Meetings

76th AOCS Annual Meeting

Philadelphia • Franklin Plaza Hotel • May 5-9, 1985

Philadelphia

Crowded agenda awaits participants

Registrants at the 76th annual AOCS meeting to be held May 5-9, 1985, in Philadelphia will find an array of technical, administrative and social activities that could fill each day from breakfast until the late night news.

There will be six simultaneous technical sessions from Monday morning through Thursday. The final schedule will be distributed to registrants when they arrive at the Wyndham Franklin Plaza Hotel. Meeting registration will open at noon Sunday on the hotel's mezzanine level. Abstracts for the more than 300 technical papers are published in this issue of *JAOCS*; the tentative technical program appeared in the January 1985 *JAOCS*.

Among the many technical sessions will be two memorial symposia. The Brian L. Walker Memorial Symposium—Lipids and Cancer—will be held Wednesday. H.H. Draper, chairman of the Department of Nutrition at the University of Guelph, will open the symposium with a brief commentary about Dr. Walker, who was a faculty member in that department. The H.W. Kircher Memorial Symposium—Chemistry, Biosynthesis and Function of Sterols—also will be Wednesday. Dr. Kircher was a professor in the Department of Nutrition and Food Science at the University of Arizona. He had been a member of AOCS since 1963 and served as an associate editor of *Lipids*, as had Dr. Walker. Dr. Kircher died during January 1984; Dr. Walker died during April 1984.

Administrative events—primarily committee meetings—actually begin Saturday afternoon, May 4, when the AOCS Governing Board convenes. Other committees will meet through the day Sunday, May 5, through Wednesday, May 8, with the Governing Board scheduled to meet again Thursday, May 9. A tentative committee schedule appears in this issue of *JAOCS*. Virtually all committee meetings are open to any AOCS member. The only exceptions are such committees as the Examination Board which considers applications for "approved chemist" standing.

There will be two plenary breakfasts and two morning plenary lectures during the meeting. On Monday, May 6, there will be a plenary breakfast which will include the annual AOCS business meeting. Spouses' program registrants will receive tickets to this breakfast. On Monday Nobel laureate Melvin Calvin will give a keynote address. The annual inaugural/awards breakfast will be Wednesday, May 8. Recipients of 1985 AOCS Awards will be recognized, including the Supelco AOCS Research Award winner. The acceptance address for that award will be presented in a plenary lecture Wednesday morning.

Social events abound. The opening mixer on Sunday evening, May 5, will be held at the Franklin Institute Science Museum, within easy walking distance of the Franklin Plaza Hotel. Shuttle bus service will be available for those who don't wish to walk. On Monday evening, May 6, the First Annual Fat People's Fun Run or Walk will be held. This five-kilometer route will wind through a park area and conclude on the steps of the municipal art museum. The event is being administered by a local runner's club. Participants will receive tee shirts. Tuesday evening will be the first of two bus trips to Atlantic City to visit gambling casinos there. Cost is $5 per person for the round trip bus ride. The Tuesday bus will leave at 5:30 p.m., arriving back in Philadelphia after midnight; on Thursday the bus will leave at 1 p.m., arriving back in Philadelphia sometime between 6 and 9 p.m.

The traditional AOCS banquet will be held Wednesday evening, May 8. Unlike past years, the cost of tickets was not included in the 1985 meeting registration price. Therefore, persons who wish to attend must order tickets separately. Cost is $25 per person for tickets ordered before the Philadelphia meeting, or $35 per person for tickets purchased in Philadelphia. The entertainment will be the Fabulous Greaseband, featuring music of the 1950s, including songs by many of the famous entertainers who grew up in Philadelphia during that era.

In addition to the opening mixer and the Monday plenary breakfast, spouses' program registrants will participate in two tours. On Monday, May 6, they will have a walking bus tour, "America's Most Historic Square Mile," including the house where Thomas Jefferson drafted the Declaration of Independence, early meeting places of America's colonial legislatures, and the Betsy Ross House. Lunch is included. On Tuesday, May 7, there will be a bus trip to Longwood Gardens outside Philadelphia. Once a du Pont estate, Longwood Gardens is modeled on gardens found in France and Italy. Following lunch at Mendenhall Inn, the spouses will tour the Franklin Mint, the world's largest private mint. If the tours are not filled by spouses' program registrants,

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tickets will be available for the individual tours at $30 per day on a space-available basis.

Also new in the meeting registration this year will be a computerized message center. Registrants will be able to check at a computer terminal to see whether a message has been left for them at the registration desk. If a registrant's name is listed, that person should inquire at the registration desk for the message. Anyone who has lost an item in a meeting room should check at the registration desk, as session chairmen are asked to bring all "found" articles there at the conclusion of each session.

Persons who have not yet made travel arrangements are reminded that AOCS has arranged for discount airfares on domestic United Airlines flights through ITS, a Chicago-area travel agency. Discounts of 40% off normal coach fare are offered on travel between April 28 and May 19. Persons outside Illinois may call 800-621-1083 toll-free between 9 a.m. and 5 p.m. Central Standard Time; Illinois residents should call 312-645-1034 and will be billed by their telephone companies at normal intra-state telephone rates.

A special inaugural

The annual installation of officers at AOCS meetings is relatively routine, but this year's installation of Joyce Beare-Rogers as president will mark two important firsts.

She will be the first person residing outside the United States to be elected president of the AOCS and she is the first woman president. AOCS' first president, Felix Paquin, was born in Canada, but moved to the United States as a relatively young man many years before he became one of the society's founding members. AOCS records do not indicate if Paquin became a U.S. citizen, but he was living in Galveston, Texas, during his tenure as society president.

Dr. Beare-Rogers is chief of nutritional research in the nutritional science division of the Health Protection Branch of Canada's Health and Welfare department. She previously has served on the Governing Board as vice president, secretary and member-at-large. She has helped organize short courses, served as an associate editor of *Lipids*, and been active on numerous AOCS committees.

She will succeed Nicholas Pelick of Supelco Inc., Bellefonte, Pennsylvania, as AOCS President during the Philadelphia meeting.

Merit Award to Khym

The recipient of the 1985 AOCS Award of Merit will be Frank P. Kyhm, an active member of the society since 1944. Khym served as national secretary of AOCS in 1975-76 and was general chairman of the 1974 AOCS meeting in Mexico City.

Khym retired a few years ago as manager for Anderson Clayton & Co. S.A. in Monterrey, Mexico. He now lives in San Antonio. He has been active as a consultant since his retirement.

The success of the 1974 meeting in Mexico City encouraged AOCS to develop its series of international conferences. Khym was a nominee for AOCS vice-president in 1977.

Khym has served on numerous AOCS committees, including meetings logistics, national program planning, local sections, society improvement, international relations and on organizing committees for world conferences. He helped found an AOCS section in Monterrey.

Khym also helped plan the first AOCS Latin American short course held in 1967.

6 named Honored Students

Six graduate students from throughout the United States will be recognized as 1985 AOCS Honored Students during the annual meeting in Philadelphia.

Each will present a technical paper on his or her research that earned the award. The Honored Students receive complimentary registration for the meeting and funds to help pay travel and housing costs. The money is contributed by firms from the fats and oils industries.

The 1985 Honored Students, their universities, and the topics for their presentations are:

Robert Chapkin, University of California, Davis, "Inability of Skin Enzyme Preparation to Biosynthesize Arachidonic Acid from Linoleic Acid."

Evan S. Deneris, University of California, Los Angeles, "Isoprene: Biosynthesis and Role in Polyisoprenoid Metabolism."

N.Z. Hassanen, Texas A&M University, College Station, "Sequential Extraction Process for Extracting Oil and Aflatoxins from Cottonseed."

M.A. Linne, Lehigh University, Bethlehem, PA, "Simultaneous Interpenetrating Networks Prepared from Special Functional Group Triglyceride Oils: Castor Oil, *Lesquerella palmeri* and Other Wild Plant Oils."

K.L. Rho, Kansas State University, Manhattan, "Retardation of Rancidity in Deep-Fried Instant Noodle (Ramyon)."

K.L. Wiese, University of Arkansas, Fayetteville, "Factors Influencing Soybean Oil Extraction Rates."

Among the donors to the 1985 Honored Student program were Akzo Chemie America; Anderson Clayton Foods; Atlas Refinery Inc.; Best Foods, CPC North America; Bowers-Siemon; Cargill Inc.; CasChem Inc.; Colgate-Palmolive Company; Eagr Development Group; Fabrica de Jabon la Corona S.A., and The French Oil Mill Machinery Co. Inc. Also Kraft Inc.; Lehn and Fink Products Group; Mettler Instrument Corp.; Nabisco Brands Inc.; Nu-Chek-Prep Inc.; Howard Roth; Shell Chemical Company; Travenol Laboratories Inc.; Union Camp Corp.; U.S. Borax Research, and Van Dyk & Co. Inc.

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76th annual meeting local committee

Gerhard Maerker
General Chairman

Thomas A. Foglia
Registration Chairman

Glen Jacobson
Technical Program Chairman

Frank Scholnick
Local Facilities Chairman

Ed Saggese
Hotel Arrangements Chairman

Bill Marmer
Entertainment Chairman

Benne Marmer
Spouses' Program Chairperson

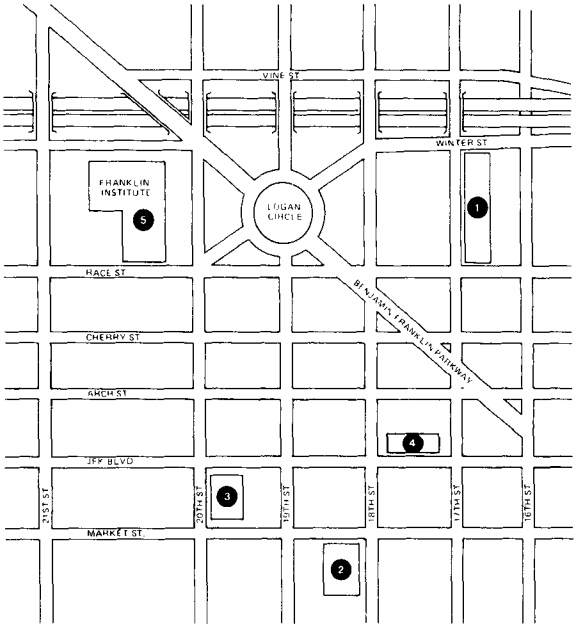
Abner Eisner
Finance Chairman

Ivan A. Wolff
Publicity Chairman

Joe Fioriti, Chairman
First Annual Fat People's
Fun Run or Walk

AOCS Hotel Locations

1. Wyndham Franklin Plaza
   (Headquarters hotel)
2. Holiday Inn—Center City
3. Penn Center Inn
4. Philadelphia Centre Hotel
5. Franklin Institute Science Museum
   (Mixer site)



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Tentative Committee schedule

Day and Committee	Time
SATURDAY, May 4	
Governing Board	2 p.m.
SUNDAY, May 5	
Committee on Program Evaluation	10 a.m.–noon
NMR	1–2 p.m.
Examination Board	1–4 p.m.
Education	2–4 p.m.
Hydrogenated Oils	3–4 p.m.
International Relations	3–4 p.m.
Advertising	4–5 p.m.
Aflatoxin	4–5 p.m.
MONDAY, May 6	
Soap & Synthetic Detergent Analysis	10–11 a.m.
1986 Annual Meeting	10 a.m.–noon
Monograph	Noon–3 p.m.
Membership Development	3–4 p.m.
Lecithin & Co-Products Analysis	3–4 p.m.
Commercial Fats & Oils Analysis	3–4 p.m.
Lipids Advisory	3–5 p.m.
Technical Safety & Engineering	3–5 p.m.
Chromatography	4–5 p.m.
Cellulose Yield	4–5 p.m.
Dibasic Acids	4–5 p.m.
Flavor Nomenclature	4–5 p.m.
Nutrition	5–6 p.m.
Atomic Absorption Spectroscopy	5–6 p.m.
TUESDAY, May 7	
Bleaching Methods	8–9 a.m.
Publications	8–10 a.m.
Fiber Determination	9–10 a.m.
Seed and Meal Analysis	10–11 a.m.
1985 World Conference Steering	10 a.m.–noon
Public Relations	11 a.m.–noon
Surfactants & Detergents Steering	Noon–2 p.m.
Membership Admission	1–2 p.m.
Approved Chemists & Certified Laboratory Program	1–3 p.m.
World Conference Planning	2–3 p.m.
Awards Administration	3–4 p.m.
Environmental	3–4 p.m.
National Program Planning	3–5 p.m.
Honored Student	4–5 p.m.
Investment	4–5 p.m.
Smalley	4–6 p.m.
Finance	5–6 p.m.
WEDNESDAY, May 8	
Commercial Fatty Acids	10–11 a.m.
1987 World Conference Steering	10 a.m.–noon
Mycotoxins	10 a.m.–noon
Protein & Co-Products Luncheon	Noon–2 p.m.
1986 World Conference Steering	1–3 p.m.
Uniform Methods	2–4 p.m.
Meeting Logistics	3–4 p.m.
AOCS Foundation	4–5 p.m.
Protein & Co-Products Session Chairmen	4–5 p.m.
THURSDAY, May 9	
Governing Board	9 a.m.–3 p.m.

Potts Award to Golich

Timothy Golich, graduate student in chemistry at the University of Wyoming, has been named the 1985 recipient of the Ralph Potts Memorial Fellowship Award.

The award, administered by AOCS through a program sponsored by Akzo Chemie America, recognizes the pioneering oleochemical research of the late Ralph Potts. It consists of a $1,000 honorarium and a plaque.

Golich will present a paper, "'Destructible' Surfactant-Based Vesicles for Controlled Delivery," during the AOCS annual meeting in Philadelphia. His research has involved synthesizing destructible double-chain surfactants that can be used to form pH-sensitive vesicles, which might be used for target-specific or time-released pharmaceuticals.

Golich received his bachelor's degree in chemistry from Whitman College in 1981 and expects to finish work on his doctorate in 1986.

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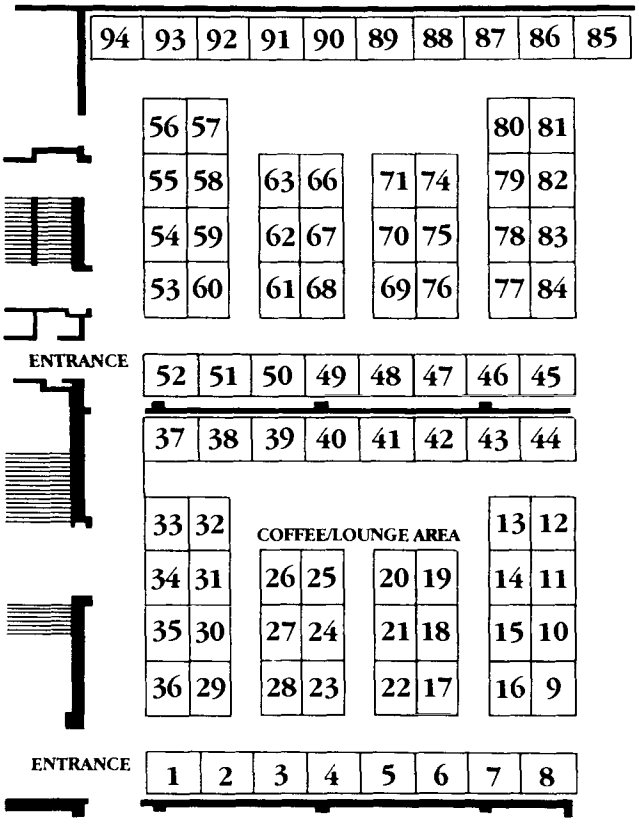
Meeting exposition

One of the largest exhibits ever held by suppliers of equipment and services to the fats and oils related industries will be held as part of the 76th annual meeting of the American Oil Chemists' Society.

The exhibit will open at noon Sunday, when the meeting registration area opens. The registration area and the exposition area both will be on the mezzanine level of the Wyndham Franklin Plaza Hotel. The exhibit also will be open Monday, Tuesday and Wednesday. It will not be open Thursday.

A list of firms that had reserved space as of mid-February follows. Persons who are unable to attend the meeting may obtain further information from exhibiting firms by writing to the addresses provided with the descriptions of individual exhibits.



ACCURATE CHEMICAL & SCIENTIFIC CORP. (Booth 25). Information will be available on reference mixtures, standards, kits for detection of lipids, glycerides, phospholipids, fatty acids, fat and oil mixtures, NIH-type mixtures, custom production of high purity lipids, fatty acids.Address: 300 Shames Dr., Westbury, NY 11590.

ALFA-LAVAL INC. (Booths 10 & 11). Alfa-Laval will highlight its high-efficiency centrifugal separators for vegetable oil refining, from Sullivan Systems. Contherm scraped-surface heat exchangers for margarine production also will be displayed. Address: 2115 Linwood Ave., P.O. Box 1316, Fort Lee, NJ 07024.

ANCO/VOTATOR DIVISION OF CHERRY-BURRELL (Booths 82 & 83). The Votator equipment lines for the fats and oils industry will be shown in photo displays. This includes oil deodorization plants along with shortening and margarine processing equipment. Address: P.O. Box 35600, Louisville, KY 40232.

ARTISAN INDUSTRIES INC. (Booth 46). The exhibit will include information on process engineering, liquid/liquid extraction, continuous filtration, washing and/or thickening, vacuum ejectors, pumps, condensers, lecithin drying, Soxhelet extractors, computer engineering-microcomputer control systems. Address: 73 Pond St., Waltham, MA 02154.

BERICO INDUSTRIES INC. (Booth 2). Information will be available on fuel-efficient Berico Soybean/Sunflower Process Driers, featuring cleaned recycle air system, insulation, tapered column design with "Turn-Flo" column mixing action for production capacity to 320 tons per hour with low noise levels in 24-hour duty cycle. Address: P.O. Box 12285, Overland Park, KS 66212.

BRINKMANN INSTRUMENTS CO. (Booth 81). Exhibited will be the Metrohm Rancimat 617, a rancidity analyzer. Address: Cantiague Road, Westbury, NY 11590.

THE CAMBRIAN ENGINEERING GROUP LIMITED (Booth 30). A model of the 'campro' horizontal deodorizer will be displayed. Specialists from Cambrian Processes, Division of The Cambrian Engineering Group Limited, will be available to explain other 'campro' deodorizer systems. Address: 2465 Cawthra Rd., Ste. 112, Mississauga, Ontario, Canada L5A 3P2.

CEM CORPORATION. (Booth 9). The exhibit will feature the CEM Moisture/Solids Analyzer AVC-80 which is designed to provide rapid, accurate determinations for all types of products. The instrument uses microwave drying and is applicable to liquids, solids and slurries covering a full range of moisture levels. The system is described as simple to operate and rapid enough to provide real time data for improved process control. Address: P.O. Box 9, Indian Trail, NC 28079.

THE CHEMITHON CORP. (Booth 84). Information will be available and photographs displayed of Chemithon's sulfonation/sulfation plants and equipment, detergent spray drying and agglomeration equipment. Production samples of products produced in Chemithon plants will be displayed. Address: 5430 W. Marginal Way S.W., Seattle, WA 98106.

CRITICAL FLUID SYSTEMS INC. (Booth 54). Critical Fluid Systems Inc. will feature pictures of its first oilseed extraction system, sold in November 1984. Representatives of the company will be available to discuss operation of the system and review with prospective customers recent pilot plant experience. Address: 25 Acorn Park, Cambridge, MA 02140.

CROWN IRON WORKS COMPANY (Booth 33). Crown Iron Works Company designs and manufactures solvent

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extraction equipment. Photos, brochures and technical literature will be displayed. Address: 1229 Tyler St. NE, P.O. Box 1364, Minneapolis, MN 55440.

DE SMET USA CORPORATION (Booths 13 & 14). The De Smet USA display will focus on energy saving in extraction and refining processes and illustrate the "MTD" deodorizing system. Staff members will be available for technical and commercial discussion. Address: 2625 Cumberland Pkwy., Suite 200, Atlanta, GA 30339.

EASTMAN CHEMICAL PRODUCTS INC. (Booth 77). Information will be available on food grade antioxidants, food grade emulsifiers and food grade chemicals. Address: 1133 Avenue of the Americas, New York, NY 10036.

EQUIPMENT ENGINEERING (Booth 5). Equipment Engineering of Indianapolis, Indiana, specializes in the repair and remanufacture of high speed and decanter centrifuges. Other services include replacement parts, custom engineered electrical control panels, outside service and purchase and trade-in of unwanted centrifuge equipment. Address: 757 E. Murry St., Indianapolis, IN 46227.

FISHER SCIENTIFIC COMPANY (Booths 23 & 24). Fisher Scientific presents a full line of food technology quality control and monitoring instruments. Everything from electronic toploading balances and sample preparation mills to extraction systems, Kjeldahl systems and a computer-aided titration system will be exhibited. Address: 711 Forbes Ave., Pittsburgh, PA 15219.

THE FOXBORO COMPANY (Booths 85, 86 & 87). The Foxboro Company will demonstrate its new Exact Controller, the first packaged application of artificial intelligence for the process industries; the new model 75 FlowExpert Totalizer/Batcher, for use in single-loop systems where accuracy is critical, and the 99UC Unit Controller, a self-contained controller for small applications. Address: Bristol Park (52-1) Dept. 120, Foxboro, MA 02035.

THE FRENCH OIL MILL MACHINERY CO. (Booths 88 & 89). French will display model machinery to illustrate its extractor with a new patented rotating extractor bottom, as well as models of current pre-presses and rolls. French designs and manufactures a full line of oil mill equipment including cracking, flaking and crushing rolls, cookers, conditioners, DTs, DT/DCs, DCs, screw presses for full pressing, and pre-pressing and solvent extraction plants. Brochures will be available. Address: P.O. Box 920, 1035 W. Greene St., Piqua, OH 45356.

GROEN DIVISION/DOVER CORP. (Booth 61). Literature and photographs describing Groen's new chilling and plasticizing system for lard, shortening, margarine and peanut butter will be displayed. The system is designed as a modern, more efficient refrigeration system requiring lower capital investment, and reducing operating energy costs by as much as 80% over conventional processing methods. Address: 1900 Pratt Blvd., Elk Grove Village, IL 60007.

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HALDOR TOPSØE, INC. (Booth 68). Information will be available on the firm's hydrogen production plant automated by catalytic decomposition of methanol and on the natural fatty alcohol process. Address: 17629 El Camino Real, Suite 302, Houston, TX 77058.

HARSHAW/FILTROL PARTNERSHIP (Booths 43 & 44). The Harshaw/Filtrol booth will describe a broad line of Filtrol clay adsorbents and Harshaw hydrogenation catalysts used in refining and processing edible and inedible oils. Technical representatives will be available to discuss specific products and applications. Address: 30100 Chagrin Blvd., Cleveland, OH 44124.

HERZOG-HART CORP., PROCESS TECHNOLOGY DIVISION (Booth 76). Information on the Buss Loop Reactor System with heat recuperation will be exhibited. Address: 462 Boylston Street, Boston, MA 02116.

BC HOESCH INDUSTRIES, INC. (Booth 16). The exhibit will feature brochures and a pilot filter press. Address: P.O. Box 461, Wharton, NJ 07885.

IDREX, INC. The Idrex booth will feature state-of-the-art, fully automated (computerized) filtration systems. Address: 1018 Lambrecht Rd., Frankfort, IL 60423.

INDUSTRIAL FILTER & PUMP MFG. CO. (Booths 39 & 40). IF&P will display an array of filtration equipment used in vegetable oil refining, particularly crude oil, bleaching, hydrogenation, winterizing and polishing. Address: 5900 Ogden Ave., Cicero, IL 60650.

JOJOBA GROWERS ASSOCIATION (Booth 74). The Jojoba Growers Association exhibit will feature photos, materials and printed handouts to better acquaint the conference attendee with jojoba oil, or liquid wax. Jojoba oil, seed, meal, wax and plants will be on display. Address: 3420 E. Shea Blvd. #125, Phoenix, AZ 85028.

L.A. SALOMON & BRO. (Booth 1). Tonsil Activated Clays and Norit Activated Carbons for processing of edible and inedible fats, oils and related products will be featured. Information and literature will be available regarding efficient use of these products. Address: P.O. Box 828, Port Washington, NY 11050.

LIBRA LABORATORIES INC. (Booth 17). Mike Blumenthal will be available to answer questions concerning computerization for chemists. Address: 495 Main St., Metuchen, NJ 08840.

MARCEL DEKKER, INC. (Booth 94). Marcel Dekker, Inc. will exhibit books and journals of interest to oil chemists including: Lewin/Preston: **High Technology Fibers: Part A**; Gruenwedel/Whitaker: **Food Analysis: Principals and Techniques, Vol. 3**; Coates/Setti: **Oils, Lubricants & Petroleum Products**, and Rieger: **Surfactants In Cosmetics**. A 20% discount will be offered on books at the booth. Address: 270 Madison Ave., New York, NY 10016.

MASCHINENFABRIK REINARTZ GmbH & CO. KG (Booths 47 & 48). This exhibit will include detailed documentation on Reinartz screw presses and related equipment (capacity range from 70 kg/hr to 150 T/day) for the mechanical processing of oilseeds. Address: Industriestrasse 14, P.O. Box 137, D-4040 Neuss 1, Federal Republic of Germany.

METTLER INSTRUMENT CORP. (Booth 53). Information will be available on TA3000 Thermal Analysis System, FP800 Thermosystem for melting point, dropping point and softening point; DL40RC MemoTitrator for general purpose titrations; DL18 Karl Fischer Titrator; DL20 CompactTitrator; DMA46 Density Meter; DPR2000 Process Control Density Meter, and DPR-S density meter interface. Address: P.O. Box 71, Hightstown, NJ 08520.

MILES LABORATORIES INC. - BIOTECH PRODUCTS DIVISION (Booth 78). The Miles exhibit will feature a new detergent enzyme product line in addition to the Miles line of industrial enzymes and acidulants for the detergent, food and edible oils industries. Product Literature will be available and technical representatives will be on hand to discuss specific products and applications. Address: P.O. Box 932, 1127 Myrtle St., Elkhart, IN 46515-0932.

MILTON ROY COMPANY (Booths 27 & 28). Milton Roy will exhibit a low cost supercritical fluid screening system containing the Critical Extraction Monitor™ for in-line solute load detection. In addition, Milton Roy's pilot and production scale systems for dense gas extraction will be featured. Address: 201 Ivyland Rd., Ivyland, PA 18974.

MIROIL/CALVERT CORPORATION (Booth 18). This exhibit will feature new field-rugged, quick tests for FFA, OFA and ACM. Information also will be available on catalytic adsorptive filter powder for used frying oils, and perlitic filter aids for all applications. Address: P.O. Box 298, Allentown, PA 18105.

NATIONAL SUNFLOWER ASSOCIATION (Booth 6). The National Sunflower Association is a trade association comprised of sunflower growers, processors, refiners and researchers. NSA's purposes include promotion, education and both export and domestic market development. Address: P.O. Box 2533, Bismarck, ND 58502.

N. HUNT MOORE & ASSOC. INC. (Booths 20 & 21). This exhibit features information on the Escher Wyss energy-efficient soybean drying, conditioning and dehulling systems; Champion soybean meal screening and grinding equipment; steam-saving and distillation equipment, and mineral oil absorption systems. Address: P.O. Box 18599, Memphis, TN 38181-0599.

NEUMUNZ INC. (Booth 32). This exhibit will feature bulletins, flowsheets and pictures of installations of Neumunz vegetable oil and food processing plants. Latest developments in critical fluids and pressure extraction also will be available. Address: 117 Fort Lee Rd., P.O. Box 827, Leonia, NJ 07605.

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NEWMAN-HOWELLS ASSOCIATES LIMITED (Booth 42). The products which will be exhibited are: (1) Iatroscan TLC/FID MKIII Analyser, for fast analysis of lipid components in fats and oils; (2) Chrompres 25 OPLC System, Over-Pressure Layer Chromatography combining HPLC adsorbents with HPLC analytical conditions to provide ultra-rapid, superior-resolved separations in the planar mode, and (3) GasSpec Micro-GC, short packed column micro-GC system linked with simple UV and IR methods of detection. Address: Wolvesey Palace, Winchester, Hants. S023 9NB, England.

NEWPORT SCIENTIFIC INC. (Booth 69). Newport's exhibit includes the diaphragm-type compressor featuring non-contaminating compression of gases and pumping of liquids. The compressors are designed for use in all types of laboratory and industrial applications requiring low gas flows. The new Supercritical Fluid (SCF) extraction system is receiving considerable attention as a means of separation in the chemical, food, pharmaceutical and energy industries. Address: 8246-E Sandy Court, Jessup, MD 20794-0189.

NOVO LABORATORIES INC. (Booth 37 & 38). Novo's exhibit will feature enzymes for the biological catalysis of fats and oils for the production of specialty fats and oleochemicals as well as enzymes for the laundry detergent industry. Address: 59 Danbury Rd., Wilton, CT 06897.

NU-CHEK-PREP INC. (Booth 79). Fatty acid and ester homologs, glycerides (tri-, di-, mono-), acid chlorides, fatty nitriles, fatty alcohols, fatty acetates, cholesteryl esters, hydrocarbons, alkyl methane sulfonates, soaps, fatty acid anhydrides, wax esters and standard reference mixtures for both TLC and GLC will be featured. Address: P.O. Box 295, Elysian, MN 56028.

OM INGREDIENTS CO. (Booth 12). New flavors to assist food manufacturers in developing new products will be exhibited. Natural grill flavor provides food manufacturers a true grill flavor. Also featured will be a natural beef flavor. Literature, samples and specifications will be available. Address: 1910 Roth St., Madison, WI 53704.

OSMONICS INC. (Booth 31). Osmo Membrane Systems including ultrafiltration, microfiltration and reverse osmosis crossflow membrane separation systems will be featured. Information will be available on laboratory scale to full production for the separation and concentration of oils and other fluid media. Osmonics Inc. offers complete application testing service. Address: 5951 Clearwater Dr., Minnetonka, MN 55343.

PALM OIL RESEARCH INSTITUTE OF MALAYSIA (Booth 70). Wall charts illustrating palm oil characteristics, use, process flow diagrams, graphs and pictures will be displayed. Address: No. 6, Persiaran Institusi, Bandar Baru Bangi, Selangor, Malaysia.

PHOTOVAC INC. (Booth 19). Exhibit information will feature a new Photoionization Air Analyzer developed by Photovac. Known as TIP (Total Ionizables Present), this hand-held, flashlight-sized, direct reading analyzer is for measurement of airborne solvents and other photoionizable impurities. Address: Unit No. 2, 134 Doncaster Ave., Thornhill, Ontario, Canada L3T 1L3.

POS PILOT PLANT CORPORATION (Booth 45). Dedicated to the development of new and improved methods of processing agricultural raw materials, POS Pilot Plant Corp. provides services and pilot scale processing equipment on a fee-for-use basis. The facility specializes in handling cereal grains, oilseeds and legumes, but has the flexibility and creativity to handle other materials. This booth will provide information on these services. Address: 118 Veterinary Rd., Saskatoon, Sask., Canada.

PRATER INDUSTRIES INC. (Booth 60). The exhibit will feature Prater Flaking Mills, Prater Hammermills for meal and hulls, and Prater screening machines. Address: 1515 S. 55th Court, Chicago, IL 60650.

ROSKAMP MFG. INC. (Booth 57). A new model roller mill for cracking or grinding oilseeds using single, double or triple reduction will be featured. This new roller mill offers greater control over end product through precise, easy roll adjustment. Information also will be available on the Mark III 28 X 52-62 flakers, designed for easy operation with accurate, consistent flaking of oilseeds at high capacity. Address: 2975 Airline Circle, Waterloo, IA 50613.

REHEIS CHEMICAL COMPANY (Booth 29). Reheis supplies USP and FCC grade potassium chloride for use in prepared foods where "low sodium" levels are required. Also featured will be higher purity FCC grade $CaCl_2$. This product meets the new mandated specification for $CaCl_2$. Address: 235 Snyder Ave., Berkeley Heights, NJ 07922.

SIMON-ROSEDOWNS LIMITED (Booth 34). Using a modular display system of high quality graphics, this exhibit details a total design-manufacture, procurement, contracting service. Specialists will be available to discuss all aspects of oil milling, solvent extraction and edible oil refining. Address: Cannon St., Hull, North Humberside, HU2 OAD, England.

SVO ENTERPRISES CORP. (Booth 15). This booth will feature Trisun Oleic Sunflower Oil, an SVO product. Trisun is a natural unsaturated oil that offers previously unattained levels of oleic fatty acids. Booth personnel will review new improvements/applications emerging from SVO's continuing research program. Address: 29400 Lakeland Blvd., Wickliffe, OH 44092.

SYBRON LABORATORY PRODUCTS (Booth 66). Sybron will exhibit lab products including Barnstad laboratory water purification systems, Thermolyne controlled heating apparatus and Nalge plastic laboratory ware. Address: P.O. Box 557, Glenside, PA 19038.

S.A. FRACTIONNEMENT TIRTIAUX (Booth 80). This booth will feature enlarged photographs, samples, mobile display and film about the Tirtiaux process of fractionation

and winterization. Address: 701, Chaussée de Charleroi, B 6220 Fleurus, Belgium.

TECHNICON INDUSTRIAL SYSTEMS (Booth 49 & 50). Technicon will exhibit two products: the InfraAlyzer™ 400RL+ (w/ liquid drawer and H-P "85" computer) for near infrared reflectance analysis of oilseeds, meals and oils. It provides rapid analysis for quality control of crushing, extraction-refining and hydrogenation processes. Also, the newly introduced InfraAlyzer™ 250 low cost near infrared reflectance analyzer which requires minimal sample preparation—analyzing oilseeds in less than 20 seconds. Address: 511 Benedict Ave., Tarrytown, NY 10591.

TEKMAR COMPANY (Booth 26). Tekmar will exhibit its line of concentrators for dynamic headspace analysis, including single station and multi-station models. Also available are Contraves Viscometers/Rheometers for research, analytical applications and process control. Tekmar will introduce a new line of titration systems from Schott Instruments. Address: P.O. Box 371856, Cincinnati, OH 45222-1856.

THE TINTOMETER COMPANY (Booths 51 & 52). Color grading and measuring instruments for edible oils, fats and tallows including the Lovibond Tintometer, FAC Scale, Model E Tintometer, New American Oil Tintometer and the latest digital readout Lovibond Automatic Tintometer for Lovibond and AOCS scale with options for chlorophyll and carotene including printer and computer interface will be featured. Address: 206 Packets Court, Williamsburg, VA 23185.

UNICHEMA INTERNATIONAL LTD. (Booths 7 & 8). Unichema will feature nickel catalysts including Pricat 9912, a highly active, poison-resistant catalyst for fatty acid hydrogenation, and Pricat 9908, an economical catalyst for producing hard fats with consistent steep dilitation curves. High-purity fractionated fatty acids from C8 to C22, specialty esters, polymerized fatty acids, isostearic and fatty acids are included in the oleochemicals manufactured by Unichema from captive feedstocks. Address: 1 Sears Dr., Paramus, NJ 07652.

UOP PROCESS DIVISION, BIOLOGICAL & FOOD PRODUCTS (Booth 22). UOP will display its Sustane line of food-grade antioxidants, including BHA, BHT, TBHQ, propyl gallate and a variety of liquid antioxidant blends. Color product brochures, specification sheets and other technical literature will be available. Sample requests will be accepted. Address: 20 UOP Plaza, Des Plaines, IL 60016.

U.S.O.P. LTD./H.L.S. INDUSTRIAL ENGINEERING CO. LTD. (Booths 3 & 4). The exhibit will feature flow-sheets

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on vegetable oil processing—solvent extraction, flash desolventizing, physical refining including wet degumming, bleaching and deacidification/deodorization, a video movie on company activities, and catalogues and brochures. Address: 4 Weizman St., Tel-Aviv, Israel.

VARIAN INSTRUMENT GROUP (Booth 90). Varian will exhibit its Vista series of gas chromatographs and liquid chromatographs and new data processing equipment. Address: 25 Hanover Rd., Florham Park, NJ 07932.

WURSTER & SANGER INC. (Booth 41). Catalogs, technical literature, plant photographs and process flowsheets will be available reflecting the complete capabilities of Wurster & Sanger as process engineers for the fats and oils industry. W & S custom builds plants for oilseeds, glyceride fats and oils, fatty acids, glycerine and their by-products. Samples of products from W & S processes will be displayed. Address: 222 W. Adams St., Suite 1500, Chicago, IL 60606.

AOCS BOOK EXHIBIT (Booths 92 & 93). Display of fats and oils-related publications by various publishers, including the AOCS. All publications on display are property of the AOCS and are for display only. They may not be taken from the booth. Lists of displayed books, with prices, will be available at the booth. Several publishers offer discounts for purchases, see order list for details.

Book exhibit

The following publications will be displayed in the AOCS Book Exhibit during the annual meeting in Philadelphia. The list includes books received through mid-February. Order sheets will be available at the book exhibit which registrants may use to order books from the publishers. Persons not attending the meeting who wish to order books should contact the original publishers, not AOCS.

Academic Press Inc., Publishers

Lipids in Cereal Technology, 1984, by Barnes.
Instrumental Analysis of Foods, Vol. 1, 1983, by Charalambous.
Instrumental Analysis of Foods, Vol. 2, 1984, by Charalambous.
Analysis of Food and Beverage, 1984, by Charalambous.
Ether Lipids, 1983, by Mangold.
Food Protein Chemistry, 1984, by Regenstein.
Starch, 2nd edition, 1984, by Whistler.

Send inquiries to: Academic Press Inc., Publishers, Sea World Dr., Orlando, FL 32887.

Alan R. Liss, Inc.

Animal and Vegetable Proteins in Lipid Metabolism and Atherosclerosis, 1983, edited by Michael J. Gibney and

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David Kritchevsky, order #1607, $32.

Cell Function and Differentiation, Part A.: Erythroid Differentiation, Hormone-Gene Interaction, Clycoconjugates, Liposomes, Cell Growth, and Cell-Cell Interactions, 1982, edited by G. Akoyunoglou, A.E. Evangelopoulos, J. Georgatsos, G. Palaiologos, A. Trakatellis, and C.P. Tsiganos, order #0165, $60.

Cell Function and Differentiation, Part B.: Biogenesis of Energy Transducing Membranes and Membrane and Protein Energetics, 1982, edited by G. Akoyunoglou, A.E. Evangelopoulos, J. Georgatsos, G. Palaiologos, A. Trakatellis, and C.P. Tsiganos, order #0166, $60.

Cell Function and Differentiation, Part C.: Enzyme Structure-Mechanism, Metabolic Regulations, and Phosphorylation-Dephosphorylation Process, 1982, edited by G. Akoyunoglou, A.E. Evangelopoulos, J. Georgatsos, G. Palaiologos, A. Trakatellis, and C.P. Tsiganos, order #0167, $42.

Lipid Research Methodology, 1984, edited by Jon A. Story, order #1969, $48.

The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects, 1984, edited by Gene A. Spiller, order #5008, $78.

Membranes, Detergents, and Receptor Solubilization, Receptor Biochemistry and Methodology, Vol. 1, 1984, edited by J. Craig Venter and Len C. Harrison, order #3700, $46.

Molecular and Chemical Characterization of Membrane Receptors, Receptor Biochemistry and Methodology, Vol. 3, 1984, edited by J. Craig Venter and Len C. Harrison, order #3702, $52.

Monoclonal and Anti-Idiotypic Antibodies: Probes for Receptor Structure and Function, Receptor Biochemistry and Methodology, Vol. 4, edited by J. Craig Venter, Claire M. Fraser and Jon Lindstrom, order #3703, $46.

New Trends in Nutrition, Lipid Research and Cardiovascular Diseases, 1981, edited by Nicolas G. Bazan, Rodolfo Paoletti and James M. Iacono, order #1604, $30.

Receptor Purification Procedures, Receptor Biochemistry and Methodology, Vol. 2, 1984, edited by J. Craig Venter and Len C. Harrison, order #3702, $34.

All orders addressed to the following and mentioning this meeting will receive a 20% discount. Karen Anspach, Exhibit Manager, Alan R. Liss, Inc., 41 E. 11 St., New York, NY 10003.

American Association of Cereal Chemists

Advances in Cereal Science and Technology, Vol. 8, 1985, edited by Yeshajahu Pomeranz, $60, 362 pp., hardcover, illustrated.

Insect Management for Food Storage and Processing, 1984, edited by Fred J. Bauer, $65, 384 pp., hardcover, illustrated.

Moisture Sorption: Practical Aspects of Isotherm Measurement and Use, 1984, edited by Theodore P. Labuza, $28.50, 150 pp., spiral bound, illustrated.

Send orders and inquiries to: APS Books, 3340 Pilot Knob Rd., St. Paul, MN 55121. Call toll-free in the U.S.: 1-800-328-7560 (except in Minnesota).

McCutcheons Publications

Emulsifiers and Detergents, North American Edition, 1984, $45. Over 4000 surfactant materials are described by trade name, manufacturer, identity, concentration, type, HLB number and application. In addition to emulsifier and detergents, there are sections on food emulsifiers, formulated detergent concentrated bases, intermediates and textile surfactants.

Functional Materials, North American Edition, 1984, $45. This directory lists information on products and suppliers for materials often used in conjunction with surfactants in formulating finished goods.

Emulsifiers and Detergents—Functional Materials, International Edition, 1984, $45. This two-part international volume contains the same type of material as the North American editions for products manufactured outside the United States and Canada.

Send inquiries to: Judy Calabro, McCutcheons Publications, 175 Rock Rd., Glen Rock, NJ 07452.

National Academy Press

Prudent Practices for Disposal of Chemicals from Laboratories, 1983, by the National Research Council, ISBN

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0-309-03390-X, $16.50.

Prudent Practices for Handling Hazardous Chemicals in Laboratories, 1981, by the National Research Council, ISBN 0-309-03128-1, $16.95.

Send inquiries to: National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20418.

Plenum Publishing Corp.

Advance in Nutritional Research, Vol. 6, 1984, edited by Draper, $49.50.

Cell Membranes: Methods and Reviews, Vol. 2, 1984, edited by Elson et al., $52.50.

Myocardial Ischemia and Lipid Metabolism, 1985, edited by Ferrari et al., $49.50.

Lipid Metabolism and its Pathology, 1985, edited by Halpern, $49.50.

Handbook of Lipid Research, Vol. 3: Sphingolipid Biochemistry, 1983, edited by Kanfer/Hakomori, $59.50.

Membrane Fluidity (Biomembranes, Vol. 12), 1984, edited by Kates/Manson, $85.00.

Drugs Affecting Lipid Metabolism 8 (Advances in Experimental Medicine and Biology, Vol. 183), 1985, edited by Kritchevsky et al., $72.50.

Send inquiries to: Plenum Publishing Corp., 233 Spring St., New York, NY 10013.

13th Lipidforum

The 13th Scandinavian Symposium on Lipids will be held June 30 through July 3 in Reykjavik, Iceland. The organizing committee is headed by Sigmundur Gudbjarnason.

Invited plenary lecturers include J.L. Beare-Rogers, H. Sprecher, R.G. Ackman, C. Ratledge, H.B.W. Patterson and O. Korber. The conference language, in general, will be English.

For further information on program registration, housing and travel, contact: Prof. S. Gudbjarnason, University of Iceland, Dunhagh 3, Reykjavik, Iceland (telex ISINFO 2307) or Prof. R. Marcuse, Lipidforum, c/o SIK, Box 5401, S-402 29 Göteborg, Sweden (telex 21651 SIK).

Lipidoxidation symposium

The Scandinavian Forum for Lipid Research and Technology (Lipidforum) and the Swedish Food Institute will hold a symposium on lipidoxidation April 22-23 in Göteborg, Sweden. Biological and food chemicals aspects will be covered in the 24 presentations, which will be divided into three sections: mechanism, effects and analysis, and protection. Further information is available from Reinhard Marcuse, assistant professor, Lipidforum c/o SIK, Box 5401, S-402 29 Göteborg, Sweden (telex 21651 SIK).

Meetings

Technical Paper Abstracts

Session A — Recent Trends in the Usage of Fats and Oils in the Baking Industry
Monday morning

1

FATS AND SURFACTANTS IN EVALUATION OF HARD RED WINTER WHEATS IN PLANT BREEDING PROGRAