixture of chlorohydroxypropyl-trimethylammonium chloride salts in high yield has been developed. Cyclization of the salts to *N*-(2,3-epoxypropyl) trimethylammonium chloride is readily achieved for reaction with starch to produce cationic ethers of value in papermaking. Water is used as the solvent throughout the synthesis, and the chemical reagents required are commercially available at low cost. The process should be readily adapted for large-scale production of cationic starches.

36

SEPARATION OF WHEAT COMPONENTS IN AN ALKALINE MEDIUM. B. V. Ettling and M. F. Adams, Washington State University, Pullman, Washington.

A scheme for separating the components of whole wheat flour was developed on the basis of using Unifine flour and an alkaline medium. The flour was first defatted by several extractions with benzene. The defatted flour was extracted twice with ammonium hydroxide solution at pH 10.2. After centrifuging, the combined protein solutions were brought to pH 5.0. The precipitated proteins were collected by centrifuging, washed, and then dried. The starch-containing solids were resuspended in ammonium hydroxide and settled by gravity. The thin slurry of bran and fine starch was siphoned from the coarse starch. The coarse starch was reslurried in ammonium hydroxide and passed through a 325-mesh screen to remove the small amount of bran. After centrifuging, a layer of pentosans was removed by hand from the top of the settled coarse starch. The bran and fine starch slurry obtained by siphoning from the first starch separation was passed through a 325-mesh screen to remove the bran. The fine starch was collected by centrifuging, and a layer of pentosans was removed by hand as above. The yields of the components were starch, 68%; pentosans, 8%; protein, 7%; bran, 2.5%; oil, 1.2% and unrecovered (water-soluble components), 13%. The starch and pentosans each contained 0.35% protein. The starch was white and of high quality. The protein was pure and suitable for nonfood uses. The pentosan fraction was a new product. The bran was clean and still contained nitrogen.

37

THE DEVELOPMENT OF BROWN FAT IN THE HAMSTER. Robert L. Smalley, Judith Jarvis, and Leonore Rowe, Kansas St. Teachers College, Emporia, Kansas.

Numerous studies have demonstrated that the "signet-ring" cell develops by passing through a moruloid stage resembling brown fat. One recent report has indicated that multilocular cells of hamster brown fat develop after unilocular cells (Science 154: 4449 1967). In the hamster brown fat cells do not become multilocular until several days after their initial appearance. Prior to the appearance of lipid droplets in the brown fat cell there is significant accumulation of glycogen, but the glycogen level drops as lipid accumulation becomes pronounced. The fatty acid composition of brown fat changes as the cells become multilocular. The changes are noted as increased saturation of fatty acids of both triglyceride and phospholipid fractions. Stearic acid content is particularly interesting, its content rising and falling along a time course nearly identical with the glycogen content curve. Hamster brown fat appears to offer an important model tissue for studies relating metabolic and morphological events. The true relationships between brown and white fats can probably be established by studies of the hamster, since appearance of multilocular and unilocular cells, and their precursors, are well separated in time.

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STUDIES ON THE EFFECTS OF COLD ON BROWN FAT IN TWO SPECIES OF INSECTIVORES COMPARED WITH *TUPAIA CHINENSIS*. R. R. J. Chaffee, M. W. Sorenson, and C. H. Conaway, Univ. of Calif., Santa Barbara, Calif.

Studies have been made on the extent of brown fat development in response to cold in the shrew, *Suncus murinus*. It was found that this insectivore has much more brown fat than cold-exposed, non-hibernating rodents and more than many of the cold-exposed hibernating rodents. Also the increase in the chemical thermogenic potential of brown fat of this species is much greater than that seen in most rodents. Preliminary studies on the pigmy shrew (*Microsorex hoyi*) showed similar, but more extensive, changes. For comparative purposes, studies have also been made on the tree shrew (*Tupaia chinensis*) and no such increase in the brown fat tissue mass nor striking increase in chemical thermogenic potential was seen. Thus *Tupaia chinensis*, which is considered by some to be a "stem primate" (cf. Sorenson, M. W., and C. H. Conaway, Sabah Soc. J., *2*:77, 1964) rather than a true shrew, resembles more closely the true primate, *Saimiri sciurea* (Chaffee *et al.*, J. Appl. Physiol., *21*:151, 1966), in its response to cold than it does the true shrews, *Suncus murinus* and *Microsorex hoyi*.

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METABOLISM AND PHYSIOLOGICAL ROLE OF BROWN ADIPOSE TISSUE. C. D. Joel, Massachusetts Mental Health Center, Boston, Mass.

Data will be presented which indicate that brown adipose tissue (BAT) serves a quantitatively important role in rewarming of the body during the frequent arousals from hibernation that occur throughout the winter. For example, in the ground squirrel essentially all the BAT is located in the head, foreleg and thoracic region, and it is this portion of the animal that rewarms first. The BAT contains 60% of the total cytochrome *c* of this early-warming region and 20% of the cytochrome *c* of the entire animal. The BAT loses 1 gm or half its total lipid store during arousal. Complete oxidation of 1 gm of lipid is adequate to supply the calories required to warm the animal to 38°C from its hibernating body temperature of 5°. There appears to be heat production within the BAT itself and also release of lipid from BAT for oxidation elsewhere. When rat BAT slices are incubated with albumin and norepinephrine but in the absence of added substrate, the oxygen consumption is linear for at least 14 hours. Therefore the high rate of oxygen consumption characteristic of this tissue can be supported entirely by its own lipid stores. The demonstration of dinitrophenol-sensitive oxidative phosphorylation in isolated BAT mitochondria indicates that during the accelerated oxidative metabolism accompanying heat production in this tissue, there must be either a very active ATP-ase system or the appearance of a uncoupling agent. Most of the effects of insulin added *in vitro* to white adipose tissue can also be observed in BAT slices, including the inhibition of lipolysis in the absence of added glucose. A conspicuous difference is the stimulation of oxygen consumption of BAT by insulin in Krebs-Ringer phosphate buffer without added glucose, a phenomenon not seen with white adipose tissue.

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BIOENERGETICS OF BROWN ADIPOSE TISSUE. R. E. Smith, P. A. Herd and Barbara A. Horwitz, University of Calif., Davis, Calif.

The components of thermogenesis in brown fat are integrated through a classical neuro-somatic relationship in which the effector response is the evolution of metabolic heat. In common with other effectors, the response derives from a neuro-sensory signal, which proceeds via spinal and/or higher reflex arcs to reach the effector by neural and finally neurohumoral transducers. We have made some attempts to specify these components by studying, 1) the induction of heat by direct stimulation of the innervation to the interscapular brown fat, 2) the sensitivity of these cells to norepinephrine, as one of the most probable humoral transmitters, and principally 3) the energetics by which the heat is actually evolved. Initially we examined oxidase activities of the tissue and its mitochondria. Later we turned to the oxidative phosphorylation, using the same media which we had routinely used with liver particles. With this system, however, we were able to obtain P/O ratios which appeared to represent only substrate level phosphorylation. Hence, the initial studies suggested that brown fat mitochondria had a low phosphorylative efficiency (Lepkovsky, 1959; Smith, *et al.*, 1967; Lindberg, *et al.*, 1967). However, the fact that these particulates accumulate Ca^{++} via a respiration-dependent process, indicates that the mitochondria do possess an energy-conservation mechanism coupled to electron transport (Hittelman, *et al.*, 1967). Moreover, recent work in our laboratory as well as in others has shown that near-theoretical P/O ratios can be obtained with brown fat mitochondria in the presence of high concentrations of bovine serum albumin (BSA). If this result is interpreted as BSA protection from uncoupling by fatty acids released during the isolation procedure, the ineffectiveness of BSA addition at various stages of the preparatory procedure, is not explained. Further, liver and brown fat when homogenized together yielded mitochondria with P/O values which would have been expected if the liver particulates were coupled and the brown fat mitochondria uncoupled. The possible significance of the results *in vitro* will be discussed in terms of thermogenic behavior *in vivo*.

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CONTROL OF OXIDATIVE METABOLISM IN BROWN FAT CELLS. O. Lindberg, Wenner-Gren Institute, Stockholm, Sweden; R. H. Eisenhardt, Wenner-Gren Institute and Univ. of Penna.; S. B. Prusiner, Wenner-Gren Institute and Univ. of Penna., and Barbara Cannon, Wenner-Gren Institute.

Brown fat cells isolated from adult hamsters readily oxidize externally added succinate and alpha-glycerol phosphate. On the other hand, the rotenone-sensitive, pyridine nucleotide-linked respiration can only be elicited by norepinephrine or similar hormones, while added pyridine nucleotide-linked substrates are with out measurable effect. Uncoupling agents are capable of eliciting part of the norepinephrine-mediated oxygen uptake, but the optimal hormonally-induceable rate can not be exceeded with uncoupler, whose effects moreover are transient. The pyridine nucleotide-linked respiration—regardless whether caused by addition of hormones or uncouplers—is oligomycin sensitive, presumably because mitochondrial ATP is required for substrate activation. Succinate and alpha-glycerol phosphate respiration is unaffected by either uncouplers or oligomycin. These results show that the system is not fully coupled, and that respiratory control does not operate in the usual sense. Control of metabolism apparently depends upon substrate activation rather than upon the level of phosphate acceptor. This suggests that availability of ATP, required for substrate activation, is the primary control agent. This view is strengthened by the observation that the inhibitor of glycolytic ATP-synthesis, iodoacetate, also interferes with the pyridine nucleotide-linked oxygen uptake. Thus the control and/or activation mechanism by ATP appears to involve compartmentalized ATP sources. Nucleotide levels under different metabolic conditions, and the role of carnitine and its derivatives are under investigation. A mechanism for brown fat thermogenesis will be presented. (Supported by grant CA-06266 from the National Cancer Institute, National Institutes of Health, and by the Swedish Medical Science Foundation.)

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REACTIONS OF ISOPROPENYL STEARATE PROCEEDING THROUGH HEXADECYLKETENE. Edward S. Rothman, Eastern Regional Research Laboratory, ARS, USDA, Philadelphia, Pennsylvania.

Enol esters, such as isopropenyl stearate, are useful intermediates for functionalizing fatty acids. Under given conditions, whereas methyl stearate remains unreactive up to 400°C, by contrast, isopropenyl stearate ester efficiently liberates hexadecylketene at 170°C. with the simultaneous expulsion of "isopropenyl alcohol," i.e., acetone. In the presence of even difficultly acylatable OH or NH compounds, the liberated aldoketene combines, usually quantitatively, with these hydrogen-bearing compounds to form esters or imides. In this manner, such diverse materials as barbituric acids, hydantoins, *N*-butylstearamide, acetanilide, *t*-butanol, and succinimide may be converted to stearoylated derivatives. Di- and tri-stearoylated hydroxamic acids may be formed by hexadecylketene stearoylation of hydroxylamine. Extension of this principle to the interaction of di-isopropenyl esters with polyfunctional acylatable materials such as bis-*N*-alkylazelaiamide or sucrose gives rise to unusual linear polyimides or 3-dimensional

network polymers or oligomers. In the absence of reactable substrate, and with strong dependence on the nature of the reaction medium, liberated hexadecylketene tetramerizes to the enol stearate of trialkylate gamma pyron, forms stearone, or forms the enol stearate of the rearrangement product heneicosane-2,4-dione. The latter compound, in forming, has increased the 18-carbon chain length to 21 continuous carbon atoms.

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SYNTHETIC REACTIONS INVOLVING UNSATURATED AND HYDROXY-UNSATURATED FATTY ACIDS. B. Freedman, M. J. Diamond, and Glenn Fuller, Western Regional Research Laboratory, ARS, USDA, Albany, California, and T. H. Applewhite, Pacific Vegetable Oil Corporation.

Four reactions will be discussed: (1) The catalytic conversion of methyl ricinoleate to methyl 12-ketostearate. (2) The alkaline cleavage of methyl ricinoleate to 10-hydroxydecanoic acid. These syntheses were performed to obtain industrially useful products. (3) The synthesis of methyl lesquerolate (methyl 14-hydroxy-*cis*-11-eicosenoate) from methyl ricinoleate, which established the absolute configuration of lesquerolic acid. (4) The synthesis of *cis,cis*-6,9-octadecadiene from linoleic acid. The latter 2 syntheses were performed to gain fundamental information involving the chemistry of fats and oils.

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DIFUNCTIONAL DERIVATIVES OF CYANOETHYLATED HYDROXYSTEARATE. H. E. Kenney, G. Maerker, and E. T. Donahue, Eastern Regional Research Laboratory, ARS, USDA, Philadelphia, Pennsylvania.

The aim of this work was the conversion of cyanoethylated hydroxy derivatives of fats to the corresponding diesters. The hydrolysis of the nitrile function in the cyanoethylated derivatives was found to be complicated by the sensitivity of the ether linkage toward acid and basic catalysts. By use of cyanoethylated methyl 12-hydroxystearate as model compound, a variety of hydrolytic procedures was examined. High yields of the desired dicarboxylic acid were achieved with a 2-step procedure in which the nitrile was first converted to the amide by means of alkaline hydrogen pyroxide, and the amide was then further hydrolized in aqueous acid. A series of diesters was synthesized.

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REACTIONS OF ALDEHYDIC ESTERS. E. H. Prydé and J. C. Cowan, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Alkyl azelaaldehydates are intriguing bifunctional compounds that can be prepared readily by reductive ozonolysis of unsaturated fatty esters in reactive solvents. Advantage may be taken of the versatility and high reactivity of the aldehyde group to carry out a wide variety of transformations, including the preparation of cyclic and acyclic acetal esters and diesters, hydroxy esters, amino esters, imino diesters, aldol diesters, and azine diesters. Alternately, advantage may be taken of the higher reactivity of the ester group in acetal esters or diesters to prepare hydrazides and various poly(ester-acetals) and poly-(amide-acetals) that have latent functionality. For example, the glycerol acetal of methyl azelaaldehydate is a hydroxy ester capable of self-condensation to an elastomeric poly(ester-acetal). This cyclic acetal is actually a mixture of 4 geometric isomers (*cis*- and *trans*-dioxolanyl and dioxanyl), which have been isolated by preparative gas-liquid and column chromatography and characterized. The poly(ester-acetal) from the equilibrium isomeric mixture exhibits an unusual isomerization-crystallization phenomena. Another monomer of interest is the amino ester, which can be hydrolyzed readily by self-catalysis to form the more stable monomer for nylon-9, 9-aminononanoic acid. Yet another monomer with unusual properties is the acetal hydrazide, a water-soluble compound that precipitates polymer from its aqueous solution upon acid-catalyzed hydrolysis of the acetal group.

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CHARACTERIZATION OF NATURALLY OCCURRING α-HYDROXYLINOLENIC ACID. Cecil R. Smith, Jr.., Northern Regional Research Laboratory. ARS. USDA. Peoria, Illinois.

The seed oil of *Thymus vulgaris* L. (Labiatae) contains 13% of a previously unknown unsaturated hydroxy fatty acid (Ia). Catalytic hydrogenation of the methyl ester (Ib) of the new acid yielded a saturated hydroxy ester (II). Upon reduction with hydrogen iodide-phosphorus, II was converted to methyl stearate. The IR and NMR spectra of Ib and II, as well as their GLC retention values, strongly suggested that they were α-hydroxy esters. II proved resistant to oxidation by von Rudloff's method, but was reduced to a diol (III) upon treatment with lithium aluminum hydride. Since degradation of III with permanganate-periodate yielded heptadecanoic acid, it was proved that Ia is an α-hydroxy acid. When Ib was oxidatively cleaved with permanganate-periodate, the products identified were propionic acid and α-hydroxynonanedioic half ester. This result together with the IR and NMR spectra of Ib indicate that Ia is α-hydroxylinolenic acid. Since Ib has a plain negative ORD curve in chloroform solution, it may be tentatively assigned the D-configuration. Comparisons will be made with methods used for structural elucidation of other naturally occurring hydroxy acids.

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PHOTOLYSIS OF METHYL α-IODOSTEARATE. David R. Howton and Guey-Shuang Wu, School of Medicine, University of California.

Decarboxylation is the major consequence of γ-radiolysis of stearic acid, but there are also formed considerable amounts of polymeric material, believed to be dimeric in nature initially (before modification by secondary processes), and arising via union of radicals (I) produced by loss of hydrogen atoms from the α-methylene group. Various properties of the α,α′-dimer (II) of stearic acid are therefore of interest, and we have explored possibilities of obtaining this substance by photolysis of α-iodostearic acid (and/or its methyl ester), anticipating that C-I homolysis would yield fragments which would have little likelihood of reacting in any other way (aside from recombination) than to give iodine and the desired product:

$$C_{16}H_{33}CHICOOH \overset{h\nu}{\rightleftarrows} C_{16}H_{33}\dot{C}HCOOH + I$$

I

$$\overset{x2}{\rightarrow} \begin{matrix} C_{16}H_{33}CHCOOH \\ C_{16}H_{33}CHCOOH \end{matrix} + I_2$$

II

Exposure of methyl α-iodostereate in nonpolar solvent to light yields iodine readily, but silicic-acid-chromatographic analysis reveals production of little of the methyl ester of II, major products being instead methyl 2-octadecenoate (III) and stearate; some α-hydroxystearate is also formed. Although this finding suggests that radicals of type I disproportionate in preference to coupling, products formed by pyrolysis of di-t-butyl peroxide in methyl stearate (shown by Wheeler et al., *J. Am. Oil Chem. Soc. 42:* 2, 1965, to yield the methyl ester of II, undoubtedly via intermediates of type I) and by γ-radiolysis of stearic acid (presumably leading likewise to I), have been shown to contain negligible amounts of III. An attempt will be made to rationalize the courses of these photolytically initiated reactions.

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SAMPLE PREPARATION FOR AFLATOXIN ASSAY, THE NATURE OF THE PROBLEM AND APPROACHES TO A SOLUTION. L. Stoloff, A. D. Campbell, S. Nesheim and A. C. Beckwith, Food and Drug Administration, Washington, D. C.

Because it has been demonstrated that a single peanut (Cucullu, et al, *J. Am. Oil Chemists' Soc. 43:* 89, 1966) or cottonseed (Whitten, *Cotton Gin and Oil Mill Press,* p. 7, Dec. 17, 1966) can be contaminated at a level of 500-600 μg of aflatoxin B_1/g. of kernel, and because it is probable that the contamination is selective, procuring a representative sample for assay becomes a formidable task. Since a representative lot sample would theoretically require taking the entire lot as a sample, it is necessary to compromise on a practicle sample. In reducing this sample to a condition in which each 50-g. portion represents the whole, consideration must be given to the extreme possibility that 1 contaminated kernel in 10,000 sound kernels could result in an aflatoxin level of 50-60 μg./kg. in the mixture. Assuming uniform contamination of the individual kernel, each 50-g. sample should contain 1/100 of that kernel, a prodigious task of dividing and mixing. Even though this extreme case may be encountered only infrequently, the more usual situation still presents difficulties. Therefore, if the extreme can be handled, one can expect to handle the more usual situation. Equipment and procedures to achieve this goal are described. The equipment includes an attrition mill (Baur), a Polytron homogenizer, and a Hobart vertical-cutter-mixer. Commodities examined are shelled peanuts and in-shell Brazil nuts, walnuts, and almonds. Comminution and mixing effectiveness were determined by particle-size analysis, distribution of kernels made radioactive by neutron activation, and, in some cases, by practical tests with naturally contaminated products.

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SAMPLING STORED CORN FOR AFLATOXIN ASSAY. Robert M. Johnson and W. T. Greenway, ARS, USDA, Beltsville, Maryland, and C. Golumbic, ARS, USDA, Hyattsville, Maryland.

Two bins of corn stored since 1955 and known to contain aflatoxin were probed extensively in 1965. One 200-g. sample was drawn at each 2 ft level in each of 4 quadrants. About 64% of these samples were found to contain aflatoxin. Although individual samples differed widely, the samples representing quadrant and level positions, did not differ significantly in aflatoxin content. In 1967 the tests were repeated. In addition, single-probe composite and double-probe composite samples were drawn from the entire depth of each quadrant in each bin for a total of 4 2½-lb. and 4 5-lb. samples respectively. About 85% of the 200-g. samples contained aflatoxin. However, we found aflatoxin in all composite samples representing each quadrant of both bins. Approximately 95% work and time could have been saved in this instance by drawing a double-probe composite sample of corn (about 2,000 g.) from any quadrant, grinding it into meal, blending thoroughly, and then testing triplicate 50-g. portions.

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SURVEY OF CEREAL GRAINS AND SOYBEANS FOR THE PRESENCE OF AFLATOXINS. O. L. Shotwell, W. F. Kwolek, H. R. Burmeister, G. M. Shannon, M. L. Goulden, E. Vandegraft, M. S. Milburn, H. H. Hall (deceased) and C. W. Hesseltine, Northern Utilization Research and Development Division, ARS, USDA, Peoria, Illinois.

A total of 3,545 samples of grain sorghum, wheat, oats, soybeans, and corn, including samples from all grades, were analyzed for the presence of aflatoxins. Samples were extracted by the procedure, slightly modified, developed by the Food and Drug Administration for the analysis of peanuts and peanut products. Extracts were assayed for the presence of aflatoxins by thin-layer chromatography. The sensitivity limit of the analysis as carried out was 3-5 ppb of the metabolites. Results were confirmed by the duckling test, but low levels of toxin are difficult to detect by biological methods. Few samples contained aflatoxins, and in these, toxins were in low levels. With the exception of 3 out of 1,311 corn samples analyzed, all positive samples were in the poorer grades.

51

SCREENING COTTONSEED FOR AFLATOXINS. Marion E. Whitten, ARS, USDA

A rapid screening method for detecting aflatoxins in cottonseed is presented. Results of a 3-year program of screening several thousand samples of cottonseed from all cotton growing regions of the U. S. is discussed.

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REMOVAL OF AFLATOXINS FROM OILSEED MEALS BY EXTRACTION WITH AQUEOUS ISOPROPANOL. Eric T. Rayner and Frank G. Dollear, Southern Utilization Research and Development Division, ARS, USDA, New Orleans, Louisiana.

Aqueous isopropanol was found to be an effective solvent for removal of aflatoxins from contaminated cottonseed and peanut meals. Extraction with 80% aqueous isopropanol at 70°C. resulted in essentially complete removal of aflatoxins, as measured by thin-layer chromatography, after 6 washes with this solvent. The isopropanol-water azeotrope, 88% isopropanol by weight, removed about 90% of the aflatoxins under the same conditions. Lower temperatures were less effective with both solvent systems.

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TEMPERATURE-DOSE RELATIONSHIPS WITH AFLATOXIN ON THE BRINE SHRIMP, *ARTEMIA SALINA*. R. F. Brown, J. D. Wildman and R. M. Eppley, Food and Drug Administration, Washington, D. C.

Temperature-dose relationships with aflatoxin B_1 have been conducted using the brine shrimp, *Artemia salina*. Increased sensitivity by the brine shrimp to aflatoxin took place with increase in temperature. Optimum sensitivity occurred at

35-37.5°C. Positive results were obtained at 0.5μg. aflatoxin B_1/ml. artificial sea water with a mortality of over 60%. Greater than 90% mortality occurred at dose levels of 1.0 μg./ml. and above. The test can be conducted in 24 hrs.; highly trained personnel are unnecessary.

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MYCOTOXIN INHIBITION OF *BACILLUS MEGATERIUM*. NANCY L. CLEMMENTS, Food and Drug Administration, Washington, D. C.

Zone inhibition using *Bacillus megaterium* NRRL-B-1368 provides a simple and rapid assay to supplement thin-layer chromatographic analysis for aflatoxin and ochratoxin. Less than 1 hr. is required to set up the test, and results are obtained after overnight incubation (16-18 hrs.). Sensitivity to aflatoxin is from 4 to 6 μg., and to crude ochratoxin from 4 to 16 μg.

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AFLATOXIN-PRODUCING ABILITY OF FUNGI. HARRY W. SCHROEDER and R. A. BOLLER, ARS, USDA.

Difference in the ability of strains of *Aspergillus flavus* to produce aflatoxin can be a major factor in determining whether or not the toxins reach significant concentrations in food or feed crops. The ability of strains of these fungi to produce and accumulate aflatoxins was tested by inoculation of Czapek's medium, fortified by the addition of various natural substrates, or on autoclaved moist seeds. The toxins were extracted and concentrated with the aqueous acetone technique prior to quantification by thin-layer chromatography. Toxin-producing and non-producing strains were found on peanuts, cottonseed, rice, corn, and Pinto beans. Although strains did not necessarily produce toxins most efficiently on the substrate from which they were isolated, a substrate-strain interaction was suggested. Strains differing widely in their ability to produce the toxins were found within a single-mass isolate.

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THE BIOASSAY OF AFLATOXINS AND RELATED SUBSTANCES WITH *BACILLUS MEGATERIUM* SPORES AND CHICK EMBRYOS. A. JAYARAMAN, E. J. HERBST and MIYOSHI IKAWA, University of New Hampshire, Durham, New Hampshire.

Using the "paper disc plate" method of antibiotic assay, aflatoxins and other toxic mold metabolites and related compounds were tested for their toxicities to the spores of *Bacillus megaterium* (University of New Hampshire strain obtained from the Dept. of Microbiology), and the toxicities compared with those of known antibiotics. Penicillin G inhibited the germination of spores at 0.2-2 μg./disc. Dicoumarol was twice as active as penicillin, and inhibited at 0.1-1 μg./disc. Significant inhibitions were obtained in the range 1-4 μg./disc for aflatoxin B_1, 4-8 μg./disc for aflatoxin G_1 and 8 μg./disc for an aflatoxin mixture. Bioautographic detection of these compounds separated on thin-layer chromatographic plates is under investigation. Nine-day-old white Leghorn chick embryos incubated during this period, in egg cartons in a laboratory incubator with the eggs being turned manually twice daily, were more sensitive than embryos incubated for the same length of time in a commercial incubator with automatic egg turning (Jamesway Model 252). The LD_{50} of aflatoxin B_1 to carton-incubated embryos was 0.01 μg. while to automatically turned embryos the LD_{50} was 1.0 μg. Carton-incubated embryos were also much more sensitive to other toxins than were embryos incubated in the commercial incubator. The hatchability of carton-incubated eggs was only 4%.

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QUANTITATIVE DETERMINATION OF PHOSPHOLIPIDS AND GLYCOLIPIDS: THIN-LAYER AND COLUMN CHROMATOGRAPHY WITH SPECTROPHOTOMETRIC ASSAY. GEORGE ROUSER, City of Hope Medical Center, Duarte, California.

Accurate and precise determination of polar lipids of blood cells and plasma is possible using chromatographic procedures for separation followed by spectrophotometric assay. Sephadex column chromatography is used for removal of nonlipid contaminants from lipid extracts and separation of gangliosides from other lipids. The lipid mixture may then be separated into individual components by thin-layer chromatography (TLC) and the amounts determined by spectrophotometric assay. Use of diethylaminoethyl and triethylaminoethyl ion exchange cellulose column chromatography prior to TLC is preferable in some cases. Determination of individual lipid classes by spectrophotometric procedures after chromatographic separation combines speed, accuracy, and sensitivity with specificity. Some of the advantages and limitations of the procedures will be described.

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THE DETERMINATION OF THE INDIVIDUAL PHOSPHOLIPIDS IN ERYTHROCYTES AND PLASMA OF VARIOUS MAMMALIAN SPECIES. GARY J. NELSON, University of California, Livermore, California.

The determination of the individual phospholipids in blood presents many problems, but recent methods using chromatographic techniques can now provide accurate and detailed analyses on small samples of whole blood. In this work the phospholipids in the plasma and erythrocytes of several common species, including cat, cow, dog, goat, guinea pig, horse, pig, rabbit, rat and sheep, were analyzed using such procedures. Plasma and cells were carefully separated (and the cells were thoroughly washed) to avoid interference between the plasma and cellular lipids during the subsequent analyses. The lipids were extracted with 2:1 chloroform-methanol (v/v) and freed of nonlipid material by passage through a Sephadex column. The phospholipids were separated by 2-dimensional thin-layer chromatography (TLC). The spots were identified by spray reagents and infrared spectrophotometry. The distribution of the phospholipids was determined by phosphorus assay of the spots scraped off the TLC plates. Analyses obtained by these procedures show that major differences exist in the phospholipid distributions between plasma and erythrocytes in the same species as well as between different species. The phospholipid composition of plasma, however, did not show large species variations. Lecithin (56-83%), lysolecithin (8-23%), and sphingomyelin (6-15%) generally account for more than 95% of the phospholipids in plasma, except in rodents. Phosphatidyl ethanolamine and phosphatidyl inositol are the only noncholine-containing phospholipids in plasma and together are usually less than 5% of the total, except for the plasma of rodents which contain up to 21.7% phosphatidyl ethanolamine (guinea pig). The phospholipid composition of erythrocytes appears to be unrelated to that of plasma, and in contrast to the finding in plasma, the phospholipid distribution in erythrocytes shows marked species variation. The major phospholipids common to the erythrocytes of all species studied in this work are phosphatidic acid, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, and sphingomyelin. Lecithin is absent from erythrocytes of cow, goat, and sheep, but is found in the cells of the other species. Some factors affecting the accuracy and reproducibility of the procedures will be discussed, with emphasis on problems associated with deterioration of the phospholipids during the analysis. (This work was performed under the auspices of the U. S. Atomic Energy Commission.)

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LIPID COMPOSITION OF HUMAN ERYTHROCYTES. JOHN D. TURNER, Baylor University College of Medicine, Waco, Texas, and GEORGE ROUSER, City of Hope Medical Center, Duarte, California.

Quantitative column and thin-layer chromatographic procedures were used to determine the lipid composition of human red blood cells from subjects of both sexes and from individuals with a variety of blood types, ages, and pathological conditions. Values obtained from analysis of intact erythrocytes were compared with those obtained from red blood cell membranes. The differences will be presented.

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THE EFFECT OF INCUBATION ON THE PLASMA PHOSPHOLIPID PATTERN IN HEALTHY PERSONS, AFTER HEPARIN ADMINISTRATION AND IN MYOCARDIAL INFARCTION. R. BERLIN, C. O. OLDFELT, and O. VIKROT, The Regional Hospital, Linköping, Sweden.

Plasma lipids, including individual phospholipids, were determined before and after incubation for 6 hr. at 37°C. in 39 healthy persons. Young men had higher lysolecithin levels than other groups. After incubation, lysolecithin and free fatty acids increased, while there was a decrease of lecithin, triglycerides, and unesterified cholesterol. Total cholesterol and total phospholipids, as well as sphingomyelin and phosphatidylethanolamine, did not change. In 18 persons these studies were repeated 20 min. after 25 mg. heparin i.v. After incubation for 6 hr. at 37°C. the values changed in the same direction as in native plasma, but significantly more. In 22 cases of myocardial infarction, lipid and phospholipid studies were performed in the acute stage. Lysolecithin levels were very low, phosphatidylethanolamine possibly decreased, and free fatty acids increased. After incubation, the lysolecithin increased less than in normal control subjects. Repeat examination in 11 cases after some weeks showed normalization of the lysolecithin level. The changes after incubation are considered to be due to enzymatic mechanisms, interalia alecithin-splitting activity, which is probably increased by heparin and decreased in myocardial infarction.

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METABOLISM OF LIPOPROTEIN LIPASE. JAMES FELTS, University of California Medical Center, San Francisco, Calif.

Lipoprotein lipase (E.C. 3.1.1.3; LPL) is located in the endothelium of certain capillary beds and is concerned with the hydrolysis of circulating triglycerides contained in chylomicrons or d <1.006 lipoproteins. Heparin is thought to be a "prosthetic group" of the active form of the enzyme. After the intravenous injection of heparin, LPL is displaced from the endothelium into the circulating blood, from which it rapidly disappears. Yoshitoshi et al. (*J. Clin. Invest. 42:* 707, 1963) have presented evidence that implicates the liver as the primary organ concerned with the clearance of LPL activity from the blood. We have carried out studies in a perfused rat liver preparation to examine directly the role of the liver in the removal of LPL activity from the circulation. Livers were perfused with defibrinated whole rat blood containing LPL from post-heparin plasma. Enzyme activity was analyzed by incubating aliquots of serum from the perfusate with a triglyceride emulsion and titrating the free fatty acids. LPL activity rapidly disappeared from the perfusate. Analysis of hepatic portal and hepatic vein samples of the perfusate indicated an "extraction" ratio of 0.70. In other experiments it was found that the removal mechanism could be completely inhibited when heparin was added to the perfusate in a concentration of 4 μ/ml. These studies together with other evidence suggest that the inactivating mechanism may consist of 2 processes. The 1st step may involve the removal of the "prosthetic group," heparin, from the enzyme by an hepatic heparinase. The 2nd step may involve the removal of the apoprotein itself. Both processes appear to be blocked by the addition of sufficient exogenous heparin.

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CORRELATION BETWEEN POST-HEPARIN LIPASE AND PHOSPHOLIPASE ACTIVITIES IN HUMAN PLASMA. WILLIAM C. VOGEL and EDWIN L. BIERMAN, Veterans Administration Hospital, Seattle, Washington.

Previous studies (*J. Lipid Res. 5:* 177, 1964; *8:* 46, 1967) have demonstrated phospholipase activity in normal human post-heparin plasma active upon phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC). Preliminary studies (*Fed. Proc. 24:* 439, 1965) using the physical fractionation techniques of zone electrophoresis, ultracentrifugation, and gel filtration did not separate the activities against triglyceride (TG) or PE substrate. Additional studies to separate the active component(s) of post-heparin plasma on TG, PE, and PC using gel filtration (Sephadex G-200), anion exchange gel filtration (DEAE Sephadex), and ultracentrifugation were performed. All activities were filtered together in the void volume on Sephadex G-200, and specific activities increased in parallel 5-fold. Further purification by filtration through DEAE Sephadex yielded a single peak containing all 3 activities. From the active G-200 filtrate, the activities can be separated from plasma lipoproteins at d = 1.210, but still are associated as a large molecular complex, since all activities subsequently filtered with the void volume on Sephadex G-200. Furthermore, it has been shown that enzymatic activity decreased in parallel in subjects placed on fat-free diets. An *in vivo* association of the post-heparin activity upon TG and PC was found: plasma TG and PC decreased in parallel after IV heparin in normal or hyperlipemic subjects; the major *in vivo* substrate was apparently the plasma very low-density lipoprotein TG and PC. Thus, post-heparin plasma contains an enzyme, associated with or part of a large molecular aggregate, active on both phospholipid and triglyceride substrates.

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DETERMINATION OF HEPARIN-INDUCED LIPOPROTEIN LIPASE IN BLOOD PLASMA. J. BOBERG, Konung Gustaf V:5 Forskningsintitut, Stockholm, Sweden.

There have been great difficulties in finding a method with good reproducibility for quantitative determination of heparin-induced lipoprotein lipase activity in blood. Improvements of a method described before will be presented. The importance of substrates, albumin preparations, and heparin doses used for the determination will be discussed.

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PLASMA LIPIDS OF YOUNG ADULTS ON CONTROLLED EXPERIMENTAL DIETS. A. KUKSIS, L. MARAI, and O.

STACHNYK, Charles H. Best Institute, University of Toronto, Ontario, Canada.

Amounts and classes of plasma lipids were determined in groups of university students following a transfer from high carbohydrate diet to diets containing corn oil or butterfat at 30-40% of total calories. A total of 42 males and 52 females subsisted on formula diets for 8-16 days, and blood was taken at 4-day intervals. The lipids were determined by GLC of the diazomethylated total lipid extracts, and by GLC of the individual lipid classes recovered from TLC. The results were characterized by significant subject variation and transient changes in the group averages, although all were within the normal range. On the high carbohydrate diet, a temporary increase was shown in the triglycerides of males, but not females, while the levels of lecithin and total cholesterol decreased in both sexes. Both sexes responded similarly to the fat diets, with the usual changes in the sterol levels being paralleled by the levels of triglycerides and lecithins. No significant alterations were seen in the free fatty acids or the sterol/sterol ester ratio. The characteristic composition of the dietary fatty acids only partially induced corresponding changes in the triglyceride and steryl ester patterns.

65

COLOR DETERMINATION OF SPAGHETTI BY THE TRISTIMULUS METHOD. D. E. WALSH, K. A. GILLES, and W. C. SHUEY, North Dakota State University, Fargo, North Dakota.

The relation between visual measurements and photoelectric measurement of spaghetti color was studied. Two photoelectric reflectance colorimeters equipped with tristimulus filters were evaluated by taking quadruplicate reflectance readings on 40 spaghetti samples which represented a wide spectrum of spaghetti colors. Using the C.I.E. (Commission Internationale de l'Eclairage) system, reflectance data were converted to trichromatic coordinates. Statistical analysis of these data compare the precision and accuracy of each instrument and show the relation between visual and photoelectric measurement of spaghetti color.

66

A WHEAT-HARDNESS INDEX. W. T. GREENAWAY, Market Quality Research Division, ARS, USDA, Beltsville, Maryland.

Kernel hardness is defined as the resistance of wheat to grinding, cutting, crushing, or abrasive action. Although hardness is associated with wheat quality, extremely hard wheats are not desired by millers because of the difficulty generally encountered in milling them. A simple test which would roughly determine wheat quality by measuring the relative degree of hardness and, at the same time, detect unusually hard wheat should have special significance to the wheat industry. In developing such a test a new relationship has been established between the Brabender Hardness Tester and the well-known Strong-Scott pearler. This relationship permits the use of either instrument to determine wheat hardness but expresses the results in terms of wheat-hardness index which is a value common to both but founded on the Brabender Hardness Tester. This device is a small burr mill which is attached easily to the Farinograph and determines wheat hardness while grinding. Using this instrument separately, it is first necessary to establish a high degree of correlation between one factor involving hardness and the other involving protein content on a set of samples. The results from this experiment, expressed as wheat-hardness index values, are then correlated with pearling index values determined on the same samples. A table may be prepared from the regression equation to convert pearling index to wheat-hardness index values. Wheat-hardness index is intended as a rough measure of quality and, at the same time, a detector of unusually hard wheat which might cause milling problems. When the experimental error is established, it might be used especially in borderline cases in place of the laborious and time-consuming dark, hard, and viterous kernel count test currently used to establish grades within hard wheat classes.

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ESTIMATION OF LYSINE AND METHIONINE ADDED TO FORTIFIED WHEAT FOOD PRODUCTS. R. E. FERREL, NANCY BELLARD, A. D. SHEPHERD, and J. W. PENCE, Western Regional Research Laboratory, ARS, USDA, Albany, California.

Nutritional enhancement of cereal proteins by fortifying with limiting amino acids appears to be both simple and practicable for food aid programs. Rapid and simple methods for estimating levels of the added nutrient are needed, however, if the practice is to be easily used commercially. Such a method for determining added lysine and methionine is reported. The finely ground fortified material is extracted with dilute acid. Methionine content of an aliquot of the extract is determined colorimetrically with nitroferricyancide, and total ninhydrin reactants are determined colormetrically on a second aliquot. Lysine content is calculated by subtracting the determined methionine content from the combined lysine-methionine ninhydrin value.

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STABILITY DURING STORAGE OF LYSINE IN LYSINE-INFUSED WHEAT KERNELS. R. E. FERREL, A. D. SHEPHERD, D. G. GUADAGNI, and J. W. PENCE, Western Regional Research Laboratory, ARS, USDA, Albany, California.

Lightly scarified but otherwise intact wheat kernels can be infused with up to 15% lysine hydrochloride. This highly fortified grain can then be blended with untreated wheat to provide lysine enrichment at any desired practical level. In transport and storage, the fortified material may well be subjected to unfavorable conditions, however. Studies were therefore undertaken to determine the stability of the added lysine. The highly fortified grain and blends at the 0.1% fortification level were stored at 90 and 100°F. at moisture levels of 9, 11, and 13%; the control at 13% moisture was held at 0°F. Samples were withdrawn periodically and evaluated chemically for added lysine, organoleptically for possible odor or color deterioration, and biologically for physiological availability of the added nutrient (PER assay). Stability remained relatively high by all criteria throughout the storage period.

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NONPOLAR LIPIDS IN HAND-DISSECTED YELLOW CORN FRACTIONS. C. W. BLESSIN, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Five hybrid yellow corns were hand-dissected into pericarp plus tip cap (8% of the total kernel); or pericarp (4%) and tip cap (4%); total endosperm (85%), or floury endosperm (22%) and horny endosperm (63%); and germ (7%). Crude fat was extracted with normal hexane in a high-speed blender operated at room temperature. Hexane-soluble material in the whole corn averaged 4.0%; in pericarp plus tip cap, 5.3%; in pericarp, 1.9%; in tip cap (includes some germ), 9.5%; in total endosperm, 1.9%; in floury endosperm, 2.8%; in horny endosperm, 0.6%; and in the germ, 29.2%. Separation of lipids by thin-layer chromatography indicated that the 5 whole corns and their hand-dissected fractions had similar lipid class compositions. The largest single fraction was composed of triglycerides. Smaller amounts of hydrocarbons, fatty acids, monoglycerides, diglycerides, sterols, and phospholipids were also present. Triglycerides were separated on a 24-in., ⅛-in. O.D. stainless steel column packed with 3% methyl silicone gum on a silane-treated support. The various whole corns and their hand-dissected fractions had similar triglyceride compositions, averaging as: C_{50}, 4%; C_{52}, 30%; C_{54}, 65%; and C_{56}, 1%. (Subscripts refer to the number of carbon atoms in the fatty acid moiety of the triglyceride.)

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USE OF THE BIURET AND DYE-BINDING TECHNIQUES FOR ESTIMATING PROTEIN IN MILLED AND BROWN RICE. LUCILA PARIAL, L. W. ROONEY, Texas A&M University, College Station, Texas; and B. D. WEBB, USDA Rice Pasture Research and Extension Center, Beaumont, Texas.

Methods for determining protein in rice have been limited principally to the Kjeldahl procedure. A relatively quick, inexpensive method for determining protein to facilitate screening of breeding lines in rice varietal improvement projects is needed. A dye-binding technique using Acid Orange G-12 dye was investigated. Two procedures were tried. In the batch procedure, groups of 50-60 ground samples were shaken in a mechanical shaker for various time intervals. For individual determinations, the sample was vigorously shaken in a special tube for 3 min. The tube contained a sliding metal plunger and metal ends which caused intimate mixing of the rice proteins with the dye and shortened the reaction time. The quantity of dye bound by the sample was measured spectrophotometrically and related to protein content. Forty-five varieties and hybrid selections of rice, representing a range in protein content of 4.65-12.30%, grown at the same location, provided samples for use in developing and comparing the usefulness of various protein tests. Optimum conditions for the test, such as grinding methods, screen size, shaking time, temperature of the dye, and sample size for milled and brown rice, are discussed. The relative usefulness of the dye-binding and biuret techniques for determining protein is discussed.

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SCREENING FOR HIGH-PROTEIN RICE VARIETIES FROM THE IRRI WORLD COLLECTION. B. O. JULIANO, C. C. IGNACIO, V. M. PANGANIBAN, and C. M. PEREZ, International Rice Research Institute, Los Baños, Laguna, The Philippines.

As part of a program on breeding for high-protein rice, the planting material and the crop from the 1966-67 multiplication of 7419 samples of the IRRI world collection were screened for Kjeldahl protein. The results showed a modal distribution for brown rice protein with a mean level of 10.5 ± 1.6%, wet basis. Samples with a mean protein of 13.5% which numbered 101, were analyzed for total amino acids with an amino acid analyzer. Lysine and threonine levels ranged from 2.94 to 4.06 and from 3.07 to 4.22 g./16.8 g. *N* for these brown rice samples, with protein levels between 13.2 and 16.6%, wet basis. The ratio of essential to total amino acids ranged from 0.289 to 0.343. Protein screening of further plantings of these varieties will verify which of these samples are genetically high-protein. The methods used in this screening and other properties of these high-protein rices will be described. [Supported in part by a Rockefeller Foundation grant and by NIH contract PH-43-67-726.]

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HEXANAL AS A MEASURE OF STORAGE DETERIORATION OF READY-TO-EAT CEREALS. C. W. FRITSCH, J. P. NELSON, and J. L. OLSON, General Mills, Inc., Minneapolis, Minnesota.

A large number of ready-to-eat cereals were stored at 0°F., 100°F., room temperature, and under high-humidity conditions. The changes in flavor and texture of the test samples compared to the 0°F. control sample were periodically evaluated by an experienced taste panel. The same samples were analyzed by gas chromatography for their hexanal content in the vapors formed after adding boiling water. In most stored cereals, increases in the hexanal peak area were found to be in good agreement with the degree of off-flavor as judged by the taste panel.

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SCREENING METHOD FOR LYSINE ASSAY. R. PALTER, L. WHITE, M. A. GAUGER, and G. O. KOHLER, Western Regional Research Laboratory, ARS, USDA, Albany, California.

The need for nutritionally balanced protein in foods for a rapidly expanding world population has led to strong interest in improving the amino acid balance of foods by genetic means. Emphasis is on increasing lysine content since this is the first limiting amino acid in most cereals and oilseeds. The genetic approach requires running literally thousands of analyses. In this paper a screening procedure has been developed which is suitable for handling these large numbers of samples. In developing the method, many of the time-consuming steps in the hydrolysis procedure have been eliminated and the lysine and total ninhydrin reactive nitrogen are simultaneously determined by an Autoanalyzer. With this system of analysis, 300 samples can be analyzed per week by 2 operators.

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SOME PROPERTIES OF THE PROTEINS OF THE ENDOSPERM, SUB-ALEURONE LAYER, AND ALEURONE LAYER OF WHEAT. G. A. H. ELTON and J. PACE, Flour Milling and Baking Research Association, Chorleywood, Hertfordshire, England.

The amino acid compositions and starch-gel electrophoretic patterns of proteins located in various parts of the wheat grain are reported and discussed. Work on the isolation and examination of individual proteins or narrow fractions has had some success with proteins in the globulin, albumin, and gliadin groups.

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PEPTIDES FROM ALPHA-GLIADIN. J. E. BERNARDIN, University of Oregon, Eugene, Oregon; MAUREEN T. O'SULLIVAN, and D. K. MECHAM, Western Regional Research Laboratory, ARS, USDA, Albany, California.

Alpha-gliadin was found to have unusual stability to heat and a surprising sensitivity to shear, in addition to its property of forming fibrillar aggregates. In an attempt to determine whether amino acid sequences of high-charge density, or other unusual groupings of amino acids occur that could be related to known properties of alpha-gliadin, polypeptides were separated from enzymic digests. The electrophoretic methods of Hartley and coworkers (*Biochem. J. 101:* 214, (1966) in particular) were used to identify and separate peptides containing specific amino acid residues. For example, alpha-gliadin contains 2 disulfide bridges per molecule. By Hartley's techniques, the polypeptides in enzymic digests containing these 2 bridges, and the peptides formed therefrom by performic acid oxidation, were identified. These and

corresponding results with other amino acid residues will be reported.

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COMPARATIVE STUDIES ON GLUTENINS FROM DIFFERENT CLASSES OF WHEAT. F. R. HUEBNER, Northern Regional Research Laboratory, USDA, Peoria, Illinois.

Although differences in gluten quality among wheat varieties are well known, the exact causes have not been elucidated, partly because there is not yet sufficient knowledge of variations which occur in the gluten proteins from different varieties. Previous work with 11 varieties from 5 different classes of wheats commonly grown in the U. S. showed that gliadin proteins in wheats from different classes were significantly different. Wheats of the same class were similar but not identical even though the wheats varied widely in gluten quality or baking properties. These studies have now been extended to glutenin portions of the same 11 varieties. The glutenins were isolated by precipitation from 70% ethanol and by gel filtration. The glutenins were reduced, alkylated, and then compared by electrophoresis in starch gel. Variations in disulfide content, solubility, viscosity, and carbohydrate content were also assessed. Whereas the amount of gluten obtained from the different wheats varied from about 11% for durums and from 9-11% for hard wheats to 5-7% for soft wheats, the ratio of gliadin to glutenin was similar in all varieties investigated: generally 55% gliadin to 45% glutenin. Electrophoretic patterns of the reduced-alkylated glutenins showed more variation between varieties of the same class than was apparent from the previous work with the gliadin fraction. Again, however, the greatest differences were between classes. In particular, reduced-alkylated glutenin from durum wheats contained little or none of the slowest-moving components present in all other wheats analyzed. In addition, the durum wheats and some soft wheats, which showed little tendency to form solid dough balls, yielded glutenins that contained up to 25% carbohydrate. This tendency to associate with carbohydrate was not observed with the hard wheats. Eventually, it may be possible to relate differences in gluten quality to individual protein constituents.

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ISOLATION AND CHEMICAL COMPOSITION OF PROTEIN BODIES AND MATRIX PROTEINS FROM CORN ENDOSPERM. D. D. CHRISTIANSON, H. NIELSEN, U. KHOO, M. J. WOLF, and J. S. WALL, Northern Regional Research Laboratory, USDA, Peoria, Illinois.

Microscopic examination of corn endosperm cells reveals a network of matrix protein in which protein bodies and starch granules are embedded. To release these protein-containing subcellular structures, corn endosperm tissue, obtained 25 days after hand pollination, was gently homogenized in phosphate buffer. The different particulate cell components were then separated from the homogenate by zonal centrifugation on a sucrose density gradient. Fractions were taken from different points of the gradient and examined microscopically. Layers containing clumped protein bodies and free single protein bodies were removed from near the bottom of the tube. The matrix protein was present in a layer near the op of the tube and was incompletely separated from soluble enzymes in solution in that region. Proteins in the isolated components were characterized by their amino acid composition and gel electrophoretic mobilities. Albumins, globulins, glutelin, and zein were extracted by classical procedures from whole endosperm for comparison. The analyses indicate that protein bodies contained primarily zein, whereas matrix proteins consisted mostly of glutelin. These findings provide information on the nature of the proteins which maintain the integrity of the endosperm cell during dry milling and which must be disrupted during wet milling. They also have important implications for understanding genetic variations of protein composition in corn, which are related to structural changes in endosperm tissue.

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EXTRACTION AND STRUCTURE STUDIES OF CORN GLUTELIN PROTEINS. H. C. NIELSEN, J. W. PAULIS, C. JAMES, and J. S. WALL, Northern Regional Research Laboratory, USDA, Peoria, Illinois.

The composition and properties of corn glutelin were investigated because this protein contains 35% of the endosperm nitrogen and is nutritionally and functionally important in milled products. After removal of salt-soluble and alcohol-soluble proteins from defatted endosperm meal, glutelin was extracted with 0.1*N* sodium hydroxide. Moving-boundary electrophoresis in pH 3.5 aluminum lactate buffer containing 6*M* urea showed that the protein was heterogeneous; a minor component had low mobility like zein, while other components migrated faster like globulins or albumins. The high molecular weight of the protein prevented its movement during starch gel electrophoresis. When disulfide bonds in this glutelin extract were cleaved by reduction and alkylation, the resulting smaller proteins migrated during electrophoresis on starch gel in 8*M* urea-aluminum lactate buffers. However, the diffuse patterns indicated that alkaline extraction caused some decomposition. Other solvents were tested for extraction of proteins from ground endosperm freed of albumins, globulins, and zein. For example, 0.1*M* dimethylaminoethanol yielded a high molecular-weight protein that appeared heterogeneous by moving-boundary electrophoresis with components having mobilities comparable to globulins. After reduction and alkylation the protein migrated in starch gel electrophoresis in several sharp bands whose mobilities corresponded to reduced-alkylated globulins. Little protein was extracted by 8*M* urea alone. Addition of 0.2*M* thioethanol to this solvent increased protein yield. This extract contained reduced proteins which, after alkylation, gave patterns on gel electrophoresis like the reduced-alkylated dimethylaminoethanol extract. These different protein extracts were analyzed for amino acids. The analyses showed that glutelins contain proteins which resemble certain globulins in amino acid composition and electrophoretic behavior, but are crosslinked by disulfide bonds to give high molecular weights.

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INTERACTIONS BETWEEN PROTEIN AND STARCH. ITSUO TAKEUCHI, Kikkoman Shoyu and Co., Noda-shi, Chiba-ken, Japan.

Sometimes, dissolution of protein into water or enzymatic hydrolysis is inhibited by dispersed or concentrated starch gel. These phenomena are occasionally caused by nonspecific physical interactions between protein and starch.

Nonspecific physical interactions were studied by viscometric method, colloid titration method—based on stoichiometric combination between positive and negative charged polymer ions, measurements of electric conductivity confirmed the stoichiometric combination, and foaming method—this was performed to know concentrations of protein and starch transferred from bulk to foam. Interactions were attributed to (1) electrostatic force of these polymer ions, (2) sorption of protein by starch gel; that is, absorption and van der Waals adsorption by starch gel. (1) Electrostatic interaction: Measured value of intrinsic viscosity of mixture of protein and starch was compared with calculated value; difference of each value showed that there was an interaction between components. This interaction was attributed to electrostatic force, because protein and starch behaved as polyelectrolytes with regard to their viscous properties. This is confirmed by colloid titration and electric conductivity. (2) Van der Waals adsorption: Concentration of each component in foam, which was obtained by bubbling mixture solution of surface active agents (synthetic surface active agents or protein) and starch, showed that surface active agent (protein) was able to transfer starch from bulk to foam apparently. This interaction was attributed to van der Waals adsorption between starch and protein.

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EFFECT OF FLOUR FRACTIONS ON HYDROGEN SULFIDE RELEASE FROM DOUGHS MIXED UNDER NITROGEN. D. K. MECHAM and MAURA M. BEAN, Western Regional Research Laboratory, ARS, USDA, Albany, California.

The amount of hydrogen sulfide released from doughs mixed under nitrogen has been observed to vary widely among flours; values of from 10 to 65 μg. from 50 g. of flour mixed at 200 rpm in a Farinograph were found. In attempts to account for this variation in terms of flour composition, fractionation and dough reconstitution methods were used and specific flour components were added to or removed from doughs. Protein components are of primary interest and results will be given. However, it was noted also that flours from which "free" lipids had been removed by petroleum ether extraction yielded less hydrogen sulfide than the control unextracted flour.

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EFFECTS OF OXIDIZING AND REDUCING AGENTS ON CHANGES OF FLOUR PROTEINS DURING DOUGH MIXING. C. C. TSEN, American Institute of Baking, Chicago, Illinois.

The distribution of flour protein components in the acetic extract was changed upon dough mixing, as shown by gel filtration. The change became intensified when dough was mixed with one of oxidizing and reducing agents. Iodate, bromate, and *N*-ethylmaleimide (a sulfhydryl-blocking agent) all could increase the glutenin fraction in the extract. On the other hand, reducing agents including sodium sulfite, cysteine, glutathione, and dithiothreitol could increase not only the glutenin fraction but also the gliadin fraction. Mixing did not enhance the effect of reducing agents as markedly as that of oxidizing agents. Results from experiments on flour-water suspensions treated with various oxidizing and reducing agents supported the above findings. The present findings in relation to the chemical dough development process will be discussed.

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SORGHUM GRAIN: ITS WORLDWIDE SIGNIFICANCE AND POTENTIAL. K. O RACHIE, University of Nebraska, Lincoln, Nebraska.

Sorghum and millets are important food crops in many parts of Asia and Africa, ranking third after wheat and rice for direct human sustenance. It is estimated that about 100 million metric tons of grain of these crops (sorghum constitutes about half) are produced annually from 130-140 million hectares (320-345 million acres). Converted into terms of direct human sustenance, this quantity of cereals would feed 700 million people annually in a predominantly cereal-diet economy like that of India (80-90% of caloric intake). India, China-Manchuria, and the torrid zone of Africa are the most important producing regions in the old world, while the U.S. is the only significant sorghum grower in the western hemisphere. Sorghum is particularly important to agricultural economies of developing countries owing to its wide range of adaptability in respect of climate, soils, and moisture conditions; the many direct food uses for its grain; and the comparatively excellent fodder value of the vegetative residue after grain harvest. Study of the World Sorghum Collection assembled in India suggests the Indian materials comprise the largest germplasm pool of good grain types for human food; the African sorghums constitute the greatest range of genetic diversity; and the derived American stocks are most elite in terms of grain production potential. In India the white or amber pearly-seeded sorghums are preferred for making bread or cooking; in East and South Africa, the local varieties often have reddish-brown seeds associated with a bitter principle, thereby providing a measure of protection against birds, and are primarily used for making beer; in China an important traditional use of "kaoliang" was for both food and distillation. Sorghum improvement is expected to make a striking impact on food production in many developing regions of the world where this crop is extensively grown but average yields are only 5-6 quintals/hectare.

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COMPOSITION AND STRUCTURE OF SORGHUM GRAIN. J. S. WALL and C. W. BLESSIN, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Sorghum grain resembles other cereals—especially corn—in general composition. However, its kernel structure and specific constituents, while posing some problems, offer distinct benefits to the miller and feed processor. In addition, breeders are taking advantage of extensive variations in sorghum types to incorporate optimum kernel size, grain hardness and color, protein quality and quantity, and starch characteristics into new hybrids. Sorghum is composed of about 72% starch, 12% protein, 3.5% fat, and 2.6% fiber. The germ, 10% of the grain, contains 70% of the fat and 13% of the protein. The germ lipid is more saturated than corn oil but does have some linolenic acid. The bran (8% of the grain) consists of pericarp and, frequently, nucellar tissue. Pigments in the nucellus and outer pericarp may color both starch and meal. Tannins in the pericarp may cause problems in foods and feeds. Most of the bran is cellulose and pentosan, but it does contain some starch, protein, and a unique wax. The endosperm is made up of a single layer of aleurone cells, a horny layer, and a floury interior. A dense layer of protein-rich cells in the horny endosperm lies next to the aleurone. A protein matrix embeds starch granules and numerous protein bodies which contain the alcohol-soluble protein, kafirin. Kafirin, which constitutes 40% of the grain protein, is poor in lysine and methionine. While starch of common hybrids is composed of about 26% amylose and 74% amylopectin, starch in waxy types is 100% amylopectin. Normal sorghum starch granules resemble those of corn but gelatinize at slightly higher temperatures. Introduction of yellow endosperm hybrids containing xanthophylls is proceeding rapidly. Some new yellow endosperm hybrids are devoid of undesirable pigments, are large and hard, and should yield superior milled products.

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THE DRY MILLING OF GRAIN SORGHUM. R. R. HAHN, A. E. Staley Mfg. Co., Decatur, Illinois.

Modern grain processing techniques have greatly expanded the usefulness of grain sorghums. It is the purpose of this presentation to review various methods of dry milling grain sorghum. The characteristics that make dry separations possible will be outlined

and the importance of grain selection, cleaning, and conditioning discussed. Finally, the composition and uses of dry milled sorghum products will be described.

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LABORATORY WET MILLING STUDIES OF SELECTED GRAIN SORGHUM TYPES. J. E. FREEMAN and S. A. WATSON, Corn Products Company, Argo, Illinois.

Wet milling of grain sorghum encountered problems not known to corn wet milling. Laboratory studies showed that problems of poor germ separation and poor starch-gluten separation were significantly improved by dehulling prior to steeping. Yellow endosperm types produced an unexpected improvement in starch-gluten separation. Pigments were present in endosperm as well as pericarp of commercial waxy types. Off-color starches resulted which were decolorized by alkaline washing and/or sodium chlorite treatment. The endosperm pigment was found to be controlled by a gene which also controlled pigments in the vegetative organs. Starch gelatinization temperature was found to be related to environmental and genetic factors. Although sweet sorghum types produced starches of low gelatinization temperature, this property could not be transferred genetically to grain types.

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PROTEIN QUALITY STUDIES ON GRAIN SORGHUM. C. W. DEYOE, P. E. SANFORD, L. M. MURPHY, Kansas State University, Manhattan, Kansas, and D. H. WAGGLE, Ralston Purina Company, St. Louis, Missouri.

Studies have been conducted to evaluate the effect of protein variation on amino acid composition and protein quality of sorghum grain. Samples for this purpose were obtained from performance test plots and sorghum plantings designed to provide materials for these studies. Studies conducted to date covering hybrids and varieties of sorghum grain have indicated similar averages and ranges in amino acid values. Hybrids analyzed have ranged from under 6% protein to over 12%. Lysine values on the samples have ranged from 0.14 to 0.27%. Varieties analyzed ranged from 7.5 to 14.0% protein and from 0.17 to 0.28% lysine. Analyses of the samples obtained have shown that as protein content increases, the proportions of lysine, histidine, arginine, threonine, and glycine in the protein of sorghum grain tends to decrease. Biological tests have shown that limiting amino acids for chicks in sorghum grain are methionine and cystine, lysine and arginine. When amino acids were added or higher protein levels were used, significant growth differences due to sorghum grain sources were not observed. These studies indicate that analyses of sorghum samples for amino acid content are correlated to a high degree with the biological feeding value.

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AMINO ACID COMPOSITION OF MILLED SORGHUM PRODUCTS. F. K. SHOUP, C. W. DEYOE, and JOANN CAMPBELL, Kansas State University, Manhattan, Kansas.

Even though sorghum grain is a high-volume cereal grain, little information is available on the amino acid composition of milled fractions. The purpose of this study was to obtain analytical and biological data on milled products. Sorghum grain (Paymaster-Kiowa) was milled using typical dry milling procedures. Endosperm fractions ranging from 5.7 to 18.0% crude protein were obtained. High-protein fractions were composed of the hard vitreous endosperm, while the low-protein fractions were floury endosperm. Amino acid composition of the endosperm fractions in addition to a bran-germ fraction and the whole grain were obtained using an Amino Acid Autoanalyzer. As the total protein content of the endosperm fractions increased, the percentages of lysine, cystine, methionine, threonine, and tryptophan in the protein decreased. Valine, isoleucine, leucine, tyrosine, and phenylalanine increased in percentage of the protein as the total protein content of the fractions increased. The protein of the bran-germ fraction when compared to the protein in the endosperm fractions contained approximately 4 times as much lysine and 2 times as much arginine and glycine. In contrast, the percentage of glutamic acid, proline, alanine, leucine, and tyrosine in the protein of the bran-germ fraction was approximately one-half of that found in the endosperm protein. A feeding study using white rats was conducted utilizing endosperm fractions containing 5.7, 9.6, 15.0, and 18.0% protein, the bran-germ fraction, and the whole grain. The study indicated lysine to be the most limiting and methionine to be the second most limiting of the amino acids. The study also confirmed the amino acid data obtained on the mill fractions.

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PIGMENT CHARACTERIZATION IN GRAIN SORGHUMS. W. K. NIP and E. E. BURNS, Texas A&M University, College Station, Texas.

Pigment characteristics in several red varieties of grain sorghum were investigated. The work included location studies, chemical and physical characteristics, and tentative identification of pigments. Investigation of the location of the pigments in grain sorghum using freezing-microtome techniques indicated orange pigments located in the epicarp, cross-cell, and tube-cell layers of the pericarp. Orange pigmentation also occurred in the seed tip portion of the grains. Characterization and identification studies included the application of chromatographic separation and purification, spectrophotometric measurements, color reactions, and hydrolyses. Anthocyanin, flavone, and aurone-type flavonoids were observed and tentatively identified. Characterization and identification of these pigments provide information of a basic nature for the solution of problems such as discoloration during processing. Genetic patterns of color inheritance may also be revealed.

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THE LIPIDS OF SORGHUM GRAIN: 1. CHARACTERIZATION OF FREE AND BOUND LIPIDS FROM SELECTED GRAIN SORGHUM VARIETIES. L. W. ROONEY, Texas A&M University, College Station, Texas.

The grain of 10 sorghum varieties, which represented genetic types comprising most commercial grain sorghum hybrids, was found to contain from 2.66 to 3.37% petroleum ether extractable lipids (free lipids). Extraction of the residue with water saturated *n*-butanol yielded an additional 0.13-0.28% lipids (bound lipids). The free lipids were fractionated by T.L.C., and the relative proportions of 5 fractions—hydrocarbons, triglycerides, free fatty acids, mono- and diglycerides, and polar compounds—were obtained. The bound lipids were fractionated with T.L.C. and characterized by comparison with known compounds. All sorghum varieties appeared to have the same types of lipids with relatively small differences in the proportions of the various fractions between varieties. Comparisons of 4 parents and their hybrids indicated very little qualitative or quantitative difference in lipids. The varieties Martin, 7078, and Kafir-60, which were grown at the same location for 3 successive years, were found to be similar in type of free and bound lipids, although the quantity of lipids varied from year to year.

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HORMONAL ACTIVATION OF LIPOLYSIS AND RESPIRATION IN FREE BROWN FAT CELLS, J. N. FAIN and NORA REED, Brown University, Providence, R. I.

Free brown fat cells can be obtained by digestion of minced brown adipose tissue with bacterial collagenase. Electron micrographs show many mitochondria with tightly packed cristae in free brown fat cells. Lipolysis can be activated to an equal extent in free brown or white fat cells but only in brown fat cells is lipolysis accompanied by a marked stimulation of respiration. Catecholamines concurrently stimulate both lipolysis and respiration in brown fat cells after a lag period of about 20 seconds. All the effects of catecholamines can be mimicked by theophylline or dibutyryl 3′, 5′-AMP excepting the lag period which is at least twice as long. The activation of lipolysis and respiration appears to require energy derived from mitochondrial oxidative phosphorylation since there was no effect of theophylline, catecholamines or dibutyryl 3′, 5′-AMP if m-chlorocarbonyl cyanide phenylhydrazone (m-Cl-CCP), a potent uncoupler of oxidative phosphorylation, was added prior to these agents. If the m-Cl-CCP was added with the lipolytic agents, it did not effect their activation of lipolysis or respiration. The addition of the uncoupler alone stimulated brown fat cell respiration within five seconds after its addition to fat cells. The activation of respiration by catecholamines could not be mimicked by adding exogenous free fatty acid despite the finding that increased medium concentrations of fatty acid blocked catecholamine action. These results have led us to the following conclusions: 1. Respiration is coupled to phosphorylation under basal conditions in brown fat cells. 2. Energy derived from oxidative phosphorylation is required for the activation of lipolysis and respiration by lipolytic agents. 3. The effects of catechol amines on brown fat cell metabolism are mediated through 3′, 5′-AMP. 4. The activation of energy metabolism by 3′, 5′-AMP is due to either a direct uncoupling action or is secondary to the activation of lipolysis by 3′, 5′-AMP.

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COMPARATIVE ENZYMOLOGY OF FATTY ACID OXIDATION BY BROWN ADIPOSE TISSUE. R. L. DRYER and J. R. PAULSRUD, University of Iowa, Iowa City, Ia.

Comparative studies of palmitic acid-1-^{14}C oxidation to $^{14}CO_2$ by cold-adapted rat and winter bat brown adipose tissue homogenates have shown gross differences in thermal response. The rates of $^{14}CO_2$ production in the bat preparations are several fold lower at 5, 10, and 15° than are rat tissue homogenates when both are set equal at 30°. This would indicate certain enzyme(s) must have higher energies of activation in the bat than in the rat. The $E_{act.}$ of several key enzymes have been examined. The authors suggest that these enzymes represent a unique mechnism for metabolic control at the lowered body temperatures experienced by the bat during hibernation. The converse of this argument may explain why the rat does not hibernate and is rendered hypothermic only with great difficulty and only for short periods of time.

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SOME ASPECTS OF FATTY ACID METABOLISM IN BROWN ADIPOSE TISSUE. E. G. BALL, Harvard Medical School, Cambridge, Mass.

Addition of epinephrine to white (WAT) or brown (BAT) adipose tissue *in vitro* markedly stimulates its oxygen consumption. In WAT this action of epinephrine is apparently mediated by formation of cyclic AMP which in turn activates a lipase. This enzyme then catalyzes the hydrolysis of the tissue's triglyceride stores. Fatty acid so released may then undergo re-esterification if a source of glycerophosphate is available. High energy phosphate needed for this process results in an increased O_2 consumption. The evidence that supports this chain of events in WAT will be reviewed. Whether a similar pattern of events occurs in BAT in response to epinephrine is not clear. Involvement of cyclic AMP is suggested by the fact that theophylline mimics the action of epinephrine. Stimulation of lipolysis is suggested since an increase in FFA and glycerol production can be observed though of a smaller magnitude than seen in WAT. However, neither nicotinic acid nor insulin (± glucose), which affect lipolytic rates and oxygen consumption in WAT, have an effect on the respiratory increase in BAT. If esterification of fatty acids occurs in BAT in response to epinephrine it can not be detected by the incorporation of labeled glucose or glycerol into glyceride-glycerol. Homogenates of BAT respire poorly and do not respond to epinephrine. However upon the addition of ATP, coenzyme A, DL-carnitine, and fumarate a 25-fold increase in O_2 consumption occurs which can be shown to be due to oxidation of endogenous fatty acids. Esterification of added palmitate-1-^{14}C triglyceride also occurs if glycerophosphate is added. Hence the enzymatic machinery for the oxidation and esterification of fatty acids is present in BAT. Whether increase oxygen consumption in BAT is geared to a demand for high energy phosphate as in WAT is not resolved.

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METABOLIC EFFECTS IN BROWN ADIPOSE TISSUE. J. R. WILLIAMSON and B. CHANCE, Johnson Research Foundation, University of Pennsylvania, Philadelphia, Penna., and S. RUSINER and J. N. FAIN, Brown University, Providence, Rhode Island.

An investigation has been made into the metabolic properties of brown adipose tissue to determine the nature of processes controlling its rate of respiration and heat production. Studies with interscapular brown fat of hamsters *in vivo* by tissue fluorescence techniques showed that the redox state of the pyridine nucleotides was increased by hypoxia, and decreased by norepinephrine. Heat production, as measured by thermistors in the brown fat and colon, followed a reciprocal pattern to the changes of reduced pyridine nucleotides. Brown fat of arousing hamsters had a lower oxygen tension than that of normal hamsters, a higher rate of heat production, and was more sensitive to hypoxia, indicating an enhanced respiration. However, norepinephrine administration to arousing hamsters elicited an oxidation of brown fat pyridine nucleotides and a further increase of heat production. These results suggest (1) a positive correlation between the respiratory rate and rate of heat generation, and (2) that the mitochondria in brown fat are not uncoupled prior to catecholamine stimulation. Preliminary measurements of the oxidation-reduction state of the respiratory carriers in isolated brown fat cells (prepared by the method of Fain *et al*, J. Biol. Chem. *242*, 1887, 1967) showed that epinephrine produced a decrease of absorption, attributable to flavoprotein reduction. These changes were characterized by a 20 to 40 sec lag period following epinephrine addition which coincided approximately with the attainment of maximal respiratory activity (5 to 10-fold increase). Uncouplers produced a further oxidation of cytochrome *b* when added after epinephrine. The epinephrine induced stimulation of respiration was oligomycin sensitive. Palmityl carnitine or fatty acids added alone were unable to stimulate respiration. These changes are consistent with the proposal that respiration is controlled by ADP. This interpretation is supported by an observed increase of

ADP content in brown fat cells after epinephrine stimulation. However, the possibility of secondary uncoupling cannot be excluded. The rate of glycerol release after epinephrine was insufficient for the process of extramitochondrial fatty acid activation and re-esterification to account for the required rate of ADP formation needed to support the respiratory activity. Activation of an ATP-driven ion pump mechanism by epinephrine is postulated as an alternative possibility to account for the stimulation of respiration.

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IDENTIFICATION OF POLYOXYALKYLENE-TYPE NONIONIC SURFACTANTS BY PAPER CHROMATOGRAPHY. G. L. SELDEN and J. H. BENEDICT, *The Procter & Gamble Company, Cincinnati, Ohio.*

Despite the increasing importance of nonionic surfactants, methods for their identification have been limited to specific groups. Accordingly, a general analytical scheme for the identification of polyoxyalkylene-type nonionic surfactants was developed, which uses qualitative tests and 2 paper chromatographic solvent systems. This procedure differentiates between alkyl polyoxyethylene (POE) ethers, alkyl POE thioethers, alkyl aryl POE ethers, POE fatty esters, POE sorbitan fatty esters, POE fatty amides, POE and POE-POP condensates. In addition, individual members of a class can be separated and identified. The method is applicable to a variety of detergent products and to mixtures of more than 1 nonionic. As few as 7 mg. of a nonionic surfactant are sufficient for complete characterization.

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THE DEVELOPMENT OF A RADIOACTIVE PARTICULATE SOIL FOR DETERGENCY STUDIES. B. E. GORDON and E. L. BASTIN, Shell Development Company, Emeryville, California.

The use of clay to replace carbon black as the particulate component of synthetic sebum has been the subject of study by several workers. To make possible precise analyses of the residual clay present on fabric after laundering, radioisotopes have been introduced into the clay particles. To avoid loss of tracer, the clay normally has been heated to very high temperatures, thereby fixing the tracer in the clay matrix. This, however, drastically alters the surface properties of the clay, which may affect its ease of removal by the detergent. Work has been underway for some time to develop a nonsurface destructive method of labeling clay, namely, neutron irradiation. It is known from hot-atom chemistry that some fraction of radionuclides generated by the (n,γ) reaction in a nuclear reactor remain in or re-enter the parent matrix. An attempt was made to take advantage of this reaction employing a kaolinite clay exposed to a neutron flux of $\sim 10^{13}$n/cm.^{2}/sec. for several hours. After an appropriate cooling time (for decay of the short half-life components) the clay was studied for the presence of isotopes of reasonable half-life, easily detectable radiation, and resistance to extraction. The results of this study will be presented.

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THE IMPORTANCE OF LIQUID-TO-CLOTH RATIO IN DETERGENCY. W. H. SMITH and A. R. MARTIN, National Institute of Drycleaning, Silver Spring, Maryland.

In a cleaning operation, the liquid may be regarded as being in 2 phases, a free liquid phase and a cloth-bound liquid phase. During cleaning, soil is distributed between these 2 liquid phases in a ratio that is dependent on the relative phase volumes. Therefore, it is essential that the volume of free liquid be as large as is practical, if good cleaning is to result. In drycleaning, the effective volume of free liquid is increased by using continuous flow with filtration. Home laundering, a batch process, uses a much higher liquid-to-cloth ratio than commercial laundering, and is therefore a simpler and probably a more efficient process. Data are given illustrating the advantages of high liquid-to-cloth ratio.

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COMPUTERIZATION IN FABRIC DETERGENCY TESTING. J. C. ILLMAN and G. M. HARTWIG, Shell Development Company, Emeryville, California, and J. W. RODDEWIG.

An automated method is described which greatly speeds the calculation of fabric detergency test results. Laboratory determination of fabric detergency commonly involves replicated, bench scale washing of small pieces of various cloths artificially soiled with various oil-carbon black mixtures. Reflectance measurements before and after washing give a measure of the amount of soil removed. Often, in a program involving several variables, thousands of reflectance measurements may be involved. Although the calculations involve only simple arithmetic, they can be very tedious. By converting the electrical signal from the reflectometer to digital form, and feeding this value to a card punch, the reflectance values are systematically recorded on punched cards. Using an appropriate computer program, the reflectance changes for each test are calculated and tabulated. The saving in operator time is large, and additionally, statistical examination of the data can be incorporated with the program. An example of the type of data output is given.

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CHLORINE-STABLE MACHINE DISHWASHING PRODUCTS. I. R. SCHMOLKA, T. M. KANEKO, and E. A. WEIPERT, Wyandotte *Chemicals Corporation.*

The increasing use of active chlorine-containing compounds in machine dishwashing compositions in the presence of low-foaming, nonionic surfactants has led to the need for chlorine-stable formulations. In order to avoid oxidative degradation of the nonionic surfactant coupled with the simultaneous loss of available chlorine, a new basic patented formulation process has been devised. This obviates the need for protecting or eliminating a terminal hydroxyl group on some nonionic surfactants. It requires a preferred order of addition and the use of tetrasodium pyrophosphate builder as an integral part of the detergent composition. It produces a free-flowing granular product which exhibits no tendency to cake upon storage. Data are presented which show good chlorine stability in comparison with commercial products, and excellent dishwashing performance in the presence of protein soils. The comparative evaluations of several low-foaming, commercially available, nonionic surfactants are shown over a temperature range of 100-160°F. in the presence of milk and egg soil.

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HEXYLENE GLYCOL AS A COSOLVENT IN DRYCLEANING. M. WENTZ and A. R. MARTIN, National Institute of Drycleaning, Silver Spring, Maryland.

The compound 2-methyl-2,4 pentanediol, known commercially as "hexylene glycol," has been used in small quantities in drycleaning detergent formulations as a coupling agent. This paper describes its use in drycleaning as a cosolvent and at a concentration level of 10%. A 10% HG solution in perchlorethylene behaves like a solvent charged with a detergent, although HG is not a surfactant. This solution will not only dissolve water and water-soluble soil, but also has a synergistic effect when blended with a detergent. The mixture has enhanced detergent properties compared with either solution alone. For example, a 1% Aerosol OT solution removes more salt from a fabric in the presence of 10% HG than in its absence.

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SYNTHETIC FATTY ALCOHOLS AND ACIDS: NEW ADDITIONS TO THE RAW MATERIAL POOL FOR SOAPS AND DETERGENTS. J. G. MOFFETT and W. DE ACETIS, Shell Oil Company, Chicago, Illinois.

Over the past 5 years, synthetic primary fatty alcohols have become a large-scale commercial reality. Price-performance advantages of synthetic fatty alcohols are an accepted fact in many surfactant and nonsurfactant end-uses, and product approval evaluations in progress are widespread. Nevertheless, demand for coconut oil and tallow remains sufficiently strong. Synthetic fatty acids, on the other hand, present a more difficult challenge. The natural fatty acids have been in abundance and are seemingly without limit in application. However, in spite of this, their ordered and homologous structure does impose inherent limits to their utility. Chemical syntheses should offer a flexibility in design such that specific-type acids can be tailor-made to meet the requirements of new and interesting applications in industrial areas, and perhaps in edible product areas as well. Significant advances in process technology have been made such as to satisfy the demands *imposed by this flexibility.*

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THE IMPACT OF NEW FIBERS AND FABRICS AND NEW LEVELS OF CONSUMER PERFORMANCE REQUIREMENTS. FRED FORTESS, Celanese Fibers Marketing Company, New York, New York.

Any prediction of the future developments in detergents and home washer and dryer appliances must be based on the phenomenal growth of man-made fibers in textiles and clothing end-uses where cotton was previously the dominant fiber. Although the first 100% chemically synthesized man-made fiber, nylon—a polyamide—was introduced in 1938, as recently as 1960, the detergents and optical brightening agents and machine washing procedures were still predominantly designed for optimum cleansing and restoring of 100% cotton fabrics. Today, the generic families of polyamides, polyester, and polyacrylics fibers and the semi-man-made fibers—the rayons and acetates—represent more than 45% of all of the fibers consumed by the U.S. textile industry. Before 1970, the chemical fibers will represent the major portion of the family wash load. From the point of view of the detergent and home appliance manufacturer, this wash load will be much more complex then ever. Not only will there be a wide range of variants for each of the generic fiber types, but the wash load will contain a number of blends and combinations with cotton, and to some extent, with chemically modified wool. The detergent formulations now, and increasingly in the future, will be required to clean and restore to brightness of color, or to whiteness, a wash load containing various blends of cotton/polyester fibers in sheets, shirts, pants, and towels, 100% nylon, polyester, acetate, and triacetate woven and knit filament fabrics in the form of blouses, slips, panties, and various blends of acrylic fibers with wool. On the assumption that each of these man-made fibers soil differently, greater versatility of soil removal and soil suspension to prevent soil redeposition will be required of the future detergent formulations. In the future, the chemical industry and the appliance manufacturer will be called upon to do more than remove soil in the washing machine. In addition to more versatile optical brighteners for all of the fibers in the wash load, chemical and mechanical means of decreasing wrinkling, edgewear, germs, and static formation will be increasingly demanded by the consumer. The permanent press and soil release finishing development will be used as examples of the increased responsibility placed on the detergent and appliance for servicing the consumers' future needs.

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THE EFFECTS OF THE CHANGING TEXTILE TECHNOLOGY ON DETERGENT FORMULATIONS. R. T. HUNTER, Colgate-Palmolive Co.

The rapidly changing character of the laundry load has created new cleaning criteria for the modern detergent composition. The impact of synthetic fibers and blends, resin finishes, soil release agents, sizing agents, softeners, etc., on the performance of the typical laundry detergents will be discussed. Concurrently with, and related to, these textile changes, there have been changes in automatic washing machines (such as an increase in cold water washing) and an increase in the use of dryers. Techniques of physical and chemical modifications of detergent compositions, which can be made in order to cope with the changing textile technology and related changes in washing equipment, will also be reviewed.

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THE NEW TOILET SOAPS. E. JUNGERMANN and A. B. HERRICK.
Abstract not available at press time.

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CHANGES IN HARDWARE. D. T. DONAVAN.
Abstract not available at press time.

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SCREENING FOR SERUM LIPOPROTEIN ABNORMALITIES —COMPARISON OF ULTRACENTRIFUGAL, PAPER, AND THIN-LAYER STARCH-GEL ELECTROPHORETIC TECHNIQUES. LENA A. LEWIS, Cleveland Clinic Foundation, Cleveland, Ohio.

Sera of normal human beings and of patients with atherosclerosis, xanthomatosis, or demyelinating disease with known lipid abnormality were analyzed for lipoproteins by ultracentrifugal technique of Lewis, Green and Page (*Am. J. Physiol. 171*:391, 1952), by paper electrophoretic technique of Lees and Hatch (*J. Lab. and Clin. Med. 61*:518, 1963), using albuminated buffer, and by thin-layer starch-gel electrophoretic technique of Lewis (*Clin. Chem. 12*:596, 1966), and for cholesterol and triglyceride. By paper electrophoresis, lipoproteins are resolved, in order of decreasing mobility, into α_1, pre-β, β, and chylomicron fractions. On starch-gel, α-lipoprotein migrates in the post-albumin area, β-lipoprotein slightly behind slow α_2-globulin, the pre-β fraction of

paper, i.e., -S 70-400 lipoprotein appears as a band with mobility less than that of β-lipoprotein, and chylomicrons do not enter the gel, but appear as lipid stainable material at the application point. When starch-gel electrophoresis is carried out in a small horizontal cell, with distance between electrodes 14 cm. or less, excellent resolution of serum lipoproteins and proteins occurs in 2 hr. Abnormal sera which showed elevated levels of -S 70-400 lipoprotein by ultracentrifugation showed elevated pre-β-lipoprotein on paper and post-β- on starch-gel; those with high levels of -S 25-70 β-lipoprotein had high β-lipoprotein levels by paper and by starch-gel electrophoresis, while those with low levels were low by all techniques. Sera with high levels of -S > 400 lipoproteins showed an increased concentration in the chylomicron band on paper and of lipid stainable material that did not enter the starch-gel. Increased or decreased levels of α-lipoprotein were demonstrated by all techniques. For screening purposes, paper electrophoresis for lipoprotein and a cholesterol determination can indicate type of serum lipid abnormality and indicate whether additional studies are necessary. Starch-gel electrophoresis is especially valuable in analysis of serum and isolated lipoprotein fractions when interaction of lipoproteins with immune globulins or other proteins is suspected; additional starch-gel patterns can be stained for protein or studied by immunoprecipitin techniques for precise characterization of the lipoprotein complex.

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ASSAY OF LIPOPROTEINS BY RADIAL IMMUNODIFFUSION. ROBERT S. LEES, Rockefeller University, New York, New York.

Widespread use of schemes for quantitation of the several lipoproteins in plasma and plasma fractions has been hindered by the complexity and expense of available methods. The simple rapid technique of protein quantitation by radial immunodiffusion, recently described by Mancini and co-workers, is applicable to the lipoproteins. Alpha-lipoprotein can be quantitated in fresh whole plasma. For quantitation of β-lipoprotein, preliminary ultracentrifugation is required to remove very low-density lipoproteins and chylomicrons, which are immunologically cross-reactive with it. Results obtained by radial immunodiffusion analysis compare well with those obtained by alternative methods of quantitation. The author has used this method not only for the rapid quantitation of lipoproteins in normal and hyperlipidemic plasmas and plasma fractions, but also for measurement of the turnover of β-lipoprotein in a patient with abetalipoproteinemia after plasma infusion.

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COMPARISON OF LIPOPROTEIN ANALYSIS BY AGAROSE GEL ELECTROPHORESIS AND ULTRACENTRIFUGATION. R. P. NOBLE, Sharon Research Institute, Sharon, Connecticut, and F. T. HATCH, J. A. MAZRIMAS, F. T. LINDGREN, L. C. JENSEN, and G. L. ADAMSON, University of California, Berkeley, California.

Electrophoretic separation of serum lipoproteins is performed in 0.5% agarose applied upon a thin, colorless plastic strip. After separation (approximately 2 hr.) the agarose is fixed, oven-dried, and stained with Oil Red O or Sudan Black B, followed by a water wash. The resulting electrophoretogram shows bands of lipoproteins corresponding to (1) high-density α, (2) low-density β, (3) very low-density pre-β fractions, and (4) chylomicrons at the origin, if present. Because these bands are well separated upon a transparent, colorless background, the strips are ideally suited to densitometric scanning. Agarose electrophoresis was compared with analytical ultracentrifugation in nonfasting morning sera from 30 patients aged 30-65, with various disorders of lipoprotein metabolism (courtesy of Dr. E. H. Strisower). Densitometric tracings were made and integrated with the Spinco Analytrol. Zones were measured with a DuPont Curve Resolver. Correlation and regression relationships between the scans and the ultracentrifuge Schlieren patterns were calculated with a CD 6600 computer. The following comparisons were made: α with the ultracentrifugal total high-density ($F_{1.20}$ 0-9); pre-β with S_f 12-400 and 20-400; β with S_f 0-12 and 0-20; the height (altitude) of the β peak with S_f 0-12 and 0.20. The correlation coefficients (r) varied between 0.75 and 0.80. From 26 to 28 of 30 points were within ±30% of the regression line calculated for each pair of variables. In a few patterns, positive correlation was noted between slow moving pre-β and S_f 12-100; between faster moving pre-β and S_f 100-400. The electrophoretogram alone or with the scan provided a relatively easy differentiation among the 5 clinical types of hyperlipoproteinemias. For survey purposes, 16 sera may be separated on a 12-inch-wide plastic sheet. After staining, classification may be done visually, or the large sheet may be cut into its separate strips for scanning. (This work was performed under the auspices of the U.S. Atomic Energy Commission. It was also supported in part by Grant No. 350 from the Northwest Chapter of the Connecticut Heart Association, and by U.S. Public Health Service Grants 5-R01-HE-07220-6 and 5-R01-HE-1882-14.)

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CHARACTERIZATION OF HUMAN LOW-DENSITY SERUM LIPOPROTEINS. F. T. LINDGREN, L. C. JENSEN, R. D. WILLS, and N. K. FREEMAN, University of California, Berkeley, California.

Studies with normal adult populations have revealed quantitative differences between males and females in the low-density lipoprotein spectra. These differences have been evaluated by several computer analyses of the ultracentrifuge Schlieren diagrams. One important difference is the higher S_f rate of the major low-density component observed in females. Also, in both the male and female groups studied, a very significant ($p < .01$) negative correlation, $r = -0.97$ and $r = -0.92$, respectively, was observed between low-density S_f rate and lipoprotein hydrated density. These differences between the male and female, as well as the relationships between S_f rate, hydrated density, molecular weight, and lipoprotein composition will be discussed.

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EFFECTS OF HYDROGEN PEROXIDE ON LIPOPROTEINS AND ASSOCIATED LIPIDS. DALE A. CLARK, EMMETT L. FOULDS, and FREDERICK H. WILSON, JR., USAF School of Aerospace Medicine, Brooks AFB, Texas.

In studying relationships between lipoproteins, serum lipid transport, peroxidase, and atherosclerosis, effects of hydrogen peroxide on serum lipoproteins were observed. After being separated from serum by the technique of flotation in the ultracentrifuge, lipoproteins were treated with 0.17*M* hydrogen peroxide. With lipoproteins from some serums, but not all, notable changes occurred within 2 hr. High-density lipoproteins had decreased to less than 50% of their initial level. S_f 0-12 lipoproteins also had decreased to 50-75% of initial levels, but S_f 20-400 lipoproteins were not much affected. Separation of lipid classes on a micro silicic acid column showed that H_2O_2 caused small losses of phospholipid, but larger decreases of triglycerides and cholesterol. The effects on cholesterol were complex; there was an apparent loss of up to 70% of cholesterol esters and an apparent increase in free cholesterol, with a net decrease in total cholesterol. Gas chromatographic analysis of fatty acids of the phospholipid, triglyceride, and cholesterol ester fractions showed that H_2O_2 caused selective loss of polyunsaturated fatty acids. Addition of H_2O_2 to serum, however, had little effect on lipoprotein or lipid levels. The stability of lipoproteins in the serum are tentatively attributed to the presence of antioxidants in serum which are not floated off with the lipoproteins during preparative ultracentrifugation. The deleterious effects of hydrogen peroxide on the integrity of lipoproteins suggests the possibility that peroxide might significantly decrease the lipid transport capacity of serum lipoproteins, and contribute to the development of atherosclerosis.

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ULTRAVIOLET AND FAR-ULTRAVIOLET CIRCULAR DICHROISM SPECTRA OF HUMAN SERUM HIGH-DENSITY LIPOPROTEIN (HDL). A. SCANU and R. HIRZ, University of Chicago, Chicago, Illinois.

Previous studies from this laboratory employing the technique of optical rotatory dispersion (ORD) had shown that the human serum HDL of d 1.063-1.21 has a large percentage of α-helix which is to a large extent retained in lipid-free (apo HDL) preparations (Scanu, A., *Proc. Natl. Acad. Sci. 54:* 1699, 1965). Studies by the technique of circular dichroism (CD) have now shown that the α-helical parameters of HDL are qualitatively different from those of apo HDL: the ratio of the measure of molar ellipticity of the $n \rightarrow \pi^*$:$\pi \rightarrow \pi^*$ transitions at 222 and 208 mμ was larger in the products containing lipids. Near-complete restoration of the HDL spectrum was observed in apo HDL preparations that had been relipidated by the addition of phospholipid micelles. As assessed by the CD parameters, the conformation of HDL was significantly more stable than that of apo HDL to solvent changes (pH, ionic strength, sodium dodecyl sulphate, and guanidine-HCl) and to chemical modification (succinylation). The same was true for apo HDL after relipidation. The results indicate that apo HDL undergoes some conformation changes when it loses its lipids. Moreover, the persistence of a high α-helical content in these preparations suggests that the secondary structure of apo HDL is, to a large extent, independent of bound lipids. These may act as stabilizers of the helical segments of the various polypeptide chains, probably through hydrophobic interactions. (Supported by grants HE-08727 and HE-24,867 from U.S. Public Health Service; G-66-30 from Life Insurance Medical Research Fund, and RN-66-6 from the Illinois and Chicago Heart Associations.)

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PREPARATION AND PROPERTIES OF AN APOPROTEIN DERIVATIVE OF HUMAN SERUM β LIPOPROTEIN. A. M. GOTTO, R. I. LEVY, and D. S. FREDRICKSON, National Institutes of Health, Bethesda, Maryland.

The insolubility of delipidated human serum β lipoprotein in aqueous media has been a major obstacle to the study of the β apoprotein. The preparation, in high yield, of an apoprotein derivative has now been achieved by succinylation and delipidation of β lipoprotein, followed by solubilization of the protein moiety in 1×10^{-2} *M* sodium decyl sulfate. Succinylation is necessary to achieve solubilization of the delipidated protein. Succinylated apoprotein is partially soluble in aqueous media, but forms a gel. The addition of sodium decyl sulfate produces complete solubilization. The apoprotein contains no detectable cholesterol or triglyceride, and less than 2% phospholipid. It retains activity with 10 of 15 rabbit and sheep antisera to human β lipoprotein. The method presented in this communication makes use both of succinylation and of a dialyzable detergent, viz., sodium decyl sulfate, in such a way as to make possible the investigation of the apoprotein derivative by the established techniques of protein chemistry. Results of such studies will be discussed.

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PHYSICAL AND CHEMICAL CHARACTERIZATION OF LOW-DENSITY LIPOPROTEIN SUBFRACTIONS FROM HUMAN PLASMA. DIANA M. LEE, PETAR ALAUPOVIC, and ROBERT H. FURMAN, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma.

Plasma low-density lipoproteins (LDL) from normal human subjects were separated by sequential preparative ultracentrifugation into 6 subfractions (d 1.006-1.009, 1.009-1.019, 1.019-1.030, 1.030-1.040, 1.040-1.053, and 1.053-1.063 g./ml.). Contaminating α-lipoproteins were removed by immunoprecipitation. The immunochemically homogeneous subfractions, containing only apolipoprotein B, were characterized by determination of chemical composition and various physical-chemical parameters. All subfractions had practically the same diffusion coefficients ($D^{o}_{20,w} = 1.7\text{-}1.9 \times 10^{-7}$ cm.²/sec.). The LDL were divided on the basis of chemical composition and physical-chemical properties into 2 distinct groups occurring within the density ranges 1.006-1.019 g./ml. (Class I) and 1.030-1.063 g./ml. (Class II). The lipoproteins of density range 1.019-1.030 g./ml. represent a mixture of both major groups. Subfractions of both groups had a constant percentage of neutral lipids (triglyceride and cholesterol), phospholipid, and protein. Whereas the phospholipid/protein ratio remained constant, the cholesterol ester content decreased and the triglyceride content increased with decreasing hydrated density. The molecular weights of subfractions of Class I and Class II were 4.6×10^6 and 3.2×10^6 g./mole, respectively. All subfractions were characterized by an identical total amount of phospholipid and protein (1.5×10^6 g./mole). It has been found that a subfraction (S_f 20-50) of VLDL characterized by the presence of apolipoprotein B had the same phospholipid/protein ratio and the same absolute amount of phospholipid and protein, even though the percent chemical composition and physical-chemical properties (molecular weight 6×10^6 g./mole) were different from those of LDL subfractions. It has been postulated, therefore, that each segment of the polydisperse system of lipoproteins which contain apolipoprotein B consists of the same basic phospholipid-protein structural unit. The absolute amount of this unit is practically constant throughout the spectrum of this lipoprotein family, and is characterized by the capacity to maintain the structural integrity of triglyceride-cholesterol micelles of different sizes and molecular weights.

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PROTEIN QUANTITY AND QUALITY AS FACTORS IN THE EVALUATION OF BREAD WHEATS. W. BUSHUK, University of Manitoba, Winnipeg, Manitoba, Canada.

Two wheat varieties, a high-quality Canadian hard red spring variety and an experimental variety with exceptionally strong gluten, were grown together in a field of variable fertility and harvested as individual rod rows. Seed from different rows was composited by protein content into larger samples ranging in protein from about 9.5 to 14.5% in increments of about 0.5%. The samples were evaluated for breadmaking quality by standard tests. Baking quality was assessed with the Remix Test at constant and optimum mixing times. For each variety, quality

parameters such as Farinograph development time, sedimentation value, absorption, and loaf volume at constant mixing, increased with protein content. The rates of increase for the 2 varieties were different. Optimum mixing time as determined with the power input meter was different for the 2 varieties, but was not affected by protein content. The slopes of the plots of loaf volume by optimum mixing against protein content were essentially the same for the 2 varieties. The Farinograph curve reflects gluten strength, which is not always equivalent to baking quality. Wheats that yield poor bread by a particular baking procedure because they are too strong remain so over a wide protein content.

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THE PRECISION AND ACCURACY OF LABORATORY TESTS. J. P. Woolcott, H. C. Cole Milling Co., Chester, Illinois.

The development of new procedures and the surveillance of methods currently in use require an estimate of the precision and the accuracy obtained. Precision is defined as the variation in successive evaluations of the same quantity, while accuracy is the relationship between the experimentally measured value and its theoretical true value. In statistical terms, the standard deviation is the measure of precision and the standard error is a measure of accuracy. The assumptions made, the significant level used, the presence of outliers, the sample size, and the types of errors (precision and systematic) are discussed. It is proposed that a nested experimental design will meet the requirements for obtaining the precision and accuracy of analytical procedures. The analysis of variance is used to determine and partition the variability among the components. The design may be modified when appropriate to provide for conditions peculiar to the particular procedure, the substance tested, or the variability of laboratories. Although a fixed factor model is used, the results may be generalized to include all laboratories interested in the procedure. A graphical method to portray the results of collaborative tests is given. The discussion includes the interpretation of the graph in respect to the systematic and precision errors. The interpretation of the results of an individual laboratory makes use of this device.

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A MODIFIED MICRO SEDIMENTATION TEST FOR SCREENING EARLY-GENERATION WHEAT SELECTIONS. J. S. Kitterman and M. A. Barmore, Western Wheat Quality Laboratory, ARS, USDA, Pullman, Washington.

A modified micro version of the Zeleny sedimentation test is described which employs the following basic modifications: (1) a 0.40-g. sample is used instead of 0.32 g.; (2) the lactic acid reagent is added immediately after dispersing the flour in hydration water; (3) after mixing for 5 min., the samples are centrifuged, the supernatant liquid discarded, and the residue redispersed in lactic acid; (4) the total volume of solution in the redispersion is 10 ml; (5) the hydrated material is allowed to settle for 10 min. before reading. These modifications expand the range, increase the sensitivity, and eliminate the "ceiling effect." This micro test was found to evaluate the wheats grown in the Pacific Northwest area more correctly than a micro version of the standard Zeleny procedure.

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A COMPARISON OF STARCH GELATINIZATION AND OTHER PHYSICAL PROPERTIES OF 24 WHEAT FLOURS. Ruth H. Matthews, Human Nutrition Research Division, ARS, USDA, Beltsville, Maryland.

Starch gelatinization properties of wheat flours were measured with the amylograph, the Brookfield Viscometer on a flour-milk model system at 70 and 24°C., and on the heating stage microscope by measuring loss of birefringence. Measurements of pH, color, and dough strength were taken. The 24 flours included market samples of family flour purchased in 6 different cities, and flours milled from soft, hard, and blended wheats grown in different areas of the U.S. Air-classified and conventionally milled flours as well as bleached and unbleached flours were included. Flours from the different areas varied considerably in paste viscosity. Factors affecting this variation were pH of the medium, type of wheat, malting procedures, and milling practices. The relation of these physical measurements to each other and to consumer satisfaction will be discussed.

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STUDIES ON PIGMENT DESTRUCTION DURING PROCESSING OF SPAGHETTI. R. R. Matsuo, J. W. Bradley, and G. N. Irvine, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, Canada.

Some of the factors associated with pigment loss in macaroni doughs during various stages of processing were studied. Various substrates for the enzyme lipoxidase were added to dough and the pigment content determined. The effects of mixing under an atmosphere of oxygen and of adding purified wheat lipoxidase were also studied. Under normal conditions, most of the pigment loss occurs during the drying cycle of processing. The greatest pigment loss occurred with added linoleic acid mixed under oxygen. The substrate, specifically the free unsaturated fatty acid, appears to be the limiting factor in the extent of pigment loss in macaroni doughs.

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EFFECT OF GLUTEN QUALITY ON THE COOKING QUALITY OF SPAGHETTI. R. R. Matsuo and G. N. Irvine, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, Canada.

The effect of different types of gluten on the cooking quality of spaghetti was studied. Gluten characteristics were studied by the Farinograph, Alveograph, and the Kaminski-Halton gluten stretching test. The cooking quality was assessed by measuring the spaghetti tenderness with an apparatus designed in our laboratory. Gluten of medium strength, stronger than glutens of standard Canadian varieties but not as tough as gluten from hard red spring wheat, appears to produce spaghetti of optimum cooking quality.

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EXPERIMENTAL PRODUCTION OF ATTA. M. Shafiq Chaudhry, M. M. MacMasters, E. P. Farrell, and W. J. Hoover, Kansas State University, Manhattan, Kansas.

Atta is the ground wheat product used for making chapatis (unleavened bread) in Pakistan and India. An experimental milling procedure was developed for producing atta by use of 4 sets of corrugated rolls and 1 set of smooth rolls. Attas were prepared from all classes of U.S. wheats. Particle size distribution, determined by sieve analysis, lay within limits of that for attas produced in Pakistan. Physical dough properties and baking quality were investigated. All of the experimentally prepared attas except that from durum wheat gave acceptable chapatis, but color was judged to be the major factor in determining a somewhat lower score for chapatis made from red wheat attas than for those made from white wheat attas.

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AMINO ACID ANALYSES OF RICE AND RICE BYPRODUCTS. D. F. Houston, M. E. Allis, and G. O. Kohler, Western Regional Research Laboratory, ARS, USDA, Albany, California.

Data are presented from chromatographic analyses of amino acid contents of 7 U.S. milled rices and such milling byproducts as bran, polish, bran plus polish, millfeed, and hulls. Values for serine, threonine, valine, and isoleucine were corrected by factors based on earlier work. Cystine and methionine were determined following performic acid oxidation. Compositions of each material were very similar for all varieties and process differences. However, appreciable differences occurred between different milling fractions. Comparisons of amino acid compositions found for milled rice and bran, with the rather variable data reported for them, show generally higher present values for those amino acids requiring special consideration for analysis. This is especially true for sulfur amino acids, indicating that earlier values are generally too low.

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GAS-LIQUID RADIOCHROMATOGRAPHY OF INTACT NATURAL TRIGLYCERIDES. W. C. Breckenridge and A. Kuksis, Charles H. Best Institute, University of Toronto, Ontario, Canada.

Gas-liquid radiochromatography was successfully applied to the simultaneous analysis of mass and radioactivity of intact standard and natural triglycerides labelled with C^{14} in their fatty acid moieties. Quantitative measurements of mass and radioactivity were made with a Barber-Colman 5000 series gas chromatograph equipped with 3% JXR columns (2 ft. × ⅛ in. I.D.), stream splitter, combustion train, and a gas counter. Maximum sensitivity required low flow rates (40-50 ml./min.) which were not compatible with high recoveries of long chain triglycerides. With an argon flow of 130 ml./min., about 300 dpm of a glyceride could be measured with a relative error of 10%. The counting efficiency was over 90%, and the overall accuracy of the procedure was ±5%. Under these conditions, the achieved resolution of radioactivity was only slightly inferior to that of the mass. Quantitative results were obtained using C^{14}-labelled tridecanoin as internal standard. The results confirm earlier claims of nearly complete recoveries, from short columns, of both saturated and unsaturated triglycerides, in the 24-54 carbon range.

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STUDIES ON THE COURSE OF *CIS-TRANS* ISOMERIZATION OF POLYUNSATURATED FATTY ACIDS. E. C. Nickell and O. S. Privett, The Hormel Institute, Austin, Minnesota.

Studies are reported on the structures and physical properties of artifacts formed by the action of heat on long-chain polyunsaturated methyl esters during fractional distillation. Nuclear magnetic resonance, mass spectra, and infrared and ultraviolet spectral analysis showed that the initial products of the action of heat on methyl esters containing 4 or more double bonds are simple *cis-trans* isomers, in which there was no apparent shift in the positions of the double bonds. Products were isolated from heat-isomerized methyl docosahexaenoate by argentation chromatography, and their properties and structures compared with that of *cis-trans* isomers prepared by conventional techniques. The course of isomerization reactions are discussed in the light of current theories of the initial stages of thermal polymerization.

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IMPROVED PROCEDURES FOR THE PREPARATION AND PURIFICATION OF POLYUNSATURATED FATTY ACIDS. O. S. Privett, J. D. Nadenicek, F. J. Pusch, R. J. Chapman, and K. K. Beutel, The Hormel Institute, Austin, Minnesota.

The preparation of polyunsaturated faty acids in ultra-high purity is described. Polyunsaturates are separated via serial crystallization of urea-inclusion compounds, first by limiting the amount of urea, and at successively lower temperatures than with an excess of urea at room temperature. Major components of the polyunsaturates are concentrated either directly or after fractional distillation via high capacity adsorption. Final purification is effected by recrystallization until no impurities can be detected in the filrate or crystal fractions. Minor components are concentrated via low-temperature fractional crystallization via a technique that brings all components of the mixture into the same range of concentrations. The fractions enriched in minor components are fractionated by argentation chromatography. Concentration of individual components of these fractions is then carried out by either (or both) amplified fractional distillation and liquid-liquid partition chromatography, with final purification by low-temperature recrystallizations until pure.

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RADIOLYSIS OF LIPIDS. I. MODE OF CLEAVAGE *IN* TRIGLYCERIDE FATTY ACIDS. M. D. Dubravcic and W. W. Nawar, University of Massachusetts, Amherst, Massachusetts.

The effect of gamma radiation on simple triglycerides was investigated. Trilaurin, trimyristin, tripalmitin, tristearin, tripalmitolein, triolein, and trilinolenin were irradiated under vacuum at 6 megarads. The volatile breakdown products were separated by vacuum distillation and identified by gas chromatography and mass spectrometry. Qualitative and quantitative data show that the cleavage in fatty acids essentially follows a specific pattern, and is not random. The mechanisms of radiolysis will be discussed.

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PREPARATION OF SOME LONG-CHAIN 2-OXAZOLIDONES. M. E. Dyen and D. Swern, Temple University, Philadelphia, Pennsylvania.

Long-chain aliphatic compounds containing the 2-oxazolidone moiety have been prepared from *cis-* and *trans*-9-octadecene, methyl oleate and elaidate, oleyl, and elaidyl alcohols, and several long-chain terminal epoxides. The 2-oxazolidones prepared are substituted in the 4-, 4,5- or 3,5-positions. The first 2 groups of oxazolidones are obtained by pyrolysis of β-iodocarbamates prepared from terminal and internal monounsaturated compounds, respectively, by addition of iodine isocyanate, and reaction with methanol. The last group is prepared by heating terminal expoxides with organic isocyanates in DMF containing a catalytic quantity of tetramethylammonium iodide or lithium chloride. Structures have been confirmed by elemental analysis, and infrared and nuclear magnetic resonance spectroscopy.

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POSITIONAL DISTRIBUTION OF DOCOSAHEXAENOIC, DOCOSAPENTAENOIC, AND EICOSAPENTAENOIC ACIDS IN AQUATIC ANIMAL TRIGLYCERIDES. C. Litchfield, Texas Agricultural Experiment Station, College Station, Texas.

Brockerhoff and co-workers have pointed out the general tendency of long-chain polyunsaturated fatty acids (22:6, 22:5, and 20:5) to be preferentially esterified at the 2-position of fish and

invertebrate triglycerides, and at the 3-position of marine mammal triglycerides. Further study has now revealed that the positional distribution of 22:6 and 22:5 in aquatic animal triglycerides can be predicted by simple proportionality equations of the type $y = ax$. The mole percent 22:6 at the 1-, 2-, or 3-position (y) is obtained by multiplying the proportionality constant for that position (a_1, a_2, or a_3) times the percent 22:6 in the total triglycerides (x). For fish, invertebrate, and turtle triglycerides, $a_1 \approx 0.26$, $a_2 = 2.07$, and $a_3 \approx 0.66$. For marine mammal blubber triglycerides, $a_1 = 0.90$, $a_2 = 0.23$, and $a_3 = 1.87$. The same equations apply to both 22:6 and 22:5. The positional distribution of 20:5 in aquatic animal triglycerides resembles that of 22:6, but the similarity is not close enough tc allow mathematical prediction.

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LOCATION OF DOUBLE BONDS IN MIXTURES OF UNSATURATED FATTY ACIDS BY OZONOLYSIS AND THIN-LAYER CHROMATOGRAPHY. JAMES B. SADDLER, University of Washington, Seattle, Washington, and HUGO KRUEGER, IAN TINSLEY, and ROBERT LOWRY, Oregon State University, Corvallis, Oregon.

Twenty unsaturated fatty acids were identified in the lipids of juvenile coho salmon by gas-liquid chromatography. Tentative assignations of chain length and number of double bonds for the unsaturated fatty acids were derived from the positions of their peaks with reference to identified peaks from individual standard fatty acids, and by plotting the log of their retention times against the number of carbons assigned. The unsaturated fatty acid mixture was separated on silver nitrate thin-layer plates into distinct bands containing fatty acids with the same number of double bonds. Removal of the separated fractions from the TLC plates and subsequent GLC analyses ascertained the degree of separation, and confirmed the separation of the fatty acids into homologous groups with the same number of double bonds. Reductive ozonolysis of the fractions from the TLC plates cleaved the double bonds and produced mixtures of aldehydes and aldehyde-esters. Separate GLC analyses of the aldehydes and aldehyde-esters from the TLC fractions identified the individual aldehydes and aldehyde-esters comprising the fatty acids in each separated fraction. Close agreement between calculated and experimental percentages gave considerable confirmation to the identity of the aldehydes and aldehyde-esters produced, and to the positions of the double bonds in the fatty acid molecule.

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LIPIDS OF MATURING GRAIN OF CORN *(ZEA MAYS)*. EVELYN J. WEBER, Crops Research Division, USDA, Urbana, Illinois.

Corn samples were collected at intervals after the dates of hand-pollination. Three inbred strains, H51, K6, and Illinois High Oil, were examined. Illinois High Oil has been selected for increasing oil content since 1896. Total lipids were extracted from the grain with chloroform:methanol:water by the Bligh and Dyer procedure. The weights of total lipids increased most rapidly between 20 and 30 days after pollination. The lipids were separated into classes by silicic acid and thin-layer chromatography. Triglycerides constituted 10-17% of the total lipids at 10 days after pollination, and increased to 69-92% at 75 days. Polar lipids at 10 days represented 70-72% and at 75 days, 4-19%. The fatty acid composition was different in the various lipid classes, and changed as the grain matured. The changes in individual phospholipids and glycolipids were measured by phosphorus and sugar analyses of the lipids separated by 2-dimensional, thin-layer chromatography.

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REMOVAL OF CHLORINATED PESTICIDES FROM CRUDE VEGETABLE OILS BY SIMULATED COMMERCIAL PROCESSING PROCEDURES. K. J. SMITH, National Cottonseed Products, P. B. POLEN, Velsicol Chemical Corp., D. DEVRIES, Shell Chemical Company, and F. COON, Wisconsin Alumni Research Foundation.

Crude soybean and cottonseed oil were processed, using simulated commercial processing procedures to determine if oil processing would remove chlorinated pesticide contaminants of either natural or spiked origin. Two crude oil lots were spiked with Endrin, DDT, DDE, Aldrin, Dieldrin, Heptachlor, and Heptachlor Epoxide before processing. Representative samples of crude oil and products following each processing step were analyzed for pesticide contamination. Results indicated that alkali-refining or subsequent bleaching did not reduce chlorinated pesticide contamination. Hydrogenation prior to deodorization substantially reduced Endrin contamination. Deodorization, with or without hydrogenation, completely eliminated chlorinated pesticides. The results of this study indicate that normal commercial processing of crude vegetable oils for human consumption effectively removes any chlorinated pesticides which may be present in crude oils. Neither the pesticide nor its conversion products can be detected in the finished oils by the use of highly sensitive methods of analysis.

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DO MARKETING AND R & D HAVE A COMMON GROUND? W. FENTON GUINNEE, The Quaker Oats Company, Chicago, Illinois.

Industry statistics identify large costs, long lead time, and high failure rates associated with the introduction of new products. Unfortunately, success with new products is associated both with industry growth and the success of individual companies. Despite this, coordination between marketing and R & D groups is substantially less than perfect. The author suggests a possible cause of the problem, a solution, and its application.

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THE BRIDGE AND THE GAP BETWEEN MARKETING AND R & D. A. L. POWELL, The Pillsbury Company, Minneapolis, Minnesota.

The world could be beating a path to your doorstep for new products, but unless your organization is lined up to deliver them and has the atmosphere in which it is possible to invent and produce them, there is no point in even thinking about it. "But of course," you will say, and yet I will wager most organizations are their own biggest handicap, and this is the greatest block to the process of new product development. Unless management is not only dedicated to a new product program but also nimble and quick-witted enough to either make it happen, encourage it to happen, or let it happen, no new products will emerge. And there are vast differences between trying to make it happen, encouraging it to happen, and letting it happen. The threat of uncertainty in this whole field is great, and the anxiety of not knowing whether it is worthwhile to pursue a specific project tends to result in the project being ignored or turned down, sidetracked, pigeonholed, or absolutely willed to death. One of the problems is that at the early stages of a new product, the language about it is imaginative, emotional, sketchy, and impressive. Business management is not accustomed to dealing with language such as this. It wants precise definition, precise estimation, and review of performance. The only way for management to handle the nebulous and ephemeral nature of the early stage of product innovation is to translate this language into a discussion of the management of risk. Management can make bets about its chances of success not so much by looking at the product itself as in making bets about the data and criteria surrounding it. How big is the product category? How fast is it growing? What segment of the population does it appeal to? What are the bets about its eventual retail price, about the dollars necessary and the ability of your organization to produce it, and about how long it will take and how many people? This way, without having to voice an opinion on the product's excellence or on something still quite indistinct and likely to raise highly subjective opinion about its qualities, we can bring about a disciplined set of criteria for suggesting whether the project is worthwhile, rather than the product, at this stage of uncertainty as to whether it will sell. For this to come about requires considerable integration. Unless management, with its policy and direction-setting function and its financial control, is locked in totally with the marketing people, and they in turn are locked in with research and production, not only will ideas fail, the chances are that few will be generated.

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ASPECTS OF TECHNICAL SELLING. DALE W. JOHNSON, Crest Products, Inc., Park Ridge, Illinois.

Technical marketing involves having technical and scientific knowledge and a background of experience on application of the products being marketed, along with the ability to convey information, in a succinct manner, to people in product development, purchasing, and production. The marketing man, to be effective, should try to develop a reputation of trust so as to, hopefully, develop a "physician-patient" relationship with customers or potential customers in order to be of utmost assistance to his firm and that of the customer.

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THE "SNAP-CRACKLE-POP" OF OBTAINING A U.S. PATENT. W. C. DAVIS, Swift & Company, Chicago, Illinois.

We will be addressing ourselves to the task the patent attorney faces in getting the patent approved in order to satisfy the Patent Office, and at the same time still fulfill his obligation to R & D and marketing personnel. Our discussion will include a review of the various parts of the patent document and the significance that attaches to each part. As an integral part of explaining the major components of the patent, our discussion will include an outline of the original disclosure to be submitted to the patent attorney. Suggestions will be made for dealing with the 3 elements of patentability, namely, novelty, utility, and unobviousness. Special emphasis will be placed on the importance of designating those portions of the disclosure which should be considered old in the art, and why the invention would not be considered obvious to others skilled in the art. The patenting of inventions can offer several competitive advantages, such as better profit margins and increased sales. The patenting of inventions can also provide insurance against a competitor obtaining a patent for the same invention, and may enhance the bargaining position of the owner by minimizing royalty outlays and increasing a competitor's development costs. Patents demonstrate to directors, competitors, and customers the competence and capacity to advance the technology. This recognition can mean the dollars and personnel required to produce the continued emphasis of the R & D facilities, and an improved market position.

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THE ROLE OF TECHNICAL SERVICE IN THE MARKETING OF FOOD INGREDIENTS. JOSEPH RAKOSKY, JR., Central Soya, Chicago, Illinois.

Suppliers of food processors are finding it increasingly difficult to compete with one another on product performance and price. These things being equal, it is then necessary for the supplier to compete on a service basis—customer service and/or technical service. Central Soya's Chemurgy Division elected to provide its customers with both services. This paper discusses ways and means in which a technical service department becomes an integral part of the overall marketing plan involving edible products. In its original concept, the department was set up as an arm of the sales department charged with the responsibility of product demonstration and technical assistance to both the salesman and his customers. These 2 functions cover many things: the initial training of the salesman; working closely with marketing in formulating new plans; performing technical service work in the field; and taking part in many other related activities. The salesmen are encouraged to use the service of the technical department in approaching new customers and in giving help to existing customers. Guidelines in asking for help are presented. In working in a customer's plant it is imperative that the technical service representative leave the impression that he is working in the customer's best interest. For this reason, the service man at Central Soya never quotes prices or asks for the order. He leaves these jobs up to the salesman. Central Soya is a large organization with nearly 6,000 employees with varied backgrounds. If necessary, any one of the 6,000 employees may be called on to offer assistance to a customer in need. With this kind of help available, the Central Soya Technical Service Department can be considered to be 6,000 men strong.

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A SALES-MINDED RESEARCH DIRECTOR'S VIEWS ON MARKETING. JUSTIN J. ALIKONIS, *Paul F. Beich Company*, Bloomington, Illinois.

Too often, lab technicians, chemists, and research directors fail to realize that "nothing happens until there is a sale." Without sales, no wheels can turn in the production department and there will be no profits. Therefore, a sales-minded director should emphasize to the entire laboratory personnel the importance of sales in every phase of product development, which includes marketing. One should look at successfully marketed cereal products in the domestic and foreign field to find guidelines for new ideas. Since there are large percentages of failures in marketing products, one should try to eliminate as many pitfalls as possible. In looking at failures, do not look for faults, but remedies, for your new ideas. Examples of successfully marketed cereal products around the world will be presented.

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HEAT DENATURATION OF SOYBEAN 11S PROTEIN. W. J. WOLF and T. TAMURA, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Soybean 11S protein in pH 7.6, 0.5 ionic strength buffer was heated at 100°C. for varying times, cooled quickly in an icebath, centrifuged if a precipitate was present, and then analyzed in an ultracentrifuge. Heating caused conversion of 11S protein into a fast-sedimenting soluble aggregate plus a slow-sedimenting fraction (2-4S) in less than 5 min. On continued heating the soluble aggregate was precipitated, and about 40% of the total protein re-

mained soluble in the 2-4S form. Heating in buffer containing 0.01 or 0.5*M* mercaptoethanol accelerated conversion of 11S protein into the 2-4S form and an insoluble aggregate. No soluble aggregate was detected at either concentration of mercaptoethanol, and the reaction was more rapid at the higher level of reducing agent. When the protein was heated in buffer containing 0.01*M* *N*-ethylmaleimide, the aggregating reaction stopped short of the insoluble state; no precipitation occurred in up to 30 min. of heating. Heat apparently disrupts the quaternary structure of the 11S protein and releases subunits that remain soluble, plus subunits that have a pronounced tendency to aggregate. Since aggregation is promoted by high concentrations of mercaptoethanol, cleavage of disulfide bonds appears to make the interactions leading to aggregation and insolubilization more favorable.

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POLYACRYLAMIDE GEL ELECTROPHORESIS OF REDUCED AND ALKYLATED SOYBEAN TRYPSIN INHIBITORS. A. C. ELDRIDGE and W. J. WOLF, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Four soybean trypsin inhibitors (SBTI's) have been isolated in our laboratory. Polyacrylamide gel electrophoresis studies of native and reduced-alkylated SBTI's in 0.025*M* glycine buffer, pH 9.2, containing 8*M* urea showed the inhibitors to be different proteins rather than intramolecular disulfide forms or intermolecular disulfide polymers of a single protein. Reduction and reduction followed by alkylation caused one of the inhibitors (Kunitz crystalline) to migrate much slower in 8*M* urea than did the native inhibitor. This decrease in mobility is attributed to an increase in the intrinsic viscosity of the reduced protein resulting from unfolding of the polypeptide chain following cleavage of the disulfide crosslinkages normally present in the SBTI.

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DENATURATION OF SOYBEAN PROTEINS WITH ORGANIC SOLVENTS AND ENZYMATIC HYDROLYSIS OF DENATURED PROTEINS. DANJI FUKUSHIMA, Kikkoman Shoyu Co., Ltd., Noda-shi, Chiba-ken, Japan.

A systematic study was made of the denaturing abilities of organic solvents toward soybean proteins. Lower alcohols were much stronger denaturants than other solvents examined. The denaturing abilities of alcohols depended strongly upon their hydrophobicities and their concentrations. The denaturing power of alcohols at low concentrations increased with their hydrophobicities, while the reverse was found at high concentrations. Next, the enzymatic hydrolysis of alcohol-denatured soybean proteins by the proteinases of *Aspergillus sojae* was investigated in detail. Generally, the maximum degree of hydrolysis was 5-10% higher in alcohol-denatured proteins than in water-denatured proteins. During the denaturing treatments, a part of soybean flour oligosaccharides disappeared, and some new reducing sugars appeared. The loss of oligosaccharides was larger for water-denaturation than for alcohol-denaturation. The addition of reducing sugars to sugar-free flours before denaturing treatments had a striking influence on the digestibilities. Defatted soybeans were fractionated into several parts, and after denaturation, the enzymatic hydrolysis of the proteins was investigated. An increase of digestibilities through alcohol-denaturation was not observed in some minor fractions, but both the acid-precipitated fraction and the whey fraction showed an increase in the degree of maximum proteolysis after alcohol-denaturation as compared with water-denaturation. The increase in maximum hydrolysis resulting from alcohol-denaturation is discussed from the standpoint of the three-dimensional structure of protein molecules. The hydrolysis data on pepsin, trypsin, papain, *Bacillus subtilis* proteinase, *Streptomyces grecius* proteinase, *Aspergillus saitoi* proteinase, etc., are also presented.

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BIOLOGICAL EVALUATION OF CRAMBE SEED MEALS AND DERIVED PRODUCTS BY RAT FEEDING. C. H. VAN ETTEN, M. E. DAXENBICHLER, and I. A. WOLFF, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois; and A. N. BOOTH, Western Regional Research Laboratory, ARS, USDA, Albany, California.

Recent chemical results have demonstrated diverse hydrolytic reactions of the major crambe seed thioglucoside *epi*-progoitrin (*e*-PG), and have clarified the responsiveness of the reaction pathway to controllable environmental factors. Principal products of thioglucoside breakdown are a mixture of three nitriles, the oxazolidinethione (*R*)-goitrin, or some combination of these substances. Such elucidation of thioglucosidase reactions has made it possible to prepare definitively characterized crambe meals, and compounds or fractions of known chemical composition derived from them, for rat-feeding studies. Inclusion of 0.23% (*R*)-goitrin in rat diets caused growth inhibition to 82% that of a control group. Mild hyperplastic goiter and a mild degenerative nonspecific alteration of liver cells were the only microscopic changes detected. A similar retardation of growth was obtained upon feeding comparable levels of *e*-PG, either by its incorporation into a standard ration, or in a crambe meal in which thioglucosidase enzyme(s) had been inactivated. In contrast, meals autolyzed to form the nitrile mixture, or the nitrile mixture itself after isolation from the meal by extraction, were more toxic. They caused poor growth or death, and resulted in bile duct hyperplasia, fibrosis, and megalocytosis as well as hypertrophy of the tubular epithelial cells in the kidney. Rats fed meal containing intact *e*-PG plus active thioglucosidase enzyme(s) showed the same lesions as those that resulted from feeding the nitrile mixture as well as some thyroid hyperplasia. Therefore, thioglucoside hydrolysis under such conditions has probably led to a mixture containing both (*R*)-goitrin and nitriles. Protein efficiency ratio (PER) was determined on defatted crambe meal which had been thoroughly extracted with aqueous acetone without use of elevated temperatures. The PER (2.75) was higher than that of casein (2.50) or soybean meal (2.15) controls.

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USE OF RAPE SEED MEAL IN PRODUCTION OF BROILERS. R. COSTABAL and N. GONZALEZ, Instituto de Investigaciones Agropecuarias, Santiago, Chile; T. MCAULLIFE and J. MCGINNIS, Washington State University, Pullman, Washington.

Three experiments were conducted to determine whether rape seed meal could be used as a replacement for sunflower seed meal and fish meal in rations for broilers. In 2 of these experiments, sunflower seed meal was replaced on a protein basis using graded levels of rape seed meal. In the third experiment, after all of the sunflower meal was replaced, additional amounts of rape seed meal were used to replace part of the fish meal. In these experiments the replacement of sunflower meal with rape seed meal gave equivalent chick growth and improved feed efficiency. When a level as high as 32% of rape seed meal was used in the third experiment, which replaced all of the sunflower meal and a part of the fish meal, growth was depressed only slightly. Additional experiments are in progress to compare rape seed meal produced in 2 different processes of oil extraction. Also, studies are in progress, with both laying hens and turkeys, to determine what levels of rape seed meal may be used satisfactorily in diets for these birds.

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SOY PROTEIN NOMENCLATURE. W. J. WOLF, Northern Utilization Research Division, USDA, Peoria, Illinois; M. P. TOMBS, Unilever Research Laboratory, Bedford, England; N. CATSIMPOOLAS, Central Soya Co., Chicago, Illinois; I. KOSHIYAMA, Tohoku University, Sendai, Japan; K. SHIBASAKI, Noda Institute for Scientific Research, Noda City, Japan; and P. MELNYCHYN, Carnation Research Center, Van Nuys, California.

Terminology used for soybean proteins in the past is reviewed. The relationships between the classical protein fractions and recently characterized proteins are emphasized. Recommendations under consideration for future usage are discussed.

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GAS CHROMATOGRAPHIC DETERMINATION OF STRUCTURE OF LIPIDS BY HYDROGENATION, HYDROGENOLYSIS, AND OZONOLYSIS. MORTON BEROZA, ARS, USDA, Beltsville, Maryland.

Three gas chromatographic techniques that may be useful for determining the structure of compounds at levels as low as 1 μg. per determination will be described. In carbon skeleton chromatography, a compound is injected onto a hot catalyst bed situated in a hydrogen carrier gas stream; functional groups are stripped from the molecule, and multiple bonds are saturated to yield hydrocarbons that help identify the carbon skeleton. A technique that saturates multiple bonds utilizes a 6-mm. length of catalyst in the gas chromatographic pathway to hydrogenate a wide variety of compounds. An ozonolysis procedure that helps locate the position of double bonds in compounds utilizes a simple ozonizer. Its application to a wide variety of compounds and to oils will be illustrated. As a further aid in this work, a device for trapping substances from a gas chromatographic column and reinjecting them into the same or another gas chromatograph with high efficiency will be described.

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QUANTITATIVE ANALYSIS OF URINARY STEROID HORMONES BY GAS-LIQUID CHROMATOGRAPHY. BILLY G. CREECH, Methodist Hospital, Houston, Texas.

Gas-liquid chromatography provides a powerful tool for separation and determination of the individual steroidal molecules which function in human endocrine systems. Its use has provided insight into disease states that was not possible with less specific methods. Chromatography may be accomplished on a wide variety of liquid phases with either free or derivatized compounds, and quantitation is simple with the use of internal standards. The majority of applications require only ionization detectors of the usual type, but special use has been made of electron-capture detection where derivatives with these properties may be prepared, and maximum sensitivity is required. While the chromatographic operations involved in the evaluation of urinary extracts are relatively simple, the isolation of hormones from their native biological source is not. The normal detoxification mechanisms of the body result in the excretion of conjugates, primarily with sulfuric and glucuronic acid, and the hormones must be freed from their conjugated state before chromatography. Since preservation of molecular integrity is essential, the usual methods of hot acid hydrolysis and solvent extraction are not desirable. Methods involving the use of enzymes have been proposed, but extreme care must be taken to avoid poor recovery due to inhibition. Solvolysis is applicable to some compounds, but often yields incomplete hydrolysis. In some instances, it may be advisable to couple thin-layer chromatography with gas chromatography. The complex nature of urine and the close structural similarity of many of the classes of hormones makes it necessary to carry out preliminary separations.

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AUTOMATED THIN-LAYER CHROMATOGRAPHY. FRED SNYDER, Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee.

Two scraping devices (manual and automatic models) for collection of thin-layer chromatographic (TLC) zones have recently been modified to accept conventional TLC end plates. The instruments permit the collection of TLC zones in a manner analogous to the collection of fractions from silicic acid columns. High-resolution curves for both radioactive and colorimetric quantitative analyses can now be obtained from adjacent lanes on the same TLC plate, and other lanes can be used for visualization by carbonization. The system employs a pipetting device having a sample holder comparable to the turntable of the zonal scaper. This device automatically dispenses reagents for radio- or colorimetric assay in appropriate volumes. Plottings and calculations of the results are included in the computerized system. Special emphasis will be given to a new liquid scintillation-quenching technique for quantitation of chemical mass and to an automatic plate-coating device. Data obtained with this system will be shown with reference to its application in organic and biochemistry.

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X-M RICE OIL DEWAXING PROCESS. D. NORMAN KINSEY, JOHN W. HUNNELL, and LAWRENCE LYNN, Riviana Foods Inc.

In the production of rice oil by the X-M solvent extractive milling process, extraction of lipid materials is made from the entire brown rice input including the endosperm, bran, and polish fractions. The crude oil has a desirable low free fatty acid content, but a high rice wax content, 2.5-3.5% of the weight of the oil stream. Conventional refining techniques, bleaching, and deodorizing will not remove rice wax in amounts sufficient to yield a clear rice oil. The slime nature of the rice wax prevented satisfactory removal of the treatment material in bleaching. Hydration of gummy materials to make them insoluble was not effective, as the addition of water to rice oil did not bind to the wax. Several conventional methods for solids-liquid separation were investigated. Various forms of filtration, i.e., vacuum, pressure, and centrifugal, failed to produce adequate flow rates and throughputs to be economically feasible. The very slight difference in specific gravity between the wax and rice oil prevented successful use of centrifugal methods of separation. By combining an immiscible liquid, water, with rice oil at ambient temperature and adding an appropriate surface-active compound, a flocculation system was established at the liquid-solid interfaces. This adhesion was sufficiently strong to allow handling of the oil without disruption. Because of the weighting effect of the water bound to the rice wax, it was centrifugally deposited as a plastic solid which was then readily separated from the rice oil. As presently used in the X-M process, such treatment is employed both in the miscella and oil states. The 1st phase of the process consists of treating an oil-hexane miscella, previously chilled at 25°C., with a 4.5% (wt.) sodium silicate solution at 4-5% level (based on oil). This operation is followed by a short holding period followed by centrifugation. Up to 80% of the wax fraction is removed. A 2nd treatment in the oil phase after desolventizing, removes the re-

maining portion of the wax. Crude oil produced by this process will remain clear at 32°F. for 5½ hr.

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NEW RICE OILS. LAWRENCE LYNN, R. M. ANDERSON, and GARY STEEN, Riviana Foods Inc.

Crude but dewaxed X-M rice oil subjected to conventional caustic refining followed by bleaching, winterization, and deodorization yields a high quality cooking/salad oil. Heretofore, excessive refining losses were experienced due to high free fatty acid in crude oils extracted from rice bran produced by conventional milling. Now, because of several unique features of extraction concomitant with the X-M rice milling process, economical processing to top quality edible oil is commercially feasible. This paper describes briefly the conventional refining of X-M rice oil and the chemical composition and characteristics of the final oil. Also discussed are results of evaluation according to standard AOCS test methods, and several performance tests and their results.

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CATALYTIC INVESTIGATION FOR THE HYDROGENATION OF COTTONSEED OIL. S. STEFANOVIC and L. ALBRIGHT, Purdue University, Lafayette, Indiana.

Refined and bleached cottonseed oil was hydrogenated in a dead-end type laboratory autoclave using various types of catalysts. Variations in the catalysts investigated were as follows: (a) Three commercial catalysts. (b) Partial poisoning of catalyst with hydrogen sulfide. (c) Effect of adding inerts such as alumina which have sometimes been found to be hydrogenation promoters. (d) Reused catalysts. The rate of hydrogenation, selectivity ratio, and isomerization index were determined for the runs at about 140-170°C. The results of this investigation clarify the variables or properties of the catalyst that are of importance, relative to both the mechanism and commercial operation.

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OXIDATIVE DETERIORATION OF PARTIALLY PROCESSED SOYBEAN OIL. L. H. GOING, Procter and Gamble.

The oxidative stability of partially processed soybean oil has been investigated in laboratory and plant-scale tests. Oxidation, as measured by peroxide formation, has been related to storage time, temperature, and availability of oxygen. The effects of this oxidation on finished product quality have been measured. Soybean oil stores best as crude oil. After refining and bleaching, the oil is more susceptible to oxidation than at other stages in processing. Increase in peroxide content is both time and temperature dependent. Substantial improvement in product quality can be obtained by minimizing exposure to oxygen. Recommendations are made for proper storage conditions for partially-processed soybean oil.

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PROBLEMS ASSOCIATED WITH THE DEVELOPMENT OF A HIGH-PROTEIN SOY BEVERAGE IN ALLEVIATING PROTEIN MALNUTRITION. L. ROSS HACKLER, New York State Agricultural Experiment Station, Cornell University, Geneva, New York.

With food in short supply in many of the underdeveloped countries, various foundations, governmental agencies, and universities are actively engaged in developing low-cost, high-protein foods as a means of alleviating, at least in part, a nutritional problem that is prevalent in many areas of the world. Considerable money, as well as effort, is being expended to try to solve the problem. For those involved, it has been, at times, a very frustrating problem. It is planned to discuss some of the problems associated with the development of a low-cost, high-protein soy beverage, and areas which appear to offer greater possibilities for success.

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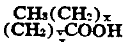
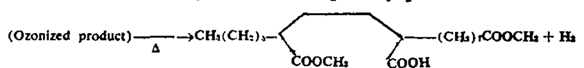
ETHYLENE ADDUCT OF CONJUGATED OCTADECADIENOIC ACIDS: OXIDATIVE DERIVATIVES. E. J. DUFEK, J. P. FRIEDRICH, and L. E. GAST, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

The previously described addition of ethylene to conjugated octadecadienoic acids gave an unsaturated cyclic fatty acid, I. where $x + y = 12$.



$CH_3(CH_2)_x$
$(CH_2)_yCOOH$
I

The ethylene adduct of *trans,trans*-9,11-octadecadienoic acid ($x = 5$, $y = 7$) was treated with 30% hydrogen peroxide in formic acid, followed by saponification of the resulting hydroxyformoxy derivative, to yield 2 crystalline glycols believed to be the *cis* and *trans* forms. The hydroxyformoxy fatty acid can be converted directly either to the glycol ester by reaction with anhydrous hydrochloric acid in methanol, or to the acetone derivative of the methyl ester by treatment with dimethoxypropane and anhydrous hydrochloric acid in methanol. Epoxidation of the methyl ester of I gave the corresponding epoxy derivative. Ozonolysis of the methyl ester of I, followed by reduction with powdered zinc, produced the expected dialdehyde ester, which was isolated as the tetramethyldiacetal. When the methyl ester of I was ozonized in methanol and the methanol removed *in vacuo* at room temperature, the ozonized product decomposed slowly with evolution of hydrogen to an acid dimethyl ester as the primary product.



$$\text{(Ozonized product)} \xrightarrow{\Delta} CH_3(CH_2)_x\text{—}\underset{COOCH_3}{\text{C}}\text{—}\underset{COOH}{\text{C}}\text{—}(CH_2)_yCOOCH_3 + H_2$$

Treatment of the ozonized product from I with hydrogen peroxide in formic acid gave as one of the major products, dihydroxystearic acid.

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HYDROFORMYLATION OF UNSATURATED FATTY ESTERS. E. N. FRANKEL and W. K. ROHWEDDER, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois, and S. MELTIN and I. WENDER, Pittsburgh Coal Research Center, Pittsburgh, Pennsylvania.

Studies of hydroformylation (oxo reaction) have pointed a way to insomerize double bonds and to introduce oxygen functionality throughout a fatty acid chain, including the terminal C_{17}-C_{18} position. Hydroformylation of unsaturated fatty esters and vegetable oils was carried out with hydrogen and carbon monoxide (3,500-4,500 psi), and catalyzed by dicobalt octacarbonyl. A mixture of fatty aldehydes was obtained at 100-110°C., and of fatty alcohols at 175-180°C. Conversion into C_{19}-oxo products varied from 40 to 90%. Distilled products contained from 50 to 90% branched isomers and from 2 to 15% linear isomers. Proportion of linear isomers increased at higher reaction temperatures and in the presence of tributylphosphine as cocatalyst. The analytical scheme involved separation of branched and linear C_{19} alcohols by silicic acid column, thin-layer, and gas-liquid chromatography. The isomer distribution of branched diester products was determined by mass spectrometry.

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HOMOGENOUS CATALYTIC HYDROGENATION OF UNSATURATED FATS: GROUP VIB—METAL CARBONYL COMPLEXES. E. N. FRANKEL and F. L. LITTLE, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Chromium tricarbonyl complexes of aromatic compounds and cycloheptatriene catalyze stereoselective hydrogenation of conjugated fatty esters (E. N. Frankel and M. Cais, unpublished). This work has now been extended to other unsaturated fatty esters and to other carbonyl complexes of chromium, molybdenum, and tungsten. Although it was previously observed that only conjugated fatty esters in a mixture were hydrogenated with the chromium complexes, we now find that under certain conditions the more active complexes can also catalyze the hydrogenation of unconjugated fatty esters. Relative catalytic activity decreases in the following order: mesitylene-$Mo(CO)_3$ cycloheptatriene-$Cr(CO)_3$, cycloheptatriene-$Mo(CO)_3$, bicycloheptadiene-$Mo(CO)_4$, methyl benzoate-$Cr(CO)_3$, mesitylene-$W(CO)_3$, mesitylene-$Cr(CO)_3$, and hexamethylbenzene-$Cr(CO)_3$.

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SOME RADIOCHEMICAL EXPERIMENTS ON MINOR CONSTITUENTS IN SOYBEAN OIL. T. L. MOUNTS, C. D. EVANS, H. J. DUTTON and J. C. COWAN, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

The fate of tracer amounts of high molecular weight hydrocarbons and insecticidal contaminants of soybean oil was studied in laboratory simulations of commercial processing procedures by radiotracer (^{14}C) techniques. To the hydrocarbon solvent used in the extraction of soybean flakes was added labeled benzo-2-pyrene, and its activity was followed during extracting, filtering, stripping, bleaching, and deodorizing, and through 2 types of liquid-liquid column chromatography. The high molecular weight hydrocarbon was retained by the oil during extraction from the flakes and was not removed by any of the processing procedures. Labeled insecticidal compounds (endrin, aldrin, dieldrin) were added to soybean oil in 1-5 ppm (by weight) amounts. The oil was subjected to bleaching and deodorizing procedures under a variety of experimental conditions; removal of the radioactive material was nearly complete.

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REACTIONS OF PEROXIDES II. REACTION OF PEROXIDES AND IODINE WITH CARBOXYLIC ACIDS. A NEW TYPE OF HUNSDIECKER REACTION. LEONARD S. SILBERT, ARS, USDA, Philadelphia, Pennsylvania.

A new reaction is described that may be classified as a "true peroxide analog" to the Hunsdiecker Reaction for synthesizing alkyl iodides in 90% yields. Aroyl peroxides (such as benzoyl peroxide) and peresters (such as t-butylperoxy isopropyl carbonate) react with aliphatic acids and iodine in relatively inert solvents like 1,3-dichloro propane. Peroxide abstracts carboxylic hydrogen in this reaction to form benzoic acid from benzoyl peroxide and t-butanol from t-butyl peresters, and the generated acyl hypoiodite decarboxylates to the alkyl iodides. In this manner, high yields of 1-iodoheptadecane are formed from stearic acid. A mechanism is presented in which the transition state bears similarity to an "identity reaction," but differs by having a hydrogen bond between an aroyloxy and an acyloxy radical that includes a charge-transfer complex of the latter with iodine. The radical of lesser stability decarboxylates, and the surviving "intermediate" radical forms a stronger acid from the weaker one. Evidence is based on conversions of a series of peroxide and acid pairs to their corresponding iodo derivatives. The reaction contradicts the currently accepted belief that carboxylic hydrogens are not transferred in free radical processes. The new method is also a usefully simple one for synthesizing iodoperfluroalkanes from perfluoroacids.

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REACTION OF CYCLOPROPENE ESTERS WITH HYDROGENATION CATALYSTS. ZIGRIDA M. ZARINS, R. K. WILLICH, and R. O. FEUGE, Southern Utilization Research and Development Division, ARS, USDA, New Orleans, Louisiana.

Methyl esters of sterculic and malvalic acids, the cyclopropene acids of cottonseed oil, were heated under nitrogen in the presence of various hydrogenation catalysts, and the effect on the cyclopropene moiety was established. Similar tests then were conducted with cottonseed oil. Palladium catalysts differed markedly from the others investigated. Heating the methyl esters of *Sterculia foetida* oil fatty acids (58.4% cyclopropenes) at 150°C. under nitrogen with 0.5% palladium in the form of 10% palladium-on-carbon catalyst decreased the cyclopropene content to 2% in 40 min. After 90 min., the Halphen test was negative, indicating the complete absence of cyclopropenes. The cyclopropenes were converted into a mixture of unsaturated condensation products and a number of unsaturated mono esters of practically unchanged molecular weight. Nickel and platinum catalysts were not nearly as active as palladium, and did not give Halphen-negative products. For example, heating the methyl esters of the *Sterculia foetida* oil fatty acids for 60 min. at 150°C. with or without 0.2% catalytic nickel lowered the cyclopropene content about 4 percentage units. Heating cottonseed oil (0.73% cyclopropenes, calculated as trimalvalin) with 0.02% palladium for 2 hr. at 150°C. gave a completely Halphen-negative oil. Palladium catalysts, freshly activated with hydrogen before being tested under nitrogen, were not as active as the same catalysts freed of adsorbed hydrogen. The effect of other operating variables was established. The palladium catalysts could be reused with only a slight decrease in activity. The cottonseed oil samples rendered cyclopropene-free by treatment with palladium were unaltered in appearance, and the noncyclopropene components were unaffected.

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THE GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF CYCLOPROPENOID FATTY ACIDS. E. L. SCHNEIDER, S. P. LOKE, and D. T. HOPKINS, Ralston Purina Company.

A method is described for the analysis of cyclopropenoid fatty acids in oils. The method consists of reacting the methyl esters of the cyclopropenoid fatty acids with silver nitrate in methanol to form ether and ketone derivatives. The derivatives formed from the cyclopropenoid fatty acids are separated from the methyl esters of the normal fatty acids by gas-liquid chromatography on a 15% diethylene glycol succinate column. The method is applicable to oils containing from 0.01 to 100% of cyclopropenoid fatty acids. The derivatives of oils containing low levels of cyclopropenoids are separated from the normal methyl esters by alumina chromatography prior to gas-liquid chromatography. Studies on the quantative aspects of the derivative formation, alumina chromatography, and gas-liquid chromatography are reported. Analyses for the total cyclopropenoid fatty acid content of cottonseed oil and *Sterculia foetida* oil by gas-liquid chromatographic and hydrobromic acid titration procedures showed good agreement. Replicate analyses of a sample

of *Sterculia foetida* oil for malvalic and sterculic acid gave coefficients of variation of 6.04 and 1.17%, respectively. (Contract #12-14-100-8882, supported by the Southern Regional Utilization and Development Division of the U.S. Department of Agriculture.)

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VOLATILES FROM SOYBEAN OIL: ISOLATION BY VACUUM DISTILLATION AND IDENTIFICATION BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY. E. SELKE, HELEN A. MOSER, and W. K. ROHWEDDER, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Commercially available soybean oil, aged under normal laboratory room conditions, was used for a time-versus-decomposition products study. Samples were taken at weekly intervals for peroxide value determinations, organoleptic evaluations, and identification of volatile decomposition products. Volatiles were collected in an all-glass, vacuum distillation system void of connecting joints. Volatiles were transferred and flame-sealed into capillary tubes by vacuum and cryogenic techniques without the use of solvents. A gas chromatograph connected in tandem with a mass spectrometer was used to separate and obtain mass spectra of the encapsuled volatiles. Various hydrocarbons, alcohols, and aldehydes were positively identified while some substituted furans, diketones, and alkyl dienals were tentatively identified. Correlations of identified compounds, peroxide values, and organoleptic evaluations will be discussed.

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GLYCERYL ETHERS IN INSECTS: IDENTIFICATION OF THE ALK-1-ENYL AND ALKYL ALKENYL ETHERS IN THE PHOSPHOLIPID FRACTION OF THREE INSECT SPECIES. EDWARD N. LAMBREMONT, Louisiana State University, Baton Rouge, Louisiana, and RANDALL WOOD, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee.

Glyceryl alkyl alkenyl and alk-1-enyl ethers have been identified in the phospholipids of 3 insect species, the American cockroach (*Periplaneta americana*), the tobacco budworm (*Heliothis virescens*), and the boll weevil (*Anthonomus grandis*). Little or no ethers were detected in the neutral lipids. The ethers were found in whole insects and in isolated fat body (adipose) tissue. The ether content is quite variable between the 3 species, and fluctuates during various developmental stages of each species. In the American cockroach, only the 1-isomer was found, however it is tentatively concluded that the 2-isomer occurs in the alkyl alkenyl ether fraction of the tobacco budworm. GLC analysis of the ether-bonded hydrocarbon side chain showed striking differences in chain length between cockroach and budworm. However, the 2 ether fractions were very similar when their side chain lengths were compared within each species. Preliminary evidence indicates biosynthesis of the alkyl alkenyl but not alk-1-enyl ethers in *Heliothis* pupae after ^{14}C-1-acetate injection.

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OXYGENATED FATTY ACIDS OF SUNFLOWER SEED OIL. K. L. MIKOLAJCZAK, R. M. FREIDINGER, C. R. SMITH, JR., and I. A. WOLFF, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

Chemical analysis of a number of sunflower (*Helianthus annuus*) seed oil samples has consistently revealed a low and variable percentage of substance(s) that react with hydrogen bromide. To characterize the compounds responsible for this reactivity, oil was obtained from selected introductions from Uruguay, Turkey, and Yugoslavia. Two epoxy fatty acids and 2 conjugated dienolic acids have been isolated by countercurrent distribution of the mixed methyl esters from the oils. The epoxy acids have been identified as *cis*-9,10-epoxystearic acid, 0.5%; and *cis*-9,10-epoxy-*cis*-12-octadecenoic (coronaric) acid, 2.2%. Characterization of the dienols revealed that they are 9-hydroxy-*trans*-10,*cis*-12-octadecadienoic acid, 1.2%; and 13-hydroxy-*cis*-9, *trans*-11-octadecadienoic acid, 1.3%. The oils of selected high-oil Russian sunflower varieties, including some currently grown in the U.S., were also screened for oxygenated fatty acids, but no more than a trace was detected by ultraviolet and thin-layer chromatographic analyses. It is not known whether the presence of these oxygenated acids is due to genetic or environmental factors, or if they may form during seed storage.

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DIHYDROSTERCULIC, A MAJOR FATTY ACID COMPONENT OF *DIMOCARPUS LONGANS* SEED OIL. R. KLEIMAN, F. R. EARLE, and I. A. WOLFF, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

The seed oil of *Dimocarpus longans*, Sapindaceae, contains 17.4% of 9,10-methyleneoctadecanoic (dihydrosterculic) acid. This identification is based on information from thin-layer chromatography (TLC), infrared analysis, gas-liquid chromatography (GLC), nuclear magnetic resonance, and mass spectroscopy. Since GLC of the oil showed components that emerged between the usual triglycerides, the cyclopropanoid acid is apparently a triglyceride constituent. The presence of smaller amounts, less than 1%, of cyclopropanoid fatty acids of different chain lengths is indicated by GLC and TLC analyses of the methyl esters. The other major fatty acids in this oil are: 16:0 (17.6%), 18:0 (7.3%), 18:1 (30.5%), 18:2 (5.9%), 18:3 (4.7%), and 20:0 (4.1%). *Dimocarpus* oil contains considerably larger amounts of cyclopropanoid fatty acids than previously reported in other seed oils.

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DECREASE, BY MILD OXIDATION, OF THE TOXICITY OF FRESH VEGETABLE OILS. H. KAUNITZ and RUTH E. JOHNSON, Columbia University, New York, New York.

In a previous long-term study, male rats of the Columbia-Sherman strain were maintained on purified diets containing commercially available vegetable oils (cottonseed, olive, corn, and soybean oils) and vegetable fats (chicken and beef fats, butter and lard). The fats were fed, at a level of 20%, either fresh or after aeration for 40 hours at 60°C., which hardly altered their peroxide values. The groups fed fresh olive oil, soybean oil, and corn oil had significantly shorter average life spans than did those fed the corresponding oxidized oils or the fresh animal fats. The incidence of spontaneous tumors was lower in the animals fed oxidized fats and oils. To confirm these findings, a second study was initiated in which groups of Columbia-Sherman males were fed fresh and aerated versions of all of the above mentioned fats and oils. In addition, fresh and aerated soybean oils were fed to C-S female rats and fresh and aerated soybean oil and lard, to males of the W/Fu strain. Soon after the start of the experiment, an epidemic of rapidly fatal lobar pneumonia occurred among the C-S males; after 37 days of the experiment, the mortality had been:

	Fresh		*Aerated*
Cottonseed oil	4		0
Olive oil	4		0
Corn oil	3		0
Soybean oil	2		3
	13	.... P < .01	3

	Fresh	*Aerated*
Chicken fat	2	1
Beef fat	0	2
Butter oil	1	2
Lard	1	1
	4	6

All C-S males were sacrificed and new groups, consisting of pathogen-free males, were placed on the diets. In all sets of males (C-S, W/Fu, and pathogen-free) those fed oxidized soybean oil were heavier than their controls fed the fresh oil (P < .01 for the pathogen-free). In view of the unchanged peroxide numbers, it is postulated that the damaging materials are of an anti-oxidant nature (perhaps biologically comparable to gossypol). Work is underway to determine the nature of the materials. (Supported by Contract PH-43-67-731 from the National Cancer Institute of the National Institutes of Health.)

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DETERMINATION OF 15-16 DOUBLE BOND IN HYDROGENATED SOYBEAN OIL. L. T. BLACK and R. E. BEAL, Northern Regional Research Laboratory, ARS, USDA, Peoria, Illinois.

A method was developed to determine the extent of hydrogenation of the carbon 15-16 double bond in soybean oil following its selective hydrogenation with a copper-chromite catalyst. Soybean oil was ozonized in carbon tetrachloride at —20°C., followed by reduction with triphenylphosphine. The ozonized-reduced sample was injected into a gas chromatograph operated at 155°C., and equipped with a 12-ft. × ¼-in. column of 100/120 mesh Porapak Q. Propionaldehyde from unhydrogenated soybean oil of known linolenate content was used as a standard. A linear relationship exists between the linolenate content of most commonly occurring unhydrogenated oils and the propionaldehyde obtained from their ozonization reduction. Propionaldehyde from hydrogenated samples, ozonized and reduced, can, therefore, be direcly related to the amount of 15-16 double bond. A stoichiometric amount of propionaldehyde results from ozonized and reduced soybean oil, as shown by comparison with a standard mixture of propionaldehyde and carbon tetrachloride. The relative standard deviation for the method is ±4.4%; for repeated injections of ozonized and reduced soybean oil, it is ±2.2%. This method has also been applied to other oils containing omega-3 double bonds.

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REACTION THIN-LAYER CHROMATOGRAPHY IN THE ANALYSIS OF PLASMALOGENS. C. V. VISWANATHAN, F. PHILLIPS, and W. O. LUNDBERG, The Hormel Institute, Austin, Minnesota.

A 2-dimensional thin-layer chromatographic method is described by which either a mixture of alkenyl acyl choline phosphatides with diacyl choline phosphatides, or a corresponding mixture of ethanolamine phosphatides, can be analyzed for its alkenyl contents, as well as for its fatty acid compositions, and, as regards the alkenyl acyl phosphatides, for its aldehyde compositions. A sample of approximately 250 μg. is spotted at the lower left and right corners of the TLC plate. After treating both corners with the vapors from concentrated hydrochloric acid, the free aldehyde, lyso-phosphatide, and diacyl phosphatide are separated in the 1st dimension, using chloroform-methanol-ammonia (70:30:5). The separated compounds on the right side of the plate (reference strip) are made visible by spraying with 5% solution of iodine in chloroform. On the basis of the position of fatty aldehydes in the reference strip, the aldehyde-containing portion on the unsprayed left side is scraped off for GLC analysis. The remainder of the left strip then is sprayed with 12% KOH in methanol, which interestifies the fatty acids of the lyso- and diacyl phosphatides in about 2 min.

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GAS-LIQUID AND THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF FATTY AMINO ACIDS. BERNARD FREEDMAN, Western Regional Research Laboratory, ARS, USDA, Albany, California.

Products from reductive amination of 12-ketostearic acid to 12-aminostearic acid have been monitored by gas-liquid chromatography (GLC) and thin-layer chromatography (TLC). The amino acid was analyzed by GLC as its trifluoroacetylated methyl ester. Conditions for the preparation of this derivative are described. Good separations of this derivative from other compounds present in the reaction mixture were achieved on a 5-ft, ⅛-in, FFAP packed column. Excellent resolution of the trifluoroacetylation reaction mixture was also obtained by TLC. Conditions for separating the free amino acid from other nonvolatile components by TLC are also described.

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AUTOMATED GC ANALYSIS OF LIQUID SAMPLES. F. G. MCCARTY, Barber-Colman Company, Rockford, Illinois.

Automatic laboratory GC analysis is now possible for most samples that can presently be handled by conventional microsyringe injection techniques. Recent developments permit the automatic GC system described to handle volatile liquid samples as well as nonvolatile crystalline or oily samples. New sample introduction techniques permit preinjection storage of samples without loss by evaporation, or cross-contamination among stored samples. The system has been successfully applied to a variety of sample types, including pesticides, methyl esters of fatty acids, sugars, and amino acids. With the system described, reliable unattended operation is possible for several days or more. This permits more efficient use of GC facilities for routine analysis, as well as yielding a greater abundance of data on research studies.

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THE EFFECT OF LIPOXIDASE ON THE LIPID COMPOSITION AND FLAVOR OF SOY MILK. W. F. WILKENS, Cornell University, Geneva, New York.

The extraction of soybeans with cold water in the production of soy milk resulted in nearly complete oxidation of polyunsaturated fatty acids and esters by lipoxidase in less than 30 min. after grinding. This amounted to almost 60% of the total lipid or 18 g. of oxidized polyunsaturated fatty acid equivalent per liter of soy milk of 7.5% solids content. High temperature extraction prevents or reduces the lipid oxidation which accounts for the primary off-flavor developed in soy milk prepared by cold water extraction.

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THE FORMATION OF 1-OCTEN-3-OL IN SOYBEANS DURING SOAKING. A. F. BADENHOP and W. F. WILKENS, Cornell University, Geneva, New York.

Soybeans, when soaked in water as a pretreatment for soy milk manufacture, were found to produce 1-octen-3-ol. Measurements indicate that at 50°C. the amount formed increases to a maximum after approximately 6 hr. of soaking. The amount formed during this period amounts to approximately 4.35 mg./100g. of unsoaked beans. A discussion of this compound with reference to its isolation and identification from soy milk, its effect on flavor of soy milk, and the effect of various soaking media on the amount formed will be presented, along with a discussion of the mechanisms which have been suggested for its formation, and evidence to support a suggested mode of formation in soaking soybeans.

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CITRUS FLAVOR TRANSFORMATIONS. J. A. ROGERS, Fritzsche Brothers, Inc., New York, New York.

The fresh peel character of citrus flavors, especially lemon, has given them preferential acceptance for use in many finished foods. Certain natural components are sensitive to conditions present in various stages of the baking process. These changes can be defined as chemical degradations, isomerizations, and transformations. Several such effects will be examined for the improvement of stability of citrus flavors in baked goods.

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THE ISOLATION AND IDENTIFICATION OF VOLATILE FLAVOR COMPOUNDS PRODUCED DURING DEEP FAT FRYING. S. S. CHANG, TSUKASA KAWADA, R. G. KRISHNAMURTHY, B. R. REDDY, J. A. THOMPSON, and KOSAKU YASUDA, Rutgers State University, New Brunswick, New Jersey.

Chemical identification of the volatile decomposition products produced by fats and oils under conditions of deep fat frying is important for the elucidation of the mechanisms of thermal oxidation, for the study of their effect upon human nutrition, and for their contribution to the deep-fat-fried flavor of foods. An apparatus which can be used for the collection of these volatile decomposition products will be described. The volatile compounds thus collected were frationated by repeated gas chromatography, and the gas chromatographic fractions were identified by the combination of infrared and mass spectrometry. A total of 95 compounds were identified as the volatile decomposition products produced by a more unsaturated corn oil. A more saturated hydrogenated cottonseed oil, when used for frying for a longer period of time under similar conditions, yielded 102 identifiable compounds. These compounds were compared with those produced by pure trilinolein under conditions of deep fat frying. The importance of some of the compounds identified to human nutrition and flavor of fried foods will be discussed.

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VOLATILE COMPONENTS OF DEEP-FAT-FRIED SOYBEANS. W. F. WILKINS, A. F. BADENHOP, and L. R. HACKLER, Cornell University, Geneva, New York.

Soybeans were soaked at 70°C. for 2 hrs., wet dehulled, and deep-fat-fried for 3-5 min. in corn oil at 190°C. The major volatile constituents were 1-octen-3-ol; 2,4-decadienal; pyrazines; and several aromatic and heterocyclic compounds.

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FLAVORING READY-TO-EAT CEREALS. J. P. LITTLEJOHN, Kellogg Company, Battle Creek, Michigan.

The topic of food flavor has long intrigued both the formulator and the consuming public. Of course, to the cereal industry, grains and/or flavors developed from grains are of most prominent interest. Perhaps the most widely used flavoring material in the cereal industry is malt. The different uses of malt and malt flavors are discussed. Processing conditions play a major role in food flavor development. Recent advances in analytical chemistry instrumentation have led to several more and better artificial flavors. Gas chromatography now allows the flavor chemist to more closely synthesize naturally occuring flavors. Adequate protection through the combined use of antioxidants and packaging must be afforded.

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FLAVORING SYNTHETIC FOODS. J. BRODERICK, H. Kohnstamm & Co., Inc., New York, New York.

The role of the flavor industry in the development of new food products will be discussed. An attempt will be made to separate fact from myth so that the food technologist can ascertain when to ask the flavor industry for help and what kind of help he can expect. The development of synthetic meat flavors will be used as one example, and the role of food company, government, university, as well as flavor research laboratories in the development of suitable meat flavors, will be outlined. The practical limitations on research by flavor organizations will be noted as well as the restrictions placed on the use of new materials by government standards and food additive regulations.

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TEXTURE AND FLAVOR RELATIONSHIPS RELATED TO SIMULATED MEAT PRODUCTS. W. E. HARTMAN, Worthington Foods, Inc., Worthington, Ohio.

This paper is in no way intended to be a technical presentation. Rather, it should be regarded as more of a historical report of the experience and problems of Worthington Foods, Inc., related to the development of acceptable plant protein food products. Pioneer work has been largely done in the areas of meat analogs and simulated dairy products, using soy bean protein isolate as the basic raw material. Flavor and textural problems have been met with stopgap or temporary solutions. It is strongly felt that this new emerging technology, and perhaps new segment of the food industry, greatly needs the contributing efforts of the cereal and oilseed chemist, the flavor expert, the process engineer, and many others to complement present technology and to present an array of acceptable consumer products to the marketplace.

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CHROMATOGRAPHIC ANALYSIS OF INDIVIDUAL MOLECULAR SPECIES OF PHOSPHATIDES AND GLYCOLIPIDS. OSSI RENKONEN, University of Helsinki, Helsinki, Finland.

The present report deals with methods for chromatographic analysis of different molecular species in classes of natural phosphatides and glycolipids, such as lecithins, cephalins, sphingomyelins, and diglyceride galactoside. The methods are based on the observation that suitable nonpolar derivatives of phosphatides and glycopids seem to be easier to separate than the native lipids themselves. They probably have a lesser tendency to form multimolecular aggregates. Successful analyses of lecithins and ethanolamine glycerophosphatides have been carried out by liquid and gas chromatography of diglyceride acetates derived from the parent phosphatides. Even highly unsaturated molecules containing up to 12 double bonds have been handled by the present methods. To separate isomeric diglycerides of 1-oleyl-2-stearin and 1-stearoyl-2-olein type, a larger group than acetyl is required to block the third hydroxyl of glycerol. The method has also been extended to sphingolipids. Sphingomyelins have been separated into several fractions by liquid chromatography of the corresponding ceramide acetates. Such derivatives have also been studied where the polar groups were not removed, but "masked" into an unpolar form. Examples are dimethyl esters of phosphatidic acids, and N-dinitrophenylated O-methylated "cephalins." Both types of derivatives have been separated on silica gel into subclasses of alkenyl-acyl, alkyl-acyl, and diacyl types of lipids. Each subclass has then been separated into several molecular species by argentation chromatography. Even glycolipids have been profitably studied by this "masking" approach. For instance, diglyceride galactosides have been separated into several fractions after conversion into fully acetylated form.

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ISOLATION AND CHARACTERIZATION OF SPHINGOLIPIDS. GERALD L. FELDMAN and A. STEWART WINDELER, Baylor University College of Medicine, Houston, Texas, and LARRY L. PITT, Mallinckrodt Chemical Works, St. Louis, Missouri.

Sphingolipids can be isolated in high purity from chromatographic columns packed with cellulose and ion-exchange celluloses. These columns are easy to pack and can be readily scaled down, affording an advantage in speed of analysis as well as an economy of solvent. Column fractions can be further purified either by conventional thin-layer chromatography, or by the use of a newly developed glass fiber paper impregnated with a high concentration of silicic acid. The latter system and the technique of its preparation will be described in detail. Following isolation, the sphingolipids are characterized by gas chromatography of their hydrolysis products. These techniques are valuable for acquisition of a maximum amount of structural data when the sample size is limited, as it is in the ocular tissues used to illustrate the basic procedures.

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ANALYSIS OF GLYCERYL-ALKYL AND -ALK-1-ENYL ETHERS BY GAS-LIQUID CHROMATOGRAPHY AND THIN-LAYER CHROMATOGRAPHY. RANDALL WOOD and FRED SNYDER, Oak Ridge Associated Universities, Oak Ridge, Tennessee.

The applicability of thin-layer and gas-liquid chromatography (TLC) (GLC) for the analysis of glyceryl-alkyl and -alk-1-enyl ethers in neutral and phospholipids of biological origin is demonstrated. Both neutral and phospholipid fractions are reduced with lithium aluminum hydride, and the glyceryl-alkyl and -alk-1-enyl ethers are analyzed simultaneously by TLC photodensitometry. The glyceryl alk-1-enyl ether composition is determined by GLC analysis of the aldehydes after hydrolysis. The glyceryl alkyl ether composition can be determined by GLC analysis of their isopropylidene, trimethylsilyl ether, or trifluoroacetate derivatives. Identification of glyceryl alkyl ethers by GLC is simplified by prior separation according to degree of unsaturation with the use of argentation TLC. Gas-liquid chromatography of the trifluoroacetate derivatives resolves the 1- and 2-isomers. Alternately, thin layers impregnated with sodium arsenite or boric acid can also be used to determine isomeric forms of glyceryl alkyl ethers and glyceryl alk-1-enyl ethers after hydrogenation. These complementary analytical techniques have been used to analyze various normal rat tissues and a number of transplantable rat and mouse tumors quantitatively for glyceryl-alkyl and -alk-1-enyl ether content, molecular weight distribution, and isomeric form. The applicability of TLC and high-temperature GLC for the analysis of intact glyceryl ether diesters, a lipid class prevalent in the neutral lipids of tumor tissue, is also demonstrated.

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ROLE OF GLUTEN PROTEIN GROUPS IN BREAD-BAKING. R. C. HOSENEY, K. F. FINNEY, M. D. SHOGREN, and Y. POMERANZ, ARS, USDA, Kansas State University, Manhattan, Kansas.

Previous work has shown that the factor(s) responsible for baking quality is located in the gluten fraction of flour. Therefore the gluten proteins were fractionated into meaningful fractions (as determined by starch gel electrophoresis) using techniques which did not denature the proteins (as measured by reconstitution and baking). Ultracentrifugation of gluten solutions at 100,000 $\times$ G separated the proteins too large to enter the starch gel from the proteins migrating into the gel. Reconstitution and baking the 2 fractions at their original as well as restricted lower and higher ratios gave normal loaf volumes. Fractionation of both good and poor quality varieties gave essentially the same ratio of nonmigrating to migrating proteins, indicating that this ratio is not responsible for quality differences. Interchanging the 2 fractions from good and poor quality varieties shows that the quality factor is located in the proteins migrating into the starch gel.

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THE ROLE OF FLOUR WATER SOLUBLES IN BREAD-BAKING. R. C. HOSENEY, K. F. FINNEY, M. D. SHOGREN, and Y. POMERANZ, ARS, USDA, Kansas State University, Manhattan, Kansas.

When gluten was washed from flour, a portion of the flour became soluble in the wash water. The amount and composition of the material solubilized was dependent upon the salt concentration in the wash water and therefore upon the flour to water ratio. The solubility of the various protein groups at different flour to water ratios is discussed. Although it has been shown conclusively that the water solubles were not responsible for quality differences, they are necessary to produce a normal loaf of breao. The water solubles were found to have a dual role by (1) contributing to gassing power and (2) modifying the physical properties of the gluten. The water solubles were fractionated into (1) dialyzate, (2) salt-soluble proteins (globulins), (3) water-soluble and heat-coagulable proteins (albumins), and (4) water-soluble and heat-noncoagulable material. The contribution of each of these fractions in baking was determined.

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DETECTION OF PROTEOLYTIC ACTIVITIES IN WHEAT PROTEINS BY STARCH GEL ELECTROPHORESIS. E. KAMINSKI and W. BUSHUK, University of Manitoba, Winnipeg, Manitoba, Canada.

A highly sensitive method for the detection of proteolytic isoenzymes by starch gel electrophoresis was developed. The substrate was hemoglobin which was incorporated into the gel. The location of the enzyme is indicated by a colorless band after treatment of the gel with nigrosin dye. This method was used to study the distribution of proteolytic isoenzymes in wheat flour proteins. Proteases in flour from the hard red spring variety Manitou can be classified into four groups: A, B, C, and

D, in order of increasing electrophoretic mobility. Isoenzymes in groups A and B are present mainly in the water- and salt-soluble fractions. Groups C and D are in the alcohol (70% ethanol)-soluble fraction. The most active enzymes are in groups A and C. In the acetic acid extract of total protein, all groups can be detected, but group C is the most active. The effect of ionic strength and pH was different on the enzymes belonging to different groups.

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WHEAT ALPHA-AMYLASES—ISOLATION AND PROPERTIES. J. H. Kruger and R. Tkachuk, Board of Grain Commissioners, Winnipeg, Manitoba, Canada.

Wheat alpha-amylase was isolated from malted Canadian hard red spring wheat. The isolation was carried out by heat-treating an extract of malted wheat, followed by acetone fractionation, complexing with glycogen, and finally by ion exchange chromatography. Four major alpha-amylase components were obtained. The total yield of components was 28% of the original alpha-amylase activity. It was shown by polyacrylamide gel disc electrophoresis, ultracentrifugation, and moving-boundary electrophoresis that the 4 components were homogenous and distinct entities. Details of the alpha-amylases will be presented.

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WATER-SOLUBLE PROTEINS OF DURUM SEMOLINA—THEIR INFLUENCE ON MACARONI QUALITY. D. E. Walsh, K. A. Gilles, and C. E. McDonald, North Dakota State University, Fargo, North Dakota.

The relation between the composition of the water solubles of durum semolina and spaghetti quality was studied using chemical analysis, DEAE cellulose column chromatography, and gel electrophoresis. Seven amber durum varieties which represented a spectrum of spaghetti quality, 1 red durum variety, and 1 common wheat variety were compared. Analytical differences in the enzyme and chemical compositions were detected between varieties. However, these differences did not appear related to spaghetti quality. DEAE column chromatography separated the water solubles of the varieties into 5-7 fractions. The unabsorbed chromatographic fraction appeared to vary with spaghetti color. Varieties showing a high protein content in this fraction made pale or brown off-color spaghetti. Gel electrophoresis of this fraction showed it to contain 2 basic proteins. Reconstitution studies were conducted to test the effect of each DEAE fraction on spaghetti quality. The unabsorbed fraction appeared to affect spaghetti color. Addition of the unabsorbed DEAE fraction from brown to off-color varieties to bright-yellow reconstituted semolina resulted in off-color spaghetti.

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CORRELATION OF ENZYMIC ACTIVITIES WITH PROCESSING CONDITIONS OF CORN PRODUCTS FOR CSM. H. W. Gardner and G. E. Inglett, Northern Regional Research Laboratory, USDA, Peoria, Illinois.

The quality maintenance of CSM (a blended food formulation of processed cornmeal soybean flour, nonfat dried milk, vitamins, and minerals, intended for feeding infants and preschool children in developing countries) is essential during storage in high humidity and temperature. Loss of food quality by enzymic action during storage is well known. For this reason, enzymic activities of the various components of CSM needed to be related to processing conditions. Considerable information is available on enzymic activities of soybeans; however, similar activities in corn and processed corn products have not been adequately investigated. A relatively heat-stable enzyme, peroxidase, was chosen as a standard to monitor the effects of corn processing on enzymes in general. The lipid-active enzymes—lipoxygenase and lipase, which are known to cause deleterious flavors—were also investigated in various corn products. Dry milling was not sufficient to destroy peroxidase activity completely, even though the fractions were processed through a drying operation of 82°C. for 20 min. Peroxidase activity in the germ fraction was much greater than in all other dry-milled fractions. After extruder or roll processing of cornmeal, peroxidase was largely destroyed. Cornmeal processed under conditions to yield consistencies acceptable for CSM specifications had no activity. Lipoxygenase was detected only with difficulty in corn endosperm, even before processing, but was found readily in the germ. Linoleate hydroperoxide, the product of lipoxygenase activity, does not accumulate in germ extracts, but it was readily decomposed by an apparent enzyme system.

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COMPUTER USE IN OPTIMIZING COMPOSITIONS OF CEREAL-BASED FOODS FOR DEVELOPING COUNTRIES. G. E. Inglett, J. F. Cavins, W. F. Kwolek, and J. S. Wall, Northern Regional Research Laboratory, USDA, Peoria, Illinois.

Supplementation of cereal grains with protein concentrates, amino acids, or a combination of the two, is the most rapid and economical means of increasing protein quality and quantity in the diet of infants and preschool children. In developing countries it is essential that maximum nutrition should be provided at minimum cost. Attainment of this objective could be aided by computer calculations, as illustrated in formulation of prototype cereal-based food products having a prescribed content of protein with optimal patterns of essential amino acids. These prototype formulas are based on mixtures of 2 or 3 protein-contributing or essential amino acid-contributing ingredients. The economics of balancing plant protein amino acid patterns to conform with an animal protein standard are evaluated. Compositional variation of the components is a major factor in programming. More extensive cereal compositional data, including variations in amino acid patterns of cereals with different protein contents and genetic backgrounds, are needed for reliable results from computer calculations.

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VARIABILITY IN THE LYSINE CONTENT OF WHEAT, RYE, AND TRITICALE. E. Villegas, C. E. McDonald, and K. A. Gilles, North Dakota State University, Fargo, North Dakota.

The lysine content of wheat, rye, and triticale was investigated to learn more about the variability of this essential amino acid in different varieties and species of wheat and a closely related genus of wheat (rye). Also, the variability of lysine in wheat kernels from different spikes of the plant and from different positions (top, center, bottom) on a spike was studied. The variation of lysine in wheat protein from different spikes and from different positions on a spike was small. The mean lysine content in protein from samples of spring wheat and samples of durum wheat was 2.68 and 2.62%, respectively. Varieties from other wheat species were found to be as much as 35% higher in lysine. An outstanding species was *T. boeoticum* because a number of varieties relatively high in lysine were also very high in protein content. Rye protein was found to be relatively high in lysine (3.39% mean). The protein of triticale, a hybrid of wheat and rye, was intermediate in lysine (3.24% mean) between that found for durum wheat and for rye.

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INFANT AND CHILD MALNUTRITION IN DEVELOPING COUNTRIES AND THE NEED FOR PROTEIN-RICH CEREAL FOODS. W. H. Sebrell, Jr., Institute of Nutrition Sciences, Columbia University, New York, New York.

The most important form of infant and child malnutrition in developing countries is that produced by deficiency in calories and protein. This is a complex syndrome that is best described as protein-calorie malnutrition of early childhood. If the principal deficiency is calories, the infant has marasmus. If the principal deficiency is protein, the infant has kwashiorkor. Marasmus is most often seen in the very young child who has been weaned too early and changed from the nutritionally good diet of human milk to a grossly inadequate food supply. This condition affects millions of babies and may produce death or inadequate growth and development, both physical and mental. The rate of increase of the population is exceeding the increase in the food supply. The prospect for the future is that the present inadequate protein and calorie supply will get worse, unless vigorous remedial measures are instituted as soon as possible. The greatest and most rapid improvement obviously must be in the kinds of food now being produced and accepted. This means essentially increased production of cereals to provide calories, and some method of improving the protein supply. In the foreseeable future, the largest supply of protein must come from improvement in the conventional foods, plants, animal and fishery resources. The addition of synthetic amino acids such as lysine to wheat or rice is one of the unconventional food supplementation methods that could be of great importance in meeting the objectives necessary. It will not be enough just to reduce excessive losses from storage, make better use of agricultural chemicals, and improve breeding practices. Problems of acceptability by the population have to be solved. These will involve increased local production of foods, increased purchasing power so that foods can be introduced into marketing channels, improved transportation so that foods can be made more readily available, and improved education so that the needs can be recognized, understood, and applied by the individuals concerned. The problems are urgent and serious and cannot be met by free distribution of foods.

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NUTRITIONAL VALUE OF CEREAL PROTEINS. D. M. Hegsted, Harvard University, Cambridge, Massachusetts.

The methods which have been used to evaluate the nutritive value of dietary proteins are briefly discussed and evaluated. The most commonly used method, PER (protein efficiency ratio), does not provide a valid estimate of differences in nutritive value and should be discarded immediately. The limitations and advantages of other methods are presented. The literature available upon the nutritive value of cereal proteins is briefly reviewed, and the apparent ability of cereal proteins and high-cereal diets to meet the protein requirements of man at various ages is estimated. The President's Scientific Advisory Committee has indicated ("The World Food Problem") that the protein supply in many of the developing nations is thought to be about 8-10% in excess of requirements, and will apparently maintain this excess if world calorie needs are met. If the latter is to be achieved, it must be largely through cereal production. The so-called "protein problem" is largely concentrated in a limited age group, and proposed methods of solution should recognize this fact. It should also be recognized that the apparent adequacy of high cereal diets for most of the population is based upon calculations rather than adequate experimental evidence. Such evidence must be obtained in the immediate future if rational approachs to the world food problem are to be developed.

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NEW OR UNCONVENTIONAL PROTEINS FOR CEREAL FOOD SUPPLEMENTATION. M. Milner, UNICEF, United Nations, New York, New York.

The so-called new or unconventional proteins, as distinguishable from traditional food proteins of animal and plant origin on the one hand, and byproduct residues fit only for fertilizer or animal use on the other, may include edible materials recovered by modification of vegetable oil processing technology, as well as materials produced by entirely new processes. Among the former are defatted peanut, cottonseed, sesame, and coconut protein concentrates. Examples of the latter include single-cell proteins (yeasts, bacteria, fungi) and defatted, bland fish protein concentrates. Soybean proteins and high-protein wheat fractions processed for edible use by new procedures may also be considered as novel sources. Plant protein isolates produced using aqueous and nonaqueous techniques are receiving increasing attention. The current stage of development of these products and their nutritional and functional value as protein supplementing agents in cereal foods will be discussed.

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FORMULATION AND TESTING OF WEANING AND SUPPLEMENTARY FOODS CONTAINING OILSEED PROTEINS. R. Bressani, Institute of Nutrition for Central America and Panama, Guatemala City, Guatemala.

Several nutritional factors must be considered in the formulation and testing of weaning and supplementary foods. Of prime importance is a knowledge of the basic diet consumed by the child population, including absolute intakes of the various food items and their chemical composition and nutritional value. The second point to be considered is the selection of formulation ingredients, based on their nutritional and chemical characteristics and on those of the diets to be supplemented. Formulations of protein-rich foods can then be accomplished in several ways, the objective being to arrive at a combination of ingredients, giving an essential amino acid pattern equal to that of high-quality protein foods. As a first approximation, a formulation can be derived by mixing the ingredients according to their respective essential amino acid content with regard to a reference pattern. This is not enough, however, and must be confirmed by biological testing with experimental animals. In this way, digestibility aspects (in protein quality) will be taken into consideration. Formulation should also include nutrients found deficient or out of balance in the human diet to be supplemented. Once the basic formula has been developed, its protein quality in experimental animals is determined. This is accomplished by feeding at several levels of protein intake, or by the use other protein quality evaluation techniques. Testing should also include experiments designed to learn of the amino acid deficiencies in the mixture. Amino acids can be added, as such or in the form of protein, to increase the supplementary value of the mixture. The quality of the formulated food as a supplement is determined by feeding experiments in combination with the basic human diet, to learn about quantities which should be ingested to

get a supplementary effect. Toxicity and longterm feeding tests could be performed if the ingredients of the mixture require such studies. Once the basic chemical and biological data are available, the formula may be tried in human subjects by means of two tests: the evaluation of the protein quality of the mixture in terms of its true protein digestibility and biological value, and the evaluation of its supplementary value to the human diet. These tests should be carried out at several levels of protein intake. The steps indicated above have been used at INCAP in the development of protein-rich foods based on corn or other cereals, and 1 or 2 oilseed protein concentrates such as cottonseed, soybean, and sesame. Some of these formulations are in commercial production under the name of Incaparina.

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THE GREAT VARIETY OF SPECTROSCOPIC TECHNIQUES AVAILABLE FOR THE ANALYSIS OF FATTY ACIDS AND LIPIDS. ROBERT T. O'CONNOR, Southern Regional Research Laboratory, ARS, USDA, New Orleans, Louisiana.

Analytical spectroscopy may be defined as "any use of any portion of the electromagnetic spectrum in any manner whatsoever for the purpose of qualitative identification, quantitative determination, or for the elucidation of molecular structure." A consideration of the electromagnetic spectrum from the high energy gamma radiation, through X-ray radiation, the electronic regions of the ultraviolet and visible, the vibrational region of infrared, the rotational region of microwaves, to the lower energy of radio frequencies now used in resonance techniques; and a consideration of how these various radiations can be used in emission, absorption, secondary emission (or fluorescence), and various scattering techniques, show that there are approximately 40 different ways in which spectra can be used as a potential tool for the analytical chemist. More surprising, probably, is the fact that a sufficient search through the literature reveals that most of these potential techniques have been introduced to the analytical chemist, and many of them are in common use. However, not all of them are applicable to the analysis of fats and lipids. To describe even the techniques which have been used by the fatty acid or lipid chemist would require a session of several days' duration. For this 2-day symposium, we have selected 6 most widely used by, or of the most current interest to, fatty acid or lipid chemistry. These 6 methods will be reviewed in detail by specialists, by experts in their respective fields, with emphasis on the most up-to-date techniques and analyses. In addition, 2 papers, not dealing directly with spectroscopic techniques, but of subject matter very closely related, are included. These are papers on gas-liquid chromatography, and a description of the important development of computer-aided chemical spectroscopy.

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NEUTRON ACTIVATION ANALYSIS AND ITS APPLICATION TO THE ANALYSIS OF FOOD PRODUCTS. VINCENT P. GUINN, Gulf General Atomic, Inc., San Diego, California.

High-flux thermal neutron activation analysis (NAA) is an extremely sensitive means of quantitatively determining most of the elements of the periodic system. The usual limits of detection, for 75 of the elements, in the absence of appreciable interferences, range from picograms (for some elements) to as high as micrograms (for less sensitive elements). A typical element can be determined down to as low as a nanogram. This high sensitivity enables one to analyze food products, for example, for numerous trace-level elements that may be present: whether natural or added beneficial essential trace elements, or deleterious elements introduced from pesticide residues (such as Br, As, and Hg), or from processing (such as Cr, Sn, Sb, and Cu). Studies to be reported include the nondestructive determination of Hg in foodstuffs down to levels as low as 0.01 ppm, and of Br in foodstuffs down to about 0.1 ppm. With radiochemical separations, these detection limits can both be extended to 0.001 ppm, if needed. By combination with paper chromatographic or solvent extraction techniques, phosphorus- and halogen-containing pesticides can be sensitively determined. The NAA method can also be used to advantage at element levels much higher than trace levels, and in such cases the very high neutron flux of a nuclear reactor may not be necessary. For example, even with a small 14 Mev neutron generator, the nitrogen content of foodstuffs can be determined nondestructively, rapidly, and accurately, down to levels of about 10 ppm. These determinations can also be made on-line, in food processing plants.

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APPLICATION OF WIDE-LINE N-M-R TO ANALYSIS OF CEREAL PRODUCTS AND FATS AND OILS. W. D. POHLE and R. L. GREGORY, Swift & Co., Chicago, Illinois.

The principles of wide-line N-M-R measurements will be outlined. The application of this type of measurement to moisture (particularly in cereals and similar products), fat in cereals, and solids in fats and oils will be reviewed and discussed.

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HIGH RESOLUTION N-M-R SPECTROSCOPY. C. Y. HOPKINS, National Research Council of Canada.

This review describes some applications of nuclear magnetic resonance (N-M-R) in the study of fatty acids and other lipids. Emphasis is placed on practical uses of this form of spectroscopy with only a brief explanation of the theory where required. Mention is also made of the usefulness of N-M-R in studies of sugars, amino acids, and other organic compounds. Applications of the 3 parameters—chemical shift, coupling constant, and peak area—are discussed. Supplementary techniques are described, namely, spin decoupling, comparison of derivatives, change of solvent, and addition of D_2O. The chief practical use of N-M-R is in the identification and determination of structure of organic compounds. Examples are given of structure determination of natural and other fatty acids, illustrated by spectra of both common and unusual acids. Glycerides and glyceryl ethers give useful spectra. Differences between isomers of mono- and diglycerides are evident in their spectra and provide good structural information. Less work has been done on the more complex phospholipids, glycolipids, sphingolipids, and plasmalogens, but possible applications of N-M-R to these compounds are indicated. Some study has been devoted to large molecules, such as the proteins and artificial polymers, but this is in a preliminary stage. N-M-R may be used in some quantitative estimations; e.g., for determining molecular weight, for analyzing mixtures, or for following the course of a reaction. It is applicable in the study of *cis-trans* isomers, in determinations of configuration and conformation, and in some cases to optical isomerism. Reference is made to some of these fields of use.

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GAS-LIQUID CHROMATOGRAPHY OF LIPIDS, CARBOHYDRATES, AND AMINO ACIDS. S. F. HERB, Eastern Regional Research Laboratory, ARS, USDA, Philadelphia, Pennsylvania.

Gas-liquid chromatography (GLC) is primarily a powerful separating tool. Qualitative and quantitative analyses are possible when certain precautions are observed. Complex mixtures of lipids are best resolved by combining thin-layer chromatography with GLC. Tentative and often correct identification of many compounds has been possible by analysis on several columns containing different stationary liquids and under different conditions of operation. Compounds can be trapped as they emerge from the GLC apparatus for analysis by mass spectrometry and infrared or ultraviolet spectrophotometry, or the emerging separated components may flow directly into one of these instruments, making positive identification of components possible. Progress has been made on the preparation of derivatives of carbohydrates and amino acids such that these compounds may soon be analyzed as readily by GLC as lipids are today. Other new developments in GLC techniques will be discussed.

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A RAPID, STABLE BREW CONCENTRATE FOR WHEY/CYSTEINE BREAD METHODS. R. G. HENIKA and MARLENE R. HENSELMAN, Foremost Foods Company, Dublin, California.

Obtaining rapid and complete gluten development with combinations of whey and L-cysteine in both batch and continuous process breadmaking has raised the question of how best to activate the yeast cell. This is essential to give a high and uniform rate of gas production during proof and early bake. Data planned and evaluated using a highly refined Box-type design and computer showed that the key factors in developing a rapid, highly concentrated and stable brew (levels of water, salt, sugar, and flour; and setting, fermenting, cooling, and holding temperatures) could be optimized to give the required loaf volume, grain quality, and uniform proof time with both batch process and continuous mix. The brew was ready in less than 1 hr., was stable for more than 2½hr. after reaching maturity, and occupied 1/5 the usual volume, provided it was teamed with the whey/cysteine reactions in a short-time breadmaking process.

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RESEARCH WITH A PILOT SCALE CONTINUOUS BREADMAKING UNIT. V. EFFECT OF BREW FERMENTATION TIME AND MAKEUP ON FLAVOR OF CONTINUOUS PROCESS BREAD. S. REDFERN, H. GROSS, R. L. BELL, and F. FISCHER, The Fleischmann Laboratories, Standard Brands Inc., Stamford, Connecticut.

It has long been considered necessary by U.S. bakers to ferment a brew to give satisfactory bread flavor in continuous process bread. The English Chorleywood process does not use any prefermented brew, but it has been argued that this does not apply to American-type bread because of the difference in formulas. We have now shown that typical American-style continuous bread can be made without any brew fermentation time, and cannot be distinguished by taste panel from bread made with a normal 2½ hr. fermentation time brew. We have also shown that if continuous mixed dough is rounded and moulded through conventional makeup equipment, the resulting bread has a texture which is the same as conventional sponge-dough bread, and is generally preferred by taste panels to bread made from the same dough but panned directly after mixing. The use of no fermentation time brews in American continuous bread production would make it truly possible to completely automate the process and handle all ingredients in bulk.

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RESEARCH WITH A PILOT SCALE CONTINUOUS BREADMAKING UNIT. VI. BUFFERING EFFECTS OF CALCIUM PROPIONATE IN NO-FLOUR BREWS. H. GROSS, S. REDFERN, R. L. BELL, and F. FISCHER, The Fleischmann Laboratories, Standard Brands Inc., Stamford, Connecticut.

In no-flour, weakly buffered brews, it was found that the interactions of calcium propionate, nonfat dry milk, and the length of brew fermentation are critical in maintaining optimum proof times. At a 0.1% level, the buffering action of calcium propionate aids in maintaining a brew pH which will protect yeast activity. Under some conditions, proof time increases of 10-20 min. are found if calcium propionate is omitted from the brew. At 0.3% calcium propionate level, the buffering action is partially negated by the antimycotic effect of this ingredient. A statistical study of the interactions of the variables, calcium propionate, nonfat dry milk, and brew fermentation time has been made. The implications of this for continuous breadmaking process are discussed.

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FARINOGRAPH STUDIES ON THE EFFECT OF VARIOUS OXIDIZING AGENTS IN THE SPONGE-AND-DOUGH SYSTEM. K. IKEZOE, Japan School of Baking, and K. H. TIPPLES, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, Canada.

A study was made of the effects of various levels of potassium bromate, potassium iodate, and ascorbic acid on the remixing characteristics of yeasted and unyeasted "sponges." The sponges, containing 70% of the total flour and variable oxidation levels, were mixed initially at slow speed in a GRL mixer and subsequently fermented or rested for periods of time ranging from 0 to 6½ hr. Portions of the sponges were then remixed in the 50-g. Farinograph bowl with tthe remainder of the flour and sufficient water to adjust the peak dough consistency to the 500-B.U. line. The well-known differences in reaction speeds between slow-acting potassium bromate and fast-acting potassium iodate were demonstrated both in the presence and absence of yeast. Ascorbic acid, on the other hand, in the presence of yeast had a completely different effect from that in unyeasted doughs. In the absence of yeast, levels of ascorbic acid up to 120 ppm caused a very pronounced increase in development time and curve stability. At higher levels of ascorbic acid, the development time became much shorter, but curves were still extremely stable. In the presence of yeast, however, ascorbic acid at all levels had very little visible effect on Farinograph curves. It is postulated that the presence of yeast and its apparent reducing effect play a large part in determining the balance between the reducing effect of ascorbic acid and the oxidation effect of its oxidation product, dehydroascorbic acid.

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WHITE LAYER CAKE STUDIES INVOLVING USE OF VARIOUS SHORTENINGS. D. D. DISCH, A. M. SWANSON, and C. H. AMUNDSON, University of Wisconsin, Madison, Wisconsin.

Butterfat in various forms was used as shortening in white layer cakes. Butterfat in dry form was prepared by drying butterfat and milk solids. The finished products contained 80.0% fat, 17.0% milk solids, and 3.0% moisture. Various lots of dried product were prepared by varying the composition of milk solids. One lot contained only skim milk solids, while other lots contained combinations of skim milk solids and buttermilk solids. White layer cakes were prepared from these dried products, from fresh butterfat with added nonfat dry milk, and from a com-

mercial-type vegetable shortening which contained added emulsifiers. Factorial designed experiments were conducted. White layer cakes containing 120% sugar were prepared with 40.0, 50.0, and 60.0% fat levels, and 116.17, 125.17, and 134.17% moisture levels. For each type of shortening, 9 batches of cake were baked using the 3 levels of fat and 3 levels of moisture. The batches were prepared in random order and in duplicate. Each daily series consisted of 6 butterfat samples and a control made from vegetable shortening. Evaluations of the various lots of batter and finished products were made, and the data were subjected to statistical analysis. The dried products containing butterfat were found to be definitely superior to fresh sweet butter and nonfat dry milk. Drying improved the shortening properties of butterfat. The dried butter products were not equal to the commercial vegetable shortening in performance. However, these products did not contain added emulsifiers. Future work will include such studies.

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EFFECT OF PROTEOLYTIC ACTIVITY OF FLOURS ON RHEOLOGY OF CONTINUOUS MIX DOUGHS. A. M. SWANSON, University of Wisconsin, Madison, Wisconsin, and R. D. MARSHALL, A. E. Staley Mfg. Co., Decatur, Illinois.

Four different flours were obtained for this study. one was a commercial sample, and the other 3 were made from various sources of wheat known to have different levels of proteolytic activity. Doughs were prepared from these flours and evaluated by means of an extensograph and a do-corder. Nonfat dry milks (NFDM) prepared from heated (185°F. for 30 min.) and unheated skim milks were used at 3 and 6% levels in the doughs. Continuous mix brews, with 12.5% flour and without flour, were used in the preparation of the doughs by means of the do-corder. The data obtained with the commercial flour showed that doughs made with flour and NFDM in the brew were more tolerant than those made without flour in the brew and with the NFDM added at the developer. When NFDM from unheated skim milk was used, the R/E values were lower than those obtained from doughs containing NFDM which had been made from heated skim milk. However, after the final determinations were made, the R/E values were quite comparable. These observations demonstrate that NFDM from unheated skim milk did not incorporate as readily as the NFDM from heated skim milk. Flour with a low level of proteolytic activity produced doughs with very high R/E values. These doughs were very tolerant of the heated and unheated milks at both the 3 and 6% levels. The R/E values of doughs made with high proteolytic activity flour were much lower. This type of flour produced very weak, slack doughs. This effect was even more pronounced when the flour was added to the brew. Doughs made from flour with medium proteolytic activity more nearly produced the type of dough that was desired.

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PROPERTIES OF WAXY AND ISOGENIC NONWAXY RICES DIFFERING IN STARCH GELATINIZATION TEMPERATURE. B. O. JULIANO, M. B. NAZARENO, and N. B. RAMOS, International Rice Research Institute, Los Baños, Laguna, The Philippines.

To minimize complicating environmental factors and differences in amylose content in the study of the implication of varietal differences in gelatinization temperature on grain properties, the samples selected were the wet and dry season crops of 12 waxy varieties and 2 pairs of isogenic nonwaxy lines differing essentially in gelatinization temperature alone. The more crystalline waxy and nonwaxy starches were found, in general, to correspond to amylopectins of lower $[\eta]$, but degree of crystallinity was not correlated with gelatinization temperature. This temperature was correlated with the rate of acid hydrolysis of the starch granule. It affected only the initial phase of the starch-water adsorption and solubility curve during heating. It also correlated with cooking time of milled rice. Cooked waxy samples of low and high gelatinization temperatures gave similar eating quality scores.

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NEW STARCHES: PROPERTIES OF FIVE VARIETIES OF COW PEA *(VIGNA SINENSIS)* STARCH. E. TOLMASQUIM, A. M. N. CORREA, and S. T. TOLMASQUIM, Instituto Nacional de Technologia, Guanabara, Brazil.

Starches were obtained from 5 varieties of cow pea: brabham, oscaroite, plumbeo, pindamonhangaba, and early red. Their Brabender viscosities were quite similar with small differences, and their iodine affinities were lower than those expected for legume starches. All the starch varieties presented single-stage swelling. The solubility of the plumbeo was more restricted than that of the others. The rate of solubilization in dimethyl sulfoxide was evaluated to determine their ionic character. All of the starches appeared to be practically nonionic.

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BARLEY STARCH. III. A STUDY OF THE STARCH PROPERTIES OF 30 BARLEY GENOTYPES. K. J. GOERING, E. ESLICK, C. A. WATSON, and JUIN KENG, Montana State University, Bozeman, Montana.

A study of the starch properties of 30 barley genotypes has been made. Some differences were found to be associated with certain phenotypic characteristics such as 2-row vs. 6-row head character, covered vs. hulless kernel, yellow-green vs. normal spike, normal vs. orange lemma, half-awned vs. full-awned spike, waxy vs. normal endosperm, and genetic thin vs. normal kernels. Although in a few instances there was a slight difference in the amylose-amylopectin ratio, these differences were not of sufficient magnitude to explain the drastic differences in the viscosity curves. If the amylose and amylopectin in these barley selections are normal, this work would suggest that the tremendous differences observed in the Brabender viscosity curves must be the result of starch granule structure.

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NEW STARCHES. IV. THE PROPERTIES OF THE STARCH FROM *TYPHA LATIFOLIA*. K. J. GOERING and YOLANDA A. RIGAULT, Montana State University, Bozeman, Montana.

Starch has been isolated from *Typha latifolia* roots by a modified wet milling technique. This starch consists of intermediate-sized granules which are packed in a capsule containing many granules. Although its pasting characteristics resemble those of corn starch, the low solubility and swelling power indicate that the granule has both strong and extensive bonding forces. The Brabender curves indicate this starch to have a high viscosity and extreme stability to cooking. This starch appears to be somewhat more readily digested with bacterial amylase than is corn starch.

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STARCH-COMPLEXED LIPIDS: QUALITATIVE DEPENDENCE UPON TWO SOLVENT SYSTEMS. S. ROGOLS, J. E. GREEN, and R. L. HIGH, The Keever Company, Columbus, Ohio.

Using the Hirsch-Ahrens technique for separation of lipids on silicic acid (*Jour. Biol. Chem.*, *233:* 311, 320, 1958) 2 cereal starches and 1 root starch were studied in terms of their lipid constituents. Regardless of the starch source, the starches were hydrolyzed and the lipids extracted using 2 solvent systems, ethyl ether according to the technique of Rogols et al. (*Die Stärke, 6:* 186, 1964), and carbon tetrachloride according to the CIRF method. Both lipid systems were then subjected to silicic acid column chromatography and the fractions collected according to the aforementioned technique of Hirsch. When a typical plot of the total solvent used (in ml) is graphed against the percent lipid present, it could be readily noted that the lipids which were obtained from the acid-hydrolyzed starch differed markedly according to the original solvent used. Carbon tetrachloride extraction yielded, quantitatively and qualitatively, a spectrum of lipids which was not similar to those extracted by the use of ethyl ether. Slides will be shown to illustrate these points.

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GLYCOLIPIDS—THEIR DISTRIBUTION, CHEMISTRY, AND ROLE IN BREADMAKING. Y. POMERANZ and K. F. FINNEY, ARS, USDA, Kansas State University, Manhattan, Kansas.

Classification, distribution, and composition of the major types of glycolipids, including glycosyl ceramides, phytoglycolipids, glycolipids of microorganisms, and glycosyl glycerides, are reviewed. A survey is presented of recent studies on changes in galactolipids in maturing wheat and on the distribution of glycolipids in various tissues of wheat and rye kernels. Thin-layer chromatograms of free lipids of Kaw wheat harvested at various stages of maturity are given. Nonpolar lipids and polar lipids, including monogalactosyl diglyceride, digalactosyl diglyceride, and phosphatidyl choline, are identified. Thin-layer chromatograms of free lipids in flour and bran of wheat, rye, barley, oats, corn, and sorghum are shown and compared with thin-layer chromatograms of total, free, and bound flour and germ lipids. The role of naturally occurring glycolipids in breadmaking is evaluated, and contributions to breadmaking performance of wheat flour and synthetic glycolipids are compared.

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VISCOSITY DEVELOPMENT IN STARCH: A METHOD FOR EVALUATING EFFECTS OF STARCH TYPE, COOKING TEMPERATURE, SLURRY CONCENTRATION, AND pH. J. E. FREEMAN and W. J. VERR, Corn Products Company, Argo, Illinois.

A new procedure has been developed for cooking starch slurries without loss of moisture and without mechanical shear. Hot and cold paste viscosities are measured with a Brookfield Viscometer. Both waxy and regular corn and sorghum starches have been studied. Slurry pH, starch concentration, cooking time, and cooking temperature have been varied over a wide range with each of the 4 starches. Some interesting effects and interactions on both hot paste viscosity and viscosity increase upon cooling have been observed. The "pasting temperature range" of a starch sample can be obtained by cooking portions of the sample for a given period of time at each of several temperatures. Starch viscosity plotted against cooking temperatures gives a sigmoid curve. Relative "pasting temperature ranges" of different starch samples can be estimated by cooking at a single temperature. This technique is useful for rapidly screening large numbers of samples.

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THE SUGARS OF WHEAT BRAN. ROBIN M. SAUNDERS and H. G. WALKER, JR., Western Regional Research Laboratory, ARS, USDA, Albany, California.

The simple sugars and oligo-saccharides of wheat bran have been reinvestigated by gel filtration and paper chromatographic techniques. Glycerol, xylose, arabinase, glucose, fructose, sucrose, raffinose, neokestose, stachyose, and a fructosyl raffinose have been found. Of these, the latter 3 have not been reported previously.

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CHARACTERIZATION OF MILK LIPASE. W. J. HARPER, The Ohio State University, Columbus, Ohio, and T. G. GAFFNEY, Cambridge University.

The nature of the lipase associated with the casein micelle was investigated by means of sephadex gel filtration, ion exchange chromatography, and polyacrylamide electrophoresis. Utilization of the substrate dibutyrylfluourescein with gel electrophoresis permitted study of the lipase activity of electrophoretically homogenous components. Dissociation of the casein micelle with rennet, salt, or freezing increased the total lipase activity, released lipase-active glycopeptides, and permitted study of the lipase associated with alpha$_s$-, beta- and kappa-caseins. Ion exchange chromatographically-purified caseins were investigated electrophoretically. All three caseins possessed lipase activity, which was associated with the specific casein component on the gel. K_m values of gel separated caseins were 18.3, 15.8, and 17.6 $\times$ $10^{-5}M$ dibutyryl-fluourescein for lipases of kappa-, alpha$_s$-, and beta-caseins. Nonlinear kinetics were obtained when mixed casein micelles were prepared artificially. A glycopeptide with lipase activity could be dissociated from the caseins by glutathione-NaCl mixtures. A mammary gland somatic cell glycoprotein lipase was isolated and shown capable of transferring lipase activity to kappa-casein under controlled conditions.

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EFFECT OF EPINEPHRINE ON CARDIAC LIPOPROTEIN LIPASE ACTIVITY. S. MOLLOY and ADAWIA ALOUSI, State University of New York, Syracuse, New York.

In previous experiments, we observed that the lipoprotein lipase (LPL) activities of homogenates of heart ventricles of rats receiving daily intramuscular injections of epinephrine suspensions in oil (0.5 mg./kg. body wt.) for periods of 2-3 weeks were significantly higher (100%) than those of controls injected with saline or oil vehicle alone. We subsequently found that even single intramuscular injections of epinephrine or norepinephrine suspensions in oil (0.5 or 1.0 mg. base/kg.) induced similar rises in LPL activity (60-150%) in periods of 2-4 hr. In addition, the same results were produced by acute ethanol intoxication or ether anesthesia, and evidence was obtained that the latter agents acted via release of catecholamines *in vivo*. Pretreatment with puromycin or with a beta-adrenergic blocking agent (Inderal) inhibited the rise in cardiac LPL activity induced by either epinephrine or ethanol intoxication. Intravenous infusion of epinephrine *in vivo*, in rats, for a short period of time (10 min.) or addition of epinephrine *in vitro* to rat heart slices or homogenates incubated for 1 hr. did not, however, induce an increase in LPL activity. In an attempt to obtain a better *in vitro* model for the study of the epinephrine effect, isolated beating rat hearts were perfused with continuously recirculating Chenoweth's solution in the presence and absence of epinephrine (0.11 and 0.22 μg. base/ml.) for periods of ½ to 3 hr. Epinephrine caused an increase in LPL activities of these hearts as measured in homogenates, but the in-

creases were smaller than those obtained *in vivo* (39.0, 37.3, 43.0, and 70.9% increases after ½, 1, 2, and 3 hr. of perfusion, respectively) and were more variable. Ouabain, used as control agent, had no such effect. Since Eagle and Robinson (*Biochem. J., 93*:10 1964) had shown that LPL synthesis occurred *in vitro* in epididymal fat bodies from starved rats when the former were incubated for periods of 3-9 hr. in a medium containing glucose, heparin, insulin, serum, amino acids, and salts, we attempted to do the same with rat heart slices incubated for 3 hr. In all cases, such incubation led to a consistent loss in LPL activity, and addition of epinephrine *in vitro* did not modify this result. The reasons for the greater positive effect of the catecholamines on cardiac LPL activities *in vivo* than *in vitro* are not clear.

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STUDIES WITH CLEARING FACTOR. 6. FRACTIONATION OF LIVER LIPASES ON SEPHADEX G-200. A. N. Payza, H. B. Eiber, and S. Walters, New York Medical College, New York, New York.

It was observed that Clearing Factor was a mixture of various lipases (Payza, et al., 1967). This conclusion was based on observations with NaCl and protamine inhibition, and on studies of heat inactivation of these enzyme mixtures or their action on various substrates. Increased activity of triglyceridase (Korn, et al., 1957), monoglyceridase (Shore, et al., 1962), (Levy, et al., 1955), lecithinase ((a) Vogel, et al., 1966) were present in the plasma of heparin injected into animals and human subjects. These enzymes were always higher in concentrations in the tissues prior to heparin injection and discharged to the plasma after the injection of heparin (Payza, et al., 1967). Whether it is the activity of the same lipase due to substrate nonspecificity, or activity of 2 separate enzymes, was not demonstrated in the case of monoglyceridase and triglyceridase. In this work, to separate the mono- and triglyceridase activity of the rat liver, fractionation was attempted. Rats were sacrificed by etherization 30 min. after intravenous injection of heparin (2 mg./kg.) by the tail vein. Pooled livers of 10 male rats were homogenized with 20 times their volume of chilled acetone at -20°C. in a Waring Blendor, for 3 min. and suction-filtered; then stored under vacuum at -10°C. Extracts were prepared as follows: 0.5 gr. liver-acetone powder and 20 ml. 0.06*M* phosphate buffer at pH 7.1 was sonicated in a Branson sonicator at 4°C. and clear supernatant collected. Lipase activity was assayed as described by Dole (Dole, 1956) on the samples from incubation mixture of 2 ml. 10% BSM, 0.2 ml. $CaCl_2$ 1*M*, 10 heparin, 1 ml. substrate (15 mg./ml.), 0.3 ml. 0.6*M* phosphate buffer at pH 7.1 and 2 ml. of enzyme solution. Sephadex G-200 columns (30/2.5 cm.) were packed and eluted with distilled water with flow rate of 20 ml./hr. Five-ml. samples were collected and assayed for lipase activity. Substrates used were triolein, α-monolein and β-monolein (Mann Research Laboratories). Alpha- and Beta-monolein were prepared by the TLC method described by A. F. Hofman from a commercial monolein preparation consisting of 85% Alpha-monolein and 15% Beta-monolein. Glyceride substrates (15 mg./ml.) were prepared as described previously and used for the assay of lipases (Eiber, et al., 1966), on the fractions eluted from Sephadex G-200 column. Protein concentrations were assayed by UV absorption at 280 mμ on a DU spectrophotometer. Monoglyceridase and triglyceridase activities and bulk of non-active protein were separated from each other on Sephadex G-200 column. Monoglyceridase was eluted right after void volume, while triglyceridase was absorbed to Sephadex G-200. Sephadex G-150 did not separate 2 enzymes, and both were eluted in the void volume. This indicated range of molecular weight of these enzymes between 150,000 and 200,000. Monoglyceridase activity was optimum in 0.06*M* ammonium chloride buffer at pH 8.5. Tris buffer is not found as effective. Triglyceridase had an optimum at pH 7 in 0.06*M* phosphate buffer. Monoglyceridase was active on both Alpha- and Beta-monoglycerides. However, Beta-monoglyceride was hydrolyzed 30% more than Alpha-monoglyceride at the end of 1 hr. incubation, assayed with appropriate substrates in 0.06*M* phosphate buffer at pH 7.1. The specific activity of monoglyceridase was 40 times higher than crude acetone powder extract of the liver. Triglyceridase specific activity was 180 times higher than rat liver acetone powder crude extracts.

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ERRORS IN DETERMINING THE STRUCTURE OF TRIGLYCERIDES BY ENZYMATIC HYDROLYSIS. F. H. Mattson and R. A. Volpenhein, The Procter & Gamble Company, Cincinnati, Ohio.

In 1956, we reported pancreatic lipase (E.C. 3.1.1.3) to be specific for the hydrolysis of long chain fatty acids esterified with the primary hydroxyl groups of glycerol. Further, it was proposed that the specificity of this enzyme made available a method by which the distribution of fatty acids esterified with the primary (1- and 3-positions) and secondary (2-position) hydroxyl groups of glycerol could be determined. Subsequently, Brockerhoff showed that if the diglycerides resulting from lipase digestion were phosphorylated and then hydrolyzed with phospholipase A, it was possible to distinguish between the fatty acids esterified at the 1- and 3-positions. In spite of the widespread use of these procedures, particularly the one involving only pancreatic lipase, there has been little discussion of possible sources of error. These include: (a) positional specificity of the enzyme, (b) presence of other lipolytic enzymes, (c) presence of lipids other than triglycerides in the substrate, (d) relative rates of hydrolysis of various triglycerides and of esters of various fatty acids, (e) isomerization of partial glycerides, and (f) isolation and analytical techniques. Specific examples of each of these categories will be presented.

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SOME FACTORS INFLUENCING ADIPOSE TISSUE LIPOLYSIS. J. N. Pereira, Chas. Pfizer & Co., Inc., Groton, Connecticut.

Since plasma free fatty acids (FFA) represent the major source of fatty acids for hepatic triglyceride synthesis, an examination of compounds which produce a net reduction in plasma FFA levels has been undertaken. Studies of the effects of adipose tissue lipolysis inhibitors on lipid metabolism have indicated that nicotinic acid, a potent inhibitor, produces biphasic effects on plasma FFA concentrations in rats, dogs, and humans. A rapid fall in plasma FFA concentrations is followed by a rise to grossly elevated levels. This sequence is accompanied by an increase in plasma corticosterone levels in rats, and requires the presence of both the pituitary and adrenal glands for full expression. Removal of the thyroid gland did not alter the time course of plasma FFA changes. The plasma FFA reduction and subsequent increase do not appear to be causally related, since low doses of nicotinic acid cause increases in plasma FFA concentrations without the primary decrease. Another potent inhibitor, 3-methylisoxazole-5-carboxylic acid, reduced FFA levels with no evidence of rebound. However, on repeated administration of this compound, a rapid loss of responsiveness is observed in rats and dogs. This resistance phenomenon involves a pituitary-adrenal mechanism, since removal of either the pituitary or adrenal gland eliminated its induction. Lipolysis in adipose tissue obtained from rats rendered resistant to this compound proceeded at a rate approximately twice as great as control tissue, and was less sensitive to the inhibitory effects of 3-methylisoxazole-5-carboxylic acid. These findings describe some of the shortcomings of available lipolysis inhibitors in testing the possibility of controlling plasma lipid levels by limiting adipose tissue lipolysis.

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ACID LIPASE OF THE CASTOR BEAN. R. L. Ory, Seed Protein Pioneering Research Laboratory, ARS, USDA.

The acid lipase of the castor bean is present in the dormant seed. It is extracted from the fat pad obtained by centrifuging a macerate of the seed in pH 7.0 buffer containing cysteine and EDTA. The pH optimum of the enzyme is 4.3; it is rather heat-stable, and is inhibited by mercurials and sulfhydryl reagents. Maximum hydrolysis of saturated triglycerides occurs with fatty acids of chain length C_4 to C_8; unsaturated C_{18} triglycerides are hydrolyzed at a slightly lower rate. This lipase is a 3-component system consisting of the apoenzyme, a lipid cofactor (found to be a cyclic tetramer of ricinoleic acid), and a protein activator. The latter is a small, heat-stable glycoprotein and appears to be related to some of the castor allergens. Maximum lipolysis requires all 3 components. Lipase activity is associated with the spherosomes, the subcellular site of oil storage in the endosperm. The purification of the enzyme and its 2 cofactors, the comparison of the protein activator to allergen fractions by various techniques, and the combined use of biochemical and histochemical techniques to localize the subcellular site of enzyme activity, will be described.

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ON THE STRUCTURE OF SOME BACTERIAL POLYGLYCERO-PHOSPHATIDES-PHOSPHOLIPIDS—A HYDROLYSIS EXPERIMENT. Robert W. Walker and Gordon L. Howard, University of Massachusetts, Amherst, Massachusetts.

A study was made of the fatty acid structure of the diphosphatidylglycerol (DPG) fractions of the following bacteria: *Mycobacterium smegmatis, Sarcina lutea, Micrococcus lysodeikticus,* and *Arthrobacter globiformis.* Phospholipase A hydrolysis in conjunction with TLC and GLC was used to determine fatty acid distribution. In the case of *S. lutea, M. lysodeikticus,* and *A. globifromis,* fatty acid distribution appeared to be nearly random between individual lipid fractions. In addition, a nearly random distribution was noted between the αα′ and ββ′ positions of the DPG molecule. In the case of *M. smegmatis,* however, fatty acid distribution was found to be quite specific. The DPG fraction lacked the long chain fatty acids (>C-19) of the total lipid extract and contained higher concentrations of branched and unsaturated fatty acids. The Alpha Alpha′ positions of DPG were occupied by tuberculostearic (12-methylstearic) and oleic acids, while the Beta Beta′ positions consisted mostly of palmitic and a branched C-17 acid. Similarly, in the phosphatidyl ethanolamine fraction of *M. smegmatis,* the Alpha position was occupied predominantly by tuberculostearic, the Beta by palmitic acids. The effect of random versus orderly fatty acid distribution upon the spacial configuration of the DPG from these 2 groups of organisms will be discussed.

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SUITABILITY OF *GEOTRICHUM CANDIDUM* LIPASE FOR THE STEREOSPECIFIC ANALYSIS OF SOME TRIGLYCERIDES. J. Sampugna and R. G. Jensen, University of Connecticut, Storrs, Connecticut.

The unique specificity of the lipase system from *G. candidum* for fatty acids with *cis*-9-unsaturation suggested the application of this enzyme as a tool in elucidating the stereospecific structure of unsaturated triglycerides. The development of a system to determine triacid triglyceride combinations containing oleic acid was especially desired; consequently, racemic and enantiomeric triglycerides containing palmitic, stearic, and oleic acids were synthesized and employed to establish the limits of the new method. The suitability of the enzyme preparation for the proposed analysis was demonstrated by the isolation of diglycerides from the *G. candidum* hydrolysis and determining that: (a) Essentially no oleic acid was present in the diglyceride when a monooleate triglyceride was digested. (b) Very little acyl migration of diglyceride products occurred during incubation periods of 30 min. or less. (c) Sufficient quantities of diglyceride were produced to allow further analysis as described below. The procedure involves a pancreatic lipase hydrolysis of 10 to 20 mg. of substrate to determine the fatty acids in the β-position, and the incubation of another aliquot of triglycerides (50-100 mg.) with *G. candidum* lipase to obtain diglyceride for further treatment. The α,α- and α,β-diglycerides are collected separately, converted to phenyl phosphatides, and digested with phospholipase A. The free fatty acids, lysophosphatides, and unreacted phosphatide (glyceryl-*sn*-2,3-diacyl-1-phenylphosphate) resulting from the phospholipase A hydrolysis are separated by thin-layer chromatography, converted to methyl esters, and analyzed by gas-liquid chromatography. The procedure developed can be considered a modification of the Brockerhoff Stereospecific Analysis, since some of the steps are identical in both methods, and the rationale behind the calculation of constituents in positions 1, 2, and 3 is somewhat similar; however, only glycerides containing fatty acids with *cis*-9 unsaturation are amenable to analysis, and a complete elucidation of the configuration of the triglyceride mixture is possible.

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ISOLATION AND STUDY OF BOVINE PANCREATIC LIPASE. I. M. Khan and K. M. Shahani, University of Nebraska, Lincoln, Nebraska.

Bovine pancreatic lipase was isolated in a pure form. The technique involved preparation of lypholized powder of fresh bovine pancreas, extraction of the enzyme with sucrose solution, fractional precipitation with ammonium sulfate and acetone, and finally chromatography on Sephadex G-100. The purity and homogeneity of the enzyme was confirmed with the gel electrophoretic, as well as chromatographic techniques. The specific activity of the purest lipase fraction was 1,510, indicating a purification of approximately 200-fold, with an overall yield of about 16%. The enzyme exhibited a single pH optimum of 8.8, its optimum temperature was 37, and its optimum substrate concentration was 10%. It hydrolyzed butterfat and vegetable oils. It lipolyzed triglycerides more rapidly than di- and monoglycerides. The enzymes showed little or no activity against *O*-nitrophenyl acetate, *p*-nitrophenyl laurate, and other simple esters, but showed high specificity against emulsified glycerol esters, indicating that it is a true lipase. By and large, the characteristics of bovine pancreatic lipase parallel those of bovine milk lipase, suggesting that the 2 enzymes are very similar and may be the same.

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THE PHYLOGENY OF LIPASE SPECIFICITY. D. L. Berner and E. G. Hammond, Iowa State University, Ames, Iowa.

Lipases have been shown to vary in their substrate specificity. Hog pancreatic lipase, for example, shows a high specificity for the 2 α-positions of glycerol. Other lipases seem to attack without

regard for the glycerol position. This study is concerned with the specificities of the digestive lipases of animals in the mammalian order and below. For vertebrates, the lipases were prepared by making an acetone powder of the pancreatic glands. For nonvertebrates, acetone powders were made of the organs suspected of being the source of digestive enzymes. The pH optima of the enzymes were determined against tributyrin and corn oil emulsions. To see if more than 1 lipase or esterase could be demonstrated, the acetone powders were subjected to disc gel electrophoresis at the pH optima. The gel column was pressed against agar gels containing dispersed triglycerides, and lipase bands were detected by clearing of the emulsion. The clear zones were compared with the bands of protein made visible by staining a duplicate gel column. Specificity studies were carried out at the pH optima of the enzymes preparations. Cocoa butter, lard, corn oil, and soybean oil emulsions were selected to detect the wide range of positional and fatty acid specificities that might exist. The pH-activity curves for the vertebrate lipases were similar to each other, but differed from that of some nonvertebrate lipases. The specificities of lipases from orders lower than mammalian tended to be more complex than the simple positional specificity of hog pancreatic lipase. Fatty acid selectivity could be demonstrated in some instances. These complex specificities can be further elucidated by studies with pure glycerides. The specificities of some of the enzymes may make them useful in studies of glyceride structure.

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THE ROHRSCHNEIDER METHOD OF COLUMN CLASSIFICATION. WALTER SUPINA, Supelco, Inc.

This method is based on the Kovats indices for 5 compounds as determined on a nonpolar squalane column. The apparent polarity of other columns is expressed as a function of the increase in Kovats indices for these same 5 compounds. As a result, it is possible to classify all stationary phases in an orderly manner. Such classification is needed to illustrate the many duplicates now in use. The method also makes possible the prediction of separations. Data will be presented showing the accuracy of the predictions.

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CONTAMINANTS IN EDIBLE FATS AND OILS. DAVID FIRESTONE, Food and Drug Administration, Washington, D. C.

Sensitive GLC methods are available for determining chlorinated pesticide and chick edema factor contaminants in fats and oils. Determination of chlorinated pesticide residues in fats and oils generally involves acetonitrile extraction, Florisil column cleanup, and analysis by electron capture cleanup. However, other procedures are available, including dimethyl sulfoxide extraction, propylene carbonate extraction and sweep co-distillation. Screening methods for chick edema factor involve alumina column fractionation of unsaponifiable or unsulfonatable matter, sulfuric acid cleanup, and examination by electron capture gas chromatography. Admixtures of animal and vegetable fats are detected by gas chromatograph'c analysis of isolated sterols, and individual vegetable oils are characterized by gas chromatographic examination of the sterols, as well as other unsaponifiable constituents.

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IDENTIFICATION OF METHYL ESTERS BY RETENTION DATA. ROBERT ACKMAN, Fisheries Research Board of Canada, Halifax, Nova Scotia, Canada.

Common saturated fatty acids with 1 methyl substituent (iso and anteiso) may be tentatively identified without much difficulty on both polar and apolar liquid phases. With several methyl substituents (isoprenoid skeletons), it is possible to predict equivalent chain lengths for the whole molecule by adjusting fractional chain lengths for each substituent in proportion to the polarity of the liquid phase. This approach breaks down when the identification of unsaturated fatty acids on polar columns is considered in detail, since the relationships between saturated and unsaturated fatty acids are variable, as indicated by chain length overlap, and are related to the polarity of the particular column. The polarity of a column is determined by the type of liquid phase, its proportion relative to the solid support, the nature of the latter, and age, and operating conditions. Irrespective of column polarity, it may be shown that there are means of tentatively identifying common unsaturated fatty acids through groups based on mutual structural features.

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THIN-LAYER AND GAS CHROMATOGRAPHIC ANALYSIS OF THE NATURAL LIPIDS THAT ARE FOUND BETWEEN THE TRIGLYCERIDES AND HYDROCARBON SPOTS IN TLC PLATES. NICHOLAS PELICK, Supelco, Inc., and L. NEWTON and M. S. SHAMMA, The Pennsylvania State University, University Park, Pennsylvania.

The use of thin-layer chromatography for the separation of neutral lipids is described. These neutral lipids, besides triglycerides and steryl esters, are of the type which are found in small amounts and usually ignored in routine analysis. These include hydrocarbons, wax esters, neutral plasmalogens, fatty aldehydes and ketones, and glyceryl ether diesters. Chemical means for derivatizing these lipids for subsequent identification will be emphasized. Some practical application will be shown on deer tarsal gland extracts, which were found to be an excellent source for the neutral lipids described in this paper.

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NUTRITIONAL EVALUATION IN INFANTS OF NEW PROTEIN FOODS. G. GRAHAM, British American Hospital, Lima, Peru.

Formulation of protein-rich foods for infants and children depends to a great extent on the cost, availability, and acceptability of their ingredients. Approximate predictions of the biological value of the resultant protein mixtures can be made on the basis of their determined amino acid compositions. Experience with cottonseed flours in particular suggests that determinations of the biological availability of essential amino acids increase the accuracy of these predictions. Testing in laboratory animals usually approximates the biological value in humans. Before such mixtures, or significant variations of these, can be used in large-scale programs, it is essential that they be submitted to critical evaluation in infants and children, the intended consumers. Because of their predictable rapid growth rate, infants recovering from severe undernutrition make ideal subjects for such evaluations. For some years now we have been involved in such testing. Following a significant period of cow's milk feeding and the establishment of a steady gain in weight, normal serum protein levels, and satisfactory clinical condition, the protein or protein mixture to be tested is used to replace cow's milk protein in the diet at isonitrogenous and isocaloric levels, previously established as adequate. Nitrogen absorption and retention, rate of weight gain, and changes in serum proteins are compared. If these are consistently found to equal or approximate those of milk or casein diets, the same exclusive protein source will be used in the diet for periods of several months. This will frequently uncover evidences of amino acid deficiency or imbalance which might not be apparent during short periods. This has been particularly true of tryptophan inadequacy. If these comparative and long-term studies give satisfactory results, the same protein will be tried in the initial dietary management of severely malnourished infants, where protein digestibility and utilization are of critical importance. We have carried out studies of the nutritional value of wheat flour enriched with fish protein concentrate, with lysine, or with milk solids, and mos recently, with wheat concentrate and toasted, defatted soy flour. We have also evaluated mixtures of corn with soy flour and methionine (Fortifex), corn with soy flour and milk solids (CSM), and corn with soy and cottonseed flours (Incaparina mixture 15). The results to be presented support the potential value of such enriched cereals in the diet of infants and children. They also give some indication of their limitations and weaknesses. If these studies have been carried out at levels which are considerably more critical than those envisioned by the developers of these mixtures, it is because we are firmly convinced that if these products are successfully introduced, they are very likely to become the only source of protein in the diets of thousands of infants and children and to be considerably diluted with carbohydrate calories in their use.

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FORMULATED CEREAL FOODS IN THE FOOD FOR PEACE PROGRAM. F. R. SENTI, ARS, USDA, Washington, D. C.

Formulated cereal foods have been added recently to the list of processed foods distributed under the USDA-AID overseas donation program in recognition of the special dietary needs of selected population groups in the less-developed countries. Primary targets for these foods are young children after weaning, school feeding programs, and for use wherever there is need for improving dietary intake. Protein is commonly deficient in diets of these recipients, but vitamins and minerals also need to be supplied. Formulated cereal foods combine precooked cereal flour or meal with a protein concentrate such as soy flour and/or nonfat dry milk, plus vitamin and mineral supplementation. The products are quick-cooking and may be served as a porridge or beverage. They are not intended to be fed to supply total calorie requirements, but rather as a supplement to provide additional amounts of nutrients, particularly protein, vitamins, and minerals. The composition is designed such that 100 g. would supply a child ⅓ to ½ of his needs for the known nutrients, except ascorbic acid. The Department's Blended Food Product, Child Food Supplement, Formula No. 2 (CSM), a corn-soy-milk mixture developed cooperatively with industry, has been purchased in greatest quantity. New formulations based on wheat fractions as the cereal component are under development. Other processed foods designed to better meet protein and vitamin requirements of recipient groups, which have been recently introduced or are ready for introduction into the Food for Peace program, are wheat flour fortified with wheat protein concentrate, vitamin A, and calcium, and rolled wheat fortified with soy flakes.

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LOW-COST PROTEIN FOODS: FORTIFIED CEREALS AND NEW PROTEIN BEVERAGES. A. M. ALTSCHUL, International Agricultural Development Service, USDA, Washington, D. C.

Fortified cereals and new protein foods are not qualitatively different in principle even though their logistics are vastly different. Fortified cereals are in fact new foods in terms of food value and cost; education is needed to emphasize their value for sensitive elements of the population, but they are already accepted in the normal food pattern. "Toned" milks will be cheaper than their natural counterparts; new protein beverages must be accepted first on the basis of taste and cost. There is a dynamics to the development of new foods: Acceptance and nutritive value alternate as the major determinants.

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PROBLEMS IN ACCEPTANCE AND MARKETING. S. CANTOR, Sidney Cantor Associates, Ardmore, Pennsylvania.

A significant number of experiences concerning foods answering to the challenges of worldwide protein malnutrition have now been recorded. Analysis of these within the framework provided by a marketing-oriented development process suggests certain conclusions. These might be regarded as critical feedback for product developers. In this paper, problems associated with cereal improvement will be considered within the total marketing context. The interrelationship of availability, acceptability, and value factors will be discussed as they are related to market segments.

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SALMONELLOSIS. JAMES H. STEELE, National Communicable Disease Center, Atlanta, Georgia.

Salmonellosis affects more people and more animals than any other single disease. It is one of the most important public health—animal health problems. Salmonellosis may vary in severity from inapparent infections to acute disease which may be fatal to the very young, the old, or the debilitated individual. It is estimated that there are 2 million persons infected each year in the U.S. During the past quarter century, reported *Salmonella* infections in man in the U.S., other than typhoid fever, have increased from 504 in 1942 to 20,867 bacteriologically proven infections in 1965. It is impossible to determine how much of the marked increase in reported human salmonellosis is due to actual increase in incidence of infections and how much is due to improved reporting. Methodology has improved during this period, but it is believed that wider application of known methods and more thorough epidemiological investigation of outbreaks have contributed most information about the occurrence and distribution of salmonellae.

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SALMONELLA ECOLOGY. V. D. FOLTZ, Kansas State University, Manhattan, Kansas.

Control of the cyclic phenomena of salmonellosis will be effective only when all sources of *Salmonella* are attacked simultaneously. Salmonella ecology must be studied to clarify the problem. Studies at the Kansas Agricultural Experiment Station related to *Salmonella* ecology have been conducted, in part, in the following areas: (1) Convenience foods studied have shown a decreased incidence of *Salmonella* contamination from 1962-65. (2) The examination of fishes recovered from sewage-polluted streams reveals a high percentage harboring salmonellae in the intestinal tract. Per OS inoculation of catfish demonstrated viability of salmonellae in the stomach and intestine for at least 29 days. We believe this indicates a demand for *Salmonella*-free fish food for commercial fish rearing. (3) Human *Salmonella* carriers can develop following contact with cold-blooded animal pets. Food workers in "critical areas" should be carefully examined as potential carriers of salmonellae. We have shown the potential danger of carrier development in our cold-blooded pet surveys.

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THE *SALMONELLA* PROBLEM FROM AN ENFORCEMENT

STANDPOINT. Kenneth R. Lennington, Food and Drug Administration, Washington, D. C.

The importance and significance of *Salmonella* contamination in foods and drugs is discussed. Products presenting greatest problems in last 2 yr. have been dried milk, egg products, drug and enzyme substances of animal origin, basic protein feed ingredients of animal origin such as fish meal, meat scrap, rendered tankage, and related substances. Prepared ready-to-eat foods, dried yeast, chocolate, and similarly processed foods have more recently been identified as potential vectors, thus compounding the problem. Gaps in knowledge concerning mechanism of man to man, man to animal, and animal to man infections present handicaps to effective control measures. Lack of data on survival of the organism, conditions favorable to proliferation, and the avenues of contamination in various food and drug processes further complicate control.

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PROFILE OF A *SALMONELLA* TESTING METHOD. James Rutledge, Lauhoff Grain Company, Danville, Illinois.

Members of the food industry are well aware of the concern expressed by regulatory officials in regard to *Salmonella* bacteria and the increased incidence of Salmonellosis. Through the effort to disrupt the cycle of transmission of this infectious bacteria, it is becoming necessary for more and more food manufacturing companies to incorporate *Salmonella* testing as a part of their overall laboratory program. A review of methodology is a necessary 1st step to be taken by persons responsible for initiating such a testing program. The purpose of this paper is to outline 1 method that is presently employed to routinely test cereal products for the presence of *Salmonella* bacteria.

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GLASSWARE APPARATUS FOR *SALMONELLA* TESTING. George J. Banwart, ARS, USDA, Beltsville, Maryland.

A glass apparatus is described for use in determining the presence of *Salmonella* or other motile types of organisms. Small numbers of salmonellae inoculated into a center wall of the apparatus grew through a semi-solid agar seal and into Brain Heart Infusion broth within 24 hr., so that biochemical and seriological reactions could be determined. Three other types of organisms inoculated with the salmonellae did not interfere with the growth or detection of the salmonellae.

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SALMONELLA RESERVOIRS IN ANIMALS AND FEEDS. E. M. Ellis, National Animal Disease Laboratory, ARS, USDA, Ames, Iowa.

Industry and government working together now and in the future need to give increasing, continuous emphasis to the prevention of contamination and recontamination with salmonellae of feed and feed ingredients in rendering plants, feed mills, and on-the-farm utilization and storage of feeds. Because increasing intensification of livestock- and poultry-raising results in greater numbers of animals on fewer highly specialized farms, producers will need to give increased attention to husbandry sanitation practices designed to aid in controlling diseases spread by fecal contamination, such as salmonellosis. The veterinary practitioner can be of professional help to his clients by counseling them on the importance of sound disease prevention and sanitation practices in the management of livestock and poultry, and in the housing and care of pets. Many believe the chain of infection could be broken by producing feeds free of salmonellae and preventing recontamination of such products. This would certainly help; however, the attack must be made in all quarters if salmonellosis in agriculture is to be successfully controlled.

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HUMANS AND PETS AS SOURCES OF *SALMONELLA*. Mildred M. Galton, National Communicable Disease Center, Atlanta, Georgia.

Reported *Salmonella* infections in man in the U.S., other than typhoid fever, have increased from 723 in 1946 to 20,040 in 1966. It has been estimated, however, that only 1% of the *Salmonella* infections are reported to public health authorities. The majority of the infections diagnosed in man are classified as sporadic cases. This situation is believed to be due largely to the lack of personnel and facilities to investigate each case thoroughly, a conclusion supported by reports from states in the *Salmonella* Surveillance Report. From these, it may be noted that frequency of isolations from multiple individuals within families vary widely from one state to another. Reports of symptomless human excretors of salmonellae without history of preceding intestinal infection vary considerably. Saphra and Winter (*New Engl. J. Med. 256:* 1128, 1957) estimated the carrier rate in the general population to be about 0.2%. It is known that the long-term "temporary" carrier occurs frequently. Felsenfeld and Young (*Am. J. Trop. Med. 29:* 483, 1949) stated that 55.7% of the outbreaks which they studied were caused by human carriers. However it should be remembered that it is frequently difficult to distinguish culprits from victims in restrospective investigations of salmonellosis. Pets most often suspected as the source of human *Salmonella* infections include dogs, cats, various avian species, and turtles. Various surveys have indicated that 15-20% of normal household dogs may be infected with salmonellae (*Am. J. Pub. Health 38:* 403, 1948; *J. Infect. Dis. 91:* 15, 1952). The rate in dogs confined to kennels is usually much higher. This potential hazard has been known to exist for about 2 decades but reports of documented human infections traced to dogs are rare. On the contrary, pet chicks and ducklings given to children at Easter-time frequently are found to be the source of infection in the recipients (*Pub. Health Rep. 78:* 1073, 1963). Parakeets in the home have been incriminated occasionally, also, as the source of human infections (*New Engl. J. Med. 264:* 868, 1961). Numerous documented cases of salmonellosis associated with pet turtles have been reported (*JAMA 192:* 347, 1965). More than 100 instances have been recorded in which the same *Salmonella* serotype has been recovered from the patient and the pet turtle involved. Studies in several states have indicated that a high percent of pet turtles for sale in retail stores are contaminated with salmonellae. However, turtle breeders in the U.S. are taking steps to reduce sources of contamination, and ultimately to eliminate infection in the baby turtles distributed.

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BACTERIOLOGICAL PROBLEMS IN THE MANUFACTURE OF OILSEED PROTEINS. R. Paul Elliott, Consumer & Marketing Service, USDA, Washington, D. C.

The Food and Agriculture Organization of the UN reports that in 1966, the population of the world continued to increase, but food production actually dropped. Whereas, in some countries, food production increased, in other critical countries such as India, many crops failed. The main product of agriculture is carbohydrate. The foods that make up most of the world's diet—the cereals, potatoes, yams, cassava, etc.—are from 1 to 12% protein on the basis of dry weight. An adult man needs 14% protein in his food. Children and pregnant or lactating women need from 16 to 20%. However great an increase there may be in the consumption of the conventional carbohydrate foods, there will be a protein defict. Increased consumption of low-protein food makes the consumer fat, but as malnourished as ever. Recognition of the importance of protein sources and their deficiency in most of the world's diet has come slowly. It is now, however, generally recognized as a principal problem.

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A METHOD OF DESTROYING *SALMONELLA*. O. H. M. Wilder, National Renderers Association, Des Plaines, Illinois.

Salmonella in animal proteins or similar feed ingredients can be killed by putting the feedstuffs through a terminal heater whereby the materials in meal form are heated to a temperature of 99°C. with a retention time of 2.5-3.0 min. at that temperature, and with a moisture content of at least 10% in the materials entering the heater. Commercial heat exchanges are modified to serve as the terminal heaters. Partial cooling takes place as moisture flashes off on emergence from the heater, or if further cooling is desired, a smaller chilled heat exchanger may be attached to the equipment.

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CONTROL OF *SALMONELLA* IN EGG PROCESSING. D. H. Berquist and Richard Forsythe, Hennington Foods, Inc., Springfield, Missouri.

Methods for destroying *Salmonella* in egg products are discussed. Several commercial methods for pasteurizing egg whites are currently employed: (1) heat-treating untreated liquid at its natural pH; (2) heat-treating liquid stabilized with an aluminum salt at pH 7.0; (3) heat-treating liquid following addition of H_2O_2; (4) heat-treating dried egg white often in combination with one of the liquid pasteurization procedures. Egg products containing whole egg and yolk are pasteurized in the liquid state at temperatures greater than 140°F. Efforts to insure the absence of *Salmonella* extend to prevent post-pasteurization contamination. Recontamination during drying and packing, sampling for quality control, and use in manufacturing plants pose serious problems. Methods of control are discussed. Sampling plan and laboratory analysis must be carefully developed to properly assay levels of organisms distributed in a nonhomogeneous manner, such as results from improper pasteurization or recontamination. Safe handling practices for egg products users are outlined.

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FACTORS AFFECTING THE HEAT RESISTANCE OF *SALMONELLA*. Henry Ng, Western Regional Research Laboratory, ARS, USDA, Albany, California.

Susceptibility of *Salmonella* to heat can vary depending on the particular strain. A *Salmonella senftenberg* designated as 775W, isolated over 20 years ago, is one of the most heat-resistant strains of *Salmonella* ever encountered. Almost 300 strains of *Salmonella*, representing 75 different serotypes, were screened for heat resistance, and none was found as resistant as 775W. One *S. blockley* strain, #2004, although 5 times more resistant than the average *Salmonella*, was only about 1/5 as resistant as 775W. Nineteen other *S. senftenberg* strains and 6 other *S. blockley* strains included in the survey were of average heat resistance. Therefore, no correlation was found between heat resistance and serotype. For any given strain of *Salmonella*, the age of a culture is found to influence its heat resistance. Cells from exponential phase cultures are more susceptible to heat than are those from stationary phase. A direct relationship was observed between heat resistance and temperature of growth—the higher the temperature of growth, the more resistant the cells. The heat resistance of cells grown in a minimal medium, however, did not markedly differ from that of cells grown in a complex medium.

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SALMONELLA IN RELATION TO AGRICULTURE. E. E. Saulmon, USDA.

Some of the more extensive programs directed to the control of salmonellosis in domestic animals, which have been conducted by the U. S. Department of Agriculture, will be identified. These have been singularly successful, insofar as the economics of production is concerned. However, they fall short of meeting the present market demand for food free from all of the 900-1,200 odd serotype species of *Salmonella* considered potentially infectious for man. The increasing incidence of salmonellosis in man, and the identification of domestic animals as a major reservoir of various species of *Salmonella*, has made it necessary to broaden the objective of existing programs, expand the research effort, and intensify testing schedules in many of the existing regulatory control programs. The activities of a special *Salmonella* task force set up to coordinate research with the needs of the various regulatory agencies specifically concerned will be reviewed. Data developed by the Animal Health Division in a market survey of feed and feed ingredients as carriers of *Salmonella* will be presented. The more pressing problems and the current programs within other Divisions will be discussed. Insofar as the findings to date may serve to identify future needs.

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ATOMIC ABSORPTION SPECTROSCOPY. Biagio Piccolo and Robert O'Connor, Southern Regional Research Laboratory, ARS, USDA, New Orleans, Louisiana.

Atomic absorption spectrophotometry has been used for the rapid determination of various metallic and nonmetallic elements in crude and refined vegetable oils and in animal fats, many present in the ppm level. Various types of crude and refined vegetable oils were analyzed for Ca, Cu, Fe, Mg, Mn, Na, P, and Zn. This method of analysis has proven to be less time-consuming, as accurate, and more convenient than other spectroscopic techniques for elemental analyses.

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THE ROLE OF CRYSTALLOGRAPHY IN THE STUDY OF TRIGLYCERIDES. C. W. Hoerr and F. R. Paulicka, SCM Corporation.

The contribution crystallographic techniques have made to the understanding of triglyceride polymorphism is reviewed. The polymorphism of these compounds is explained in terms of crystal structure. The concept of molecular packing is applied in explanation of the polymorphism of natural fats. The need for a systematic investigation of the crystal structure and molecular composition of natural fats is stressed.

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APPLICATIONS OF INFRARED ABSORPTION SPECTROSCOPY IN THE ANALYSIS OF LIPIDS. N. K. Freeman, University of California, Berkeley, California.

The uses of infrared spectroscopy in lipid chemistry are discussed, with the main emphasis on quantitative analytical methods.

In reviewing the infrared technique from an overall perspective, attention is also given to instrumental developments, sampling techniques, and qualitative applications. Some features are brought out in the context of examples that are cited. These include some significant established methods as well as recent developments, such as the use of integrated band intensities and the involvement of computers and computer techniques in infrared analysis. Current trends and future directions are indicated, and some unrealized potentialities are suggested.

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SOME APPLICATIONS OF MASS SPECTROMETRY TO THE ELUCIDATION OF STRUCTURE. RALPH T. HOLMAN and KWOK SUN, The Hormel Institute, Austin, Minnesota.

In the field of lipid chemistry, mass spectrometry has been applied primarily to simple compounds related to fatty acids. The application to complex lipids has been limited because of their insufficient volatility and the thermal decompositions experienced. The location of branches in lipid molecules via mass spectrometry is possible, because the ions resulting from fragmentation of bonds adjacent to a branch are more stable. This has been useful in elucidation of structures of naturally occurring branched fatty acids. Pyrolysis leads to similar fragmentation patterns, and pyrolograms of branched hydrocarbons have been interpreted and found to be analagous to the mass spectra of the parent substance. The location of double bonds in fatty acids has been attempted via mass spectra of a variety of derivatives. The more successful approaches have been through the isopropylidene derivatives, the keto derivatives, and via deuteration with tetradeuterohydrazine. In the latter instance, mass spectrometry of the pyrolysis products serves to locate the deuterium atoms and hence, the double bonds in the original substance, regardless of position or number of double bonds.

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A REVIEW OF COMPUTER-AIDED SPECTROSCOPY. R. O. CRISLER, The Procter & Gamble Company.

Techniques are being developed which have as their objective, improvement in the accuracy of infrared absorbance measurements, and more exact recording of the true band shapes. From these improved spectra, accurate quantitative parameters are being obtained which can supplement or even supplant the present chart recordings as the fundamental spectral representations. These techniques include methods for processing spectra to eliminate most of the instrumentally introduced artifacts, and to resolve the corrected spectra into algebraic representations in the form of sums of distribution functions. The consequences of the increasing use of these techniques will be to improve significantly our ability to do both qualitative and quantitative analysis. Descriptions of the more interesting of these methods and of some of the approaches to computer-aided qualitative analysis will be given.

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TOCOPHEROL DESTRUCTION AND PEROXIDE FORMATION IN ETHYL OLEATE, LINOLEATE, LINOLENATE, AND ARACHIDONATE. L. A. WITTING, Elgin State Hospital, Elgin, Illinois.

The molar ratio of lipid peroxide formation to tocopherol destruction was studied in the range of fatty ester to tocopherol of 700:1 to 36,000:1. As was expected, onset of rapid peroxidation was delayed in proportion to the initial antioxidant level. At the onset of rapid lipid peroxidation, however, the peroxide formed was 4, 160, 310, and 650 times the level of residual tocopherol for oleate, linoleate, linolenate, and arachidonate, respectively. These ratios were independent of the initial antioxidant level. Prior to onset of rapid peroxidation, the quantities of peroxide found per tocopherol destroyed were in the ratios of 0.025:1:2:4 for oleate, linoleate, linolenate, and arachidonate, respectively. It was necessary to develop an analytical procedure which permitted the determination of low levels of α-tocopherol, 0.05-1.0 μM/g. in highly autooxidized oils, 800-1200 μM peroxide/g. The work was conducted with a view towards formulating a model system for the study of lipid peroxidation and tocopherol destruction *in vivo*.

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VITAMIN E IN FOODS: DETERMINATION OF TOCOLS AND TOCOTRIENOLS. H. T. SLOVER, J. LEHMANN, and R. J. VALIS, USDA.

A method is described for the analysis of foods for the forms of vitamin E. Detailed procedures, chosen to minimize vitamin loss, are given for extraction, saponification, and partial purification by thin-layer chromatography. The entire procedure has been evaluated with both standards and food samples. The individual tocopherols (both tocols and tocotrienols) in the partially-purified extract are identified and estimated by a GLC method similar to that reported earlier for standard mixtures. Response factors relative to didecyl pimelate as an internal standard and overall recoveries were determined for α-tocopherol, β-tocopherol, γ-tocopherol, ꞩ-tocopherol, α-tocotrienol, and β-tocotrienol. Sample sizes depended on tocopherol content, and were usually chosen to contain 3-50 μg. of the individual tocopherols. Data for a number of seeds and oils are given. The greatest variety of forms was found in barley, which contains all the forms listed above, plus γ-tocotrienol.

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ANTIOXIDANTS IN BIOLOGICAL SYSTEMS. H. H. DRAPER, University of Illinois, Urbana, Illinois.

Mammalian and avian species apparently require exogenous antioxidants for the stabilization of tissue lipids in general, and of the lipoprotein components of cellular and subcellular membranes in particular. Tocopherols are the prime lipid-soluble antioxidants in nature, but water-soluble substances, including ascorbic acid, sulfur amino acids, and selenium compounds, also possess activity. Vitamin E deficiency leads to lesions which are grossly dissimilar in different species and organs, but which are all characterized in their recipient stages by a degeneration of cell wall or particulate membranes in the affected tissues. Some synthetic antioxidants have the ability to prevent and alleviate these lesions, the most effective so far discovered being certain aromatic amines and quinoline derivatives. Although the role of vitamin E at the molecular level therefore appears to be nonspecific, from an overall physiological standpoint, the tocopherol molecule may be unique in its ability to meet all of the specifications of a biologically active lipid antioxidant. All substitutes examined to date have been found to possess some physiological limitation, such as inefficient absorption from the intestine, inadequate transfer across the placenta, insufficient deposition in the egg to sustain reproduction, or a toxic effect on the developing embryo. The concentration of tocopherols in natural fats and oils is strongly correlated with their degree of unsaturation, and the requirement for this vitamin is related to the proportion of polyunsaturated fatty acids in the lipid consumed.

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PROGRESSIVE CHANGES IN THE COMPOSITION OF THE LIPIDS OF THE MAJOR ORGANS OF IMMATURE RATS AFTER HYPOPHYSECTOMY. B. JENSEN and O. S. PRIVETT, The Hormel Institute, Austin, Minnesota.

Male immature (25-day-old) rats were hypophysectomized (hypox) and maintained on a diet of 28% casein, 52% sucrose, 4% nonnutritive cellulose, 3% salts and vitamins, and 13% safflower seed oil. A control group of nonoperated rats was maintained on the same diet. Subgroups of rats from both the operated and control groups were sacrificed at the beginning of the experiment and at selected intervals for 40 weeks. The major organs were excised, weighed, and extracted; fatty acid and lipid class analyses were made on the extracted lipids. Six weeks after the operation, the growth of the hypox animals was only about ⅓ that of the controls; the major organs of these animals were also proportionately smaller, except for the testes, which were only 1/10 that of the controls. Essentially the same relationship existed throughout the remainder of the experiment. The major difference in fatty acid and lipid class composition of the organs of the normal and hypox animals had occurred 3 weeks after the operation with the greatest changes occurring in the testes. The main effect of hypophysectomy was on the triglyceride fraction, which was characterized by a decrease in the percentage of palmitic and docosapentaenoic acids, and an increase in the percentage of oleic and especially linoleic acid. Base-line information is reported on the effects of hypophysectomy on growth and development of the immature rat, as well as on lipid composition of the major organs.

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FATTY ACID COMPOSITION OF ADULT *SCHISTOSOMA MANSONI*. THOMAS M. SMITH, H. B. WHITE, JR., and T. J. BROOKS, JR., University of Mississippi Medical Center.

The fatty acid composition of triglyceride and total phospholipid fractions of adult *Schistosoma mansoni* has been examined. Both triglyceride and phospholipid contained fatty acids varying in chain length from 12 through 24 carbons, and trace amounts of shorter chain components were found in the triglyceride fraction. A docosahexaenoic acid in the triglyceride fraction represented the highest degree of unsaturation encountered. Branched chain fatty acids of 16 and 18 carbons were found in both phospholipid and triglyceride. Examination of fatty acids from fluke total lipid revealed the presence of small amounts of odd-numbered carbon fatty acids varying in chain length from 13 through 23 carbons.

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DETOXIFICATION OF CASTOR MEAL AND ITS UTILIZATION IN LIVESTOCK RATIONS. JIM RIDLEHUBER and CHARLES HAY, Plains Cooperative Oil Mill, Lubbock, Texas.

Castor seed (*Ricinus Communis* L.) contains a highly toxic protein compound called ricin. The solvent-extracted castor meal can be detoxified by a moist heat treatment into a safe and palatable livestock ration. Cattle-feeding tests indicate that the protein in castor meal is equal or superior to the protein in cottonseed meal for fattening rations. Other feeding tests using castor meal with known levels of ricin indicate that the ruminant animal has a high tolerance for ricin, with the toxicity apparently deactivated in the rumen. Meat from animals fed castor meal showed no trace of ricin content or toxicity when fed to mice. Organs of the animals showed no damage from the ingestion of ricin. Young Holstein calves were able to ingest whole raw castor seeds with no toxic symptoms or ill effects. Cattle fed high levels of ricin showed no ill effects other than reduced feed intake because of the unpalatability of raw castor meal. The moist heat detoxification procedure increases palatability to a level equal with other protein sources. Tests are being conducted on deallergenization of castor meal, and more extensive testing is in progress on any possible effect of castor meal on meat from animals fed castor meal.

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THE EFFECT OF TEMPERATURE ON THE ARTIFACTUAL FORMATION OF METHYL ESTERS IN MOUSE LIVER. JON J. KABARA and ETIENNE MUELLER, SR., University of Detroit, Detroit, Michigan.

The occurrence of methyl esters of fatty acids as a natural component of biological tissue has been an issue in need of clarification during the past few years. This laboratory previously found that microgram quantities of methyl esters already present in mouse liver tissue could be increased during experimental manipulation. The present study investigates various factors that might affect the presence or absence of the "natural" methyl esters. Quantitative measurements indicated that lyophilization of the tissue had no effect on the appearance or disappearance of methyl ester. Twenty-four-hr. incubations of aqueous (nonmethanolic) suspensions of the tissue at various temperatures showed a pattern of ester production strikingly similar to enzymatic-type reactions. A search for methanol in the tissue did not reveal any extractable alcohol at the level of detection necessary for the minimum amounts of ester measured. Further study is necessary to establish possible metabolic significance of these esters and the source of the methyl donor.

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THE KETOGLUTARATE SHUNT AND LIPID SYNTHESIS IN THE BRAIN OF THE NEONATAL RAT. A. F. D'ADANO and G. FRIGYESI. Albert Einstein Medical College, and J. C. SMITH. Montefiore Hospital.

Extra mitochondrial lipid synthesis requires the translocation of acetyl groups from the mitochondria where they are derived from pyruvate oxidation. A pathway has been described by which these acetyl groups are transported to the cytoplasm as carbons 4 and 5 of α-keto glutarate or glutamate (*J. Biol. Chem., 240:* 613, 1965). This pathway, which has been termed "the ketoglutarate shunt," proceeds by the following reaction sequence:

α-KG $\rightarrow$ isocitrate $\rightarrow$ citrate $\rightarrow$ acetyl CoA + oxaloacetate

Since the cellular geometry of the cells of the central nervous system changes radically during neonatal brain development, it was of interest to study the characteristics of this conversion relative to lipid synthesis during this period. Brain slices from animals 1-2 days old were incubated with DL-glutamate-5-C^{14}. Optimal conditions for conversion to radioactive lipids were determined. These are: 20 μM glucose in Krebs-Ringer bicarbonate buffer at pH 7.8 for 4 hr. Acetyl groups are provided by the precursor both for fatty acids (primarily palmitic) and for sterols.

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LIPID DEPLETION AND DNA CONTENT OF FAT CELLS FROM EPIDIDYMAL FAT PADS IN COLD-EXPOSED RATS. DONALD THERRIAULT and ROGER HUBBARD, USARIEM, Natick, Massachusetts

The mass of fat contained in adipose tissue is the resultant of 2 factors—the number of adipocytes, and the fat content of individual adipose cells. When rats are exposed to a cold environment, they initially lose weight. This loss of weight is reflected in a depletion of body fat. On prolonged exposure to cold, the animals increase their food consumption and begin to grow again. However, the rate of growth in the cold never equals that at normal room temperature. Furthermore, their lipid stores are significantly lower than the control rats maintained at normal ambient temperature. The question arises as to whether the depletion in fat stores is the result of a change in cell population of adipose tissue, or a reduction in the lipid content of the cells. In order to answer this question, measurements of the desoxyribonucleic acid and lipid content of isolated adipose cells obtained from one of the epididymal fat pads in the rat were made. In addition, total lipid analysis of the contralateral fat pad was measured. Assuming that the desoxyribonucleic acid content is a measure of the cell number, and that the lipid is primarily in the fat cell, it is possible to calculate the number of adipose cells in the tissue, and the fat content per cell. The results indicate that there is a significant depletion of fat per cell in cold-acclimated rats. However, this is partially compensated by a hyperplasia. Nevertheless, the net result is a decrease in total adipose tissue in cold-exposed rats.

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GLYCEROKINASE ACTIVITY OF THE ISOLATED EPIDIDYMAL FAT CELL OF THE RAT. ROGER HUBBARD, PAUL VOORHEIS, and DONALD THERRIAULT, USARIEM, Natick, Massachusetts.

The estimation of lipolysis in adipose tissue based upon the measurement of glycerol production assumes that there is no significant glycerokinase activity in that tissue. Using isolated epididymal fat cells from the rat, we have demonstrated a small but significant glycerokinase activity. The incorporation of C^{14} labelled glycerol into α-glycerophosphate, glyceride-glycerol, CO_2, and total fatty acids, was measured in isolated fat cells from epididymal adipose tissue. Linear progress curves were obtained and used to calculate the kinetics of the glycerokinase-catalyzed reaction. An ion-exchange chromatographic method was developed to separate glycerol from α-glycerophosphate and other acidic intermediates. The α-glycerophosphate was separated from these intermediates by hydrolyzing the α-glycerophosphate to glycerol, using ceric ammonium nitrate, and fractionating the glycerol on the ion exchange column. The specific activity of the α-glycerophosphate was obtained by counting radioactivity in a liquid scintillation counter, and determining the amount of α-glycerophosphate enzymatically. The kinetics of this reaction in the isolated fat cell and its physiological implications will be discussed.

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DESCRIPTION OF SAMPLES OF WHEATS AND WHEAT PRODUCTS FOR MULTINUTRIENT ANALYSES. F. N. HEPBURN and J. H. TULLOSS, American Institute of Baking, Chicago, Illinois.

This initial paper describes the samples, sampling plan, and procedures for procurement, preparation, and distribution of samples in the multinutrient study. To study the wheat foods available to the consumer in different sections of the country, 10 types of food products were obtained from each of 2 cities in each of 5 geographical regions of the U.S. (100 samples). Products were: enriched white bread (conventional and continuous mix), whole wheat bread, hamburger rolls, doughnuts, all-purpose flour, biscuit mix, and breakfast cereals (whole grain, wheat flakes, and shredded wheat). An additional 56 samples of wheat products were studied to determine changes in composition from grain to food product. These were comprised of 11 wheats, flours milled from those wheats, and products prepared from the flours. Both conventional and continuous-mix bread were prepared from hard wheat flours, macaroni from durum semolina, and either cake or crackers from soft wheat flours. Samples were obtained by the American Institute of Baking and after preparation (freeze-drying, grinding, blending, etc.), subsamples were distributed to participating laboratories for nutrient analyses.

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EXTRACTABLE AND "BOUND" FATTY ACIDS IN WHEAT AND WHEAT PRODUCTS. F. W. QUACKENBUSH and J. A. INKPEN, Purdue University Lafayette, Indiana.

To provide quantitative data on the fatty acids in a broadly representative group of samples of wheat and wheat products, total lipid was obtained by extraction with chloroform-ethanol-water with subsequent hot-acid hydrolysis of the extracted (CEW) solvent was equally as effective an extractant as water-residue to release "bound" lipid. The chloroform-ethanol-water saturated *n*-butanol (WSB); however, both solvents systems extracted substantial amounts of nonlipid (hexane-insoluble) substance, and neither effected complete removal of lipid material from the sample, as shown by subsequent acid hydrolysis of the extracted residue. The extractable lipid from wheat and wheat flour contained much more stearate, much less palmitate, and usually more linoleate than the "bound" lipid removable only after acid hydrolysis. One hundred fifty-six samples of wheat, wheat flour, and wheat consumer products were analyzed for total and individual fatty acids. The data indicate that individual wheat products have nearly the same fatty acid composition in all regions of the country.

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TOCOPHEROLS IN WHEATS AND WHEAT PRODUCTS. H. T. SLOVER, JOANNA LEHMANN, and R. J. VALIS, USDA, Beltsville, Maryland.

A variety of wheats and wheat products have been analyzed for the various forms of vitamin E. Data, in μg./g., are given for the amounts of α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, α-tocotrienol, and β-tocotrienol, as determined by gas-liquid chromatography of their trimethylsilyl ethers. Nine common wheats, both hard and soft, and 2 durum wheats—the flours from all of these and products made from them were analyzed in duplicate. In addition, 10 pooled consumer product samples from each of 10 representative U.S. cities have also been analyzed. The data from the wheat to flour to product series permit an assessment of the fate of the tocopherols present in wheat, and their relative contributions to the tocopherols in the products. Although all the wheats had similar amounts of the same tocopherols, the durum wheats were slightly lower in α- and β-tocopherol and higher in α- and β-tocotrienol. Processing into white flour removed the major part of all forms. The variation in the identity and quantity of tocopherols in the consumer products reflected, in most instances, differences in the nonwheat ingredients. Most baked products contained γ- and δ-tocopherols, characteristic of vegetable fats, along with variable amounts of the tocopherols from wheat.

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DISTRIBUTION OF MANGANESE, COPPER, NICKEL, ZINC, AND MAGNESIUM IN WHEATS AND WHEAT FOOD PRODUCTS. ELIZABETH G. ZOOK and FLORIDA E. GREENE, USDA, Beltsville, Maryland.

Five minerals were determined by atomic absorption spectroscopy in 11 known wheat blends, 20 commercially prepared flours from these blends, and 25 specially prepared products from the flours. The same minerals were determined in 10 consumer products from 10 cities. Zinc and copper varied significantly among the hard wheats; manganese, nickel, zinc, and magnesium among their flours. Copper and zinc were lower in breads prepared by continuous mix as compared to conventional dough breads prepared from the same flours. Nickel in breads increased 5-fold over the flour contribution, probably added by the shortening. Flour was the major source of the other minerals in bread. Manganese, copper, and zinc varied among the 4 soft wheats, most of the variation being contributed by a single very low-mineral wheat blend. Among the 13 soft wheat flours, manganese and zinc were lowest in the short patent (cake) flours; manganese, copper, zinc, and magnesium were highest in the cut-off (cracker) flours, and intermediate in the straight grade (cracker) flours. In cakes, magnesium and zinc were about twice the level in the flours, nickel was 5-fold higher; in crackers, nickel was 12-fold higher. Otherwise, mineral content of cakes and crackers was contributed mainly by the flour. A higher percentage of the durum wheat minerals appeared in the semolinas than in the flours from either hard or soft wheat. Copper was higher in macaroni than in the semolina; otherwise, mineral content of macaroni was accounted for by the semolina. No discernable pattern of mineral distribution could be found with the 100 market samples of consumer products.

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B-VITAMINS IN WHEATS AND WHEAT PRODUCTS. MARILYN M. POLANSKY and E. W. TOEPFER, USDA, Beltsville, Maryland, and F. N. HEPBURN, American Institute of Baking, Chicago, Illinois.

The amounts of thiamine, riboflavin, pyridoxine, pyridoxal, and pyridoxamine have been determined in 10 consumer products purchased in 10 cities representing 5 geographical areas of the U.S. Eleven wheats, and the flours and products made from these wheats, also were analyzed. In general, the data for the consumer products from the various geographical areas appeared to be consistent for each product. The whole wheat products contained much higher amounts of vitamin B_6 than the refined products. In the wheat to flour products (processing) studies, durum wheat contained more thiamine and vitamin B_6 than hard or soft wheats. In milling the hard and soft wheats to flour, between 20 and 35% of the thiamine was retained, about 35% of the riboflavin, and only about 15% of the vitamin B_6. The flour from durum wheat retained about the same amount of thiamine, considerably more riboflavin, and a little more B_6. Pyridoxine accounted for ¾ of the vitamin B_6 in wheat samples, over ½ in flours, about ⅔ in macaroni, and less than ½ in baked products. The increase in B-vitamins in some products and also the change in proportions of 3 vitamin B_6 components from that of flour was due to the addition of ingredients such as eggs, milk, and yeast.

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ANALYSIS OF FREE STEROLS AND SATURATED STEROL ESTERS ISOLATED FROM DIFFERENT FLOUR MILL STREAMS. C. P. BERRY, V. L. YOUNGS, and K. A. GILLES, North Dakota State University, Fargo, North Dakota.

Qualitative and quantitative determinations of free and esterified sterols in wheat are described. Determinations were carried out principally with column, thin-layer, and gas-liquid chromatography. Free sterols and sterol ester analyses were performed on U.S. and foreign wheats. The endosperm of hard red spring wheat contained small quantities of free sterols; conversely, a higher content of saturated sterol esters was observed. The endosperm of durum wheat contained an appreciable amount of free sterols, whereas the saturated sterol esters appeared only as a trace. The mill streams of several wheats were analyzed. The content of free sterols increased as ash increased; conversely, a decrease in saturated sterol ester content was observed. For hard red spring and durum wheat, the major portion of the free sterols exists in the bran region. Sitosterol, campesterol, cholesterol, and brassicasterol were identified in the free sterols and saturated sterol esters by gas-liquid chromatography. Sitosterol and campesterol were most abundant.

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REACTION OF ALDEHYDE OILS WITH A RESINOUS POLYOL STYRENE-ALLYL ALCOHOL COPOLYMER. A. E. RHEINECK, North Dakota State University, Fargo, North Dakota, and P. R. LAKSHMANAN, Gulf Oil Company.

A series of film formers based on aldehyde oils, derived by reductive ozonolysis of soyabean and linseed oils, were prepared by a 2-step reaction with a resinous styrene-allyl alcohol copolymer. The methyl acetals of the di- and monoaldehyde oils were interchanged with the hydroxyl functionality of the copolymer with potassium acid sulfate as the catalyst as follows: (a) The heterogeneous mixture was heated only to clarity in the 1st step. This consumed about 20-25% of the hydroxyl groups of the resinous polyol. (b) Films of the partially reacted products of (a) were cast and cured at various temperatures between 150 and 250°C. for variable periods of time. The films showed good hardness, scratch, and chemical resistance. The film-former in the (a) stage was compatible with melamine and urea-formaldehyde resins, and upon baking, these compositions also produced clear, hard, chemical-resistant films.

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URETHANE COATINGS BASED ON ALDEHYDE OILS. A. E. RHEINECK, North Dakota State University, Fargo, North Dakota, and P. R. LAKSHMANAN, Gulf Oil Company.

A series of polyol-acetals of aldehyde oils were prepared by an acetal interchange reaction between dimethyl- and tetramethyl acetals of aldehyde oils derived by the reductive ozonlysis of linseed and soybean oils. The acetals interchanged with pentaerythritol, trimethylol propane, and glycerol contained the equivalent of 0.93 and 1.88 aldehyde groups per oil molecule. The polyol acetals were reacted with only toluene diisocyanate in an NCO/OH of 2. These products were tested as film formers. Curing was by 2 reactions, namely, oxidation polymerization of the residual unsaturation in the oil, and moisture curing of the unreacted isocyanate group. Good quality films were formed.

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PRODUCTION OF VINYLIC UNSATURATION BY PARTIAL

DEHYDROBROMINATION OF VICINAL DIBROMO FATTY DERIVATIVES. A. E. RHEINECK, North Dakota State University, Fargo, North Dakota, and B. SREENIVASAN, Lever Brothers.

The reaction of methyl dibromostearate with metal cyanates in dimethyl formamide or dimethyl sulfoxide results in the disappearance of the peak at 548 cm.$^{-1}$ in the IR spectrum, due to the -C-Br group. Chemical analysis of the products reveals the presence of residual bromine. GLC and NMR examination of the products show the presence of olefinic unsaturation, indicating that partial dehydrobromination has occurred. The result is a double bond with 1 carbon atom attached to a bromine atom. This structure has some vinylic characteristics. The application of this process to partially brominated linseed oil yields a dehydrobrominated linseed oil, with residual bromine. This oil has drying properties very similar to raw linseed and safflower oils.

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RESIN-CATALYZED EPOXIDATION OF METHYL UNDECYLENATE. A. E. RHEINECK and JOEL BENDER, North Dakota State University, Fargo, North Dakota, and B. SREENIVASAN, Lever Brothers.

Epoxidation of methyl undecylenate by peracetic acid, produced *in situ* from acetic acid and hydrogen peroxide catalyzed by an ion-exchange resin of the sulfonic acid type, reaches a maximum of 3.8% oxirane. Examination of the products by TLC reveals unreacted undecylenate, epoxy undecanoate, and several other polar products. Residual undecylenate can be independently determined by its IR peak at 905 cm.$^{-1}$, which has been observed to obey Beer's law. GLC of the products on a polyester column yields a big peak for undecylenate and a smaller peak for the epoxy ester, even in products where the contents of the latter are high. On a silicone (DC-200) column, both components emerge with peak sizes more in line with their respective proportions.

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GLYCEROLYSIS OF LINSEED OIL: A COMPOSITIONAL STUDY. A. E. RHEINECK and RICHARD BERGSETH, North Dakota State University, Fargo, North Dakota, and B. SREENOVASAN, Lever Brothers.

The alcoholysis reaction of glycerol and AR linseed oil was studied with sodium hydroxide, sodium glyceroxide (Na O-CH_2CHOH-CH_2OH_2), and litharge, as catalysts. Reaction temperatures were varied from 150 to 225°C. with time. The samples and products were analyzed by thin-layer chromatography (TLC), gas-liquid chromatography (GLC), periodic acid determination of α-monoglycerides, and photodensitomitry of TLC spotted and charred plates. The reaction catalyzed by the sodium catalysts underwent alcoholysis which involved all fatty acids at essentially equal reaction rates. The reaction catalyzed by litharge showed a deviation, in which the linolenic acid had a slower reaction rate in the monoglyceride formation. An approximation of diglyceride and triglyceride was made by combining the results of the periodic acid determination of α-monoglycerides and the results of photodensitiometric runs of the samples on thin-layer chromatography plates.

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EFFECT OF *CIS-TRANS* ISOMERS AND RELATED PHYSICAL PROPERTIES OF MONOUNSATURATED LIPIDS ON SHORTENING POWER. JOYCE OSTRANDER and ADA MARIE CAMPBELL, University of Tennessee, Knoxville, Tennessee.

The effect of *cic-trans* isomers on the shortening power of lipids was investigated. Lipid samples studied included: a commercial vegetable oil (control), oleic acid, triolein, elaidinized oleic acid, elaidinized triolein, 2 samples in which elaidinized oleic acid was substituted at different levels for oleic acid, and 2 samples in which elaidinized triolein was substituted at different levels for triolein. Breaking strength of plain pastry wafers was used as a test for estimating the shortening power. Melting point, surface tension, interfacial tension, and viscosity measurements were made on the lipid samples. Lipid composition analyses included gas-liquid and thin-layer chromatography. Moisture determinations were made on the dough and wafers. At each level of substitution of elaidinized lipid, including the zero level, wafers containing triglycerides had a significantly higher breaking strength than samples containing fatty acids. Breaking strength showed significant correlation with the melting point of the lipid and with the percentage of *trans* isomers. Other significant correlation coefficients were found, and other statistical tests were applied.

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EXPERIENCE WITH SOME MIXTURE DESIGNS—OPTIMIZATION OF EMULSIFIER BLENDS IN BAKERS' SHORTENING. R. P. BASSON and D. T. RUSCH, Atlas Chemical Industries Inc., Wilmington, Delaware.

Extreme vertices, 2-factor composites, and simplex designs were used to determine optimum levels and proportions of emulsifiers for use in bakers' shortening. Comparisons of the respective designs were made. Volume, symmetry, grain, and organoleptic properties of white and yellow cakes were measured. Suitability of the blends for icings was determined by icing and cream filling volume and texture. A computer program was prepared to determine the response which best fitted the observations. The program used standard linear regression techniques and plotted a series of iso-response contours. These contours were used to optimize formulation with consideration given to relative cost and functionality of the total system.

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THE FUNCTIONS OF SODIUM STEAROYL-2-LACTYLATE IN YEAST-LEAVENED AND CHEMICALLY LEAVENED BAKED PRODUCTS. R. J. TENNEY and D. M. SCHMIDT, C. J. Patterson Co., Kansas City, Missouri.

The food uses of the stearoyl-2-lactylates are reviewed. The chemical and physical properties of the compounds are discussed. Studies of the effects of sodium stearoyl-2-lactylate in yeast-leavened and chemically leavened systems are reported. Sodium stearoyl-2-lactylate (SSL) is a newly developed emulsifier, dough conditioner, and whipping agent for use in nonstandardized foods, in an amount required to produce the intended effect. SSL is shown to impart tolerances to processing and ingredient variations in yeast-leavened and chemically leavened baked products. In the production of yeast-leavened baked products containing high shortening levels, SSL strengthens the dough, improving machinability, and produces breakfast rolls and coffee cakes with improved volume, texture, and keeping quality. In the production of chemically leavened baked products, SSL is shown to impart tolerances to production and formulation variables. White cake batters containing SSL are shown to produce cakes with excellent volume, symmetry, texture, and keeping quality.

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THE EFFECTS OF SODIUM STEAROYL-2-LACTYLATE ON PASTE VISCOSITY OF FOOD STARCHES. R. J. TENNEY, M. W. WARD, and R. N. VAN VACTOR, C. J. Patterson Co., Kansas City, Missouri.

The effects of the stearoyl-2-lactylates on wheat starch previously reported are reviewed. Studies of the effects that sodium stearoyl-2-lactylate (SSL) produces on wheat, potato, corn, rice, and tapioca starches are reported. Using the Brabender Visco/amylo/Graph, slurries of starches, with and without SSL, were heated to 92.5°C. and held at this temperature for 15 min. The viscosity of each starch paste, with and without SSL, was studied over a pH range of 4 through 7. An increase in hot paste viscosities of tapioca, wheat, and corn starches was noted with the addition of SSL. A decrease in hot paste viscosities of potato and rice starches was observed with the addition of SSL. In all starches of the study, the addition of SSL increased transition temperature or gelation temperature.

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CHROMATOGRAPHIC ANALYSIS OF SORBITAN FATTY ACID ESTERS. M. SAHASRABUDHE, Canadian Department of National Health and Welfare, Ottawa, Canada.

Sorbitan monostearate (SMS) is used in foods at levels of 0.1-0.6% in combination with other emulsifying agents. Although it is known that commercial SMS consists of mixed esters of a reproducible mixture of polyols derived from sorbitol, little information is available on the actual composition of these mixtures. This paper describes (1) a procedure for the analysis of the sorbitan polyols and their mono- and difatty acid esters, and (2) a method for the quantitative estimation of sorbitan fatty acid esters in foods. Polyols, fatty acids, monoesters, and diesters are separated by liquid partition-column chromatography and analysed by GLC as trimethyl silyl ethers. Individual mono- and difatty acid (palmitic, stearic, and oleic) esters of 1,4-sorbitan and isosorbide were prepared in the laboratory and used as standards. Recoveries of purified esters from foods were in the range of 92-100%. The procedure can be adapted for routine quality control analysis. A scheme of analysis for the quantitative estimation of several emulsifiers is discussed.

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THE EFFECT OF VARIOUS MESOMORPHIC PHASES OF MONOGLYCERIDE: WATER SYSTEMS IN STARCH PRODUCTS. N. KROG, Aktieselskabet, Grindstedvaerket, Denmark.

Distilled monoglycerides (GMS) in hydrated form are becoming more widely used in the food industry, particularly in the manufacture of starchy foodstuffs, such as bakery products, mashed potatoes, macaroni, etc. GMS can be hydrated in various ways. Various mesomorphic phases with very different physical behavior can be produced, depending on temperature, time, and concentration of the GMS in the water. The physical characteristics regarding viscosity, optical texture, and x-ray diffraction data of 6 different phases of GMS:H_2O are described. The technical effects of these phases have been tested in sponge cake and white bread in baking experiments. The results show remarkable differences in the effects of monoglycerides with regard to the volume and texture of sponge cake and the antistaling effect in white bread. These effects are related to the molecular structure of the particular GMS:H_2O system used. The best effect is found when the monoglycerides are added in a form in which the GMS molecules are arranged in spherical units in equilibrium with the water. The internal structure of these units is built in concentric bimolecular layers separated by concentric water layers. In such a system the GMS molecules have the greatest possible translational freedom and, therefore, can easily interact with other materials and form, for example, complexes with amylose. From a technical standpoint, it is important to use the correct form of hydrated GMS in order to obtain the optimum results in the end product.

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RAPID GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF METHYL ESTERS. M. D. JELLUM, Georgia Experiment Station, Experiment, Georgia.

Several studies were begun concerning the fatty acid composition of corn oil. These studies included the determination of oil composition of existing germplasm (released and unreleased inbred lines), inheritance of individual fatty acids, environmental influence on oil composition, and others. A need for a rapid gas-liquid chromatographic procedure was created, due to the large number of oil samples required for these studies. A stabilized preparation of diethylene glycol succinate (DEGS), which will withstand column temperatures in excess of 250°C., was used as a liquid phase. Oil from 4 corn inbred lines and their reciprocal crosses and several fatty acid standards (methyl esters) were analyzed by GLC procedures, differing in column temperature and carrier gas (helium) flow rate. Column temperatures of the different procedures varied from 180 to 235°C., and helium flow rate varied from 55 to 110 ml./min. Results of the composition of the corn oil samples and the NHI type standards were similar for all procedures. Retention time for linolenate varied from 2.5 to 25 min. A rapid analysis of corn oil samples was obtained with a column temperature of 235°C. and a helium flow rate of 110 ml./min. The rapid procedure using stabilized DEGS will not separate arachidic and eicosenoic acids. For corn oil, palmitoleic and arachidic acids can be separated by a column temperature of 220°C. and helium flow of 100 ml./min. and still retain a retention time for linolenic acid of 5 min. The rapid GLC procedure can be used for quantitative analysis of the fatty acid composition of oil from most of the common agronomic crops.

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FRACTIONATION AND ANALYSIS OF GLYCERIDES AND RELATED LIPIDS BY REVERSED-PHASE PARTITION CHROMATOGRAPHY. ORVILLE S. PRIVETT, LOWELL J. NUTTER, E. C. NICKELL, and ROLAND GROSS, The Hormel Institute, Austin, Minnesota.

Liquid-liquid partition chromatography is a very mild process, and ideally suited to the fractionation of lipids. Described here are various ramifications of reversed-phase partition column chromatography for the fractionation and analysis of triglycerides, methyl esters, and polar lipids. Continuous monitoring via hydrogen flame detection is compared to gas-liquid and thin-layer chromatographic methods for following the course of fractionation and quantification of separations. Observations are also made on the importance of various parameters of the process in experiments on the efficiency, capacity, and reproducibility of the separation of model mixtures of triglycerides, methyl esters, and polar lipids. The utility of the technique is demonstrated on natural lipid mixtures.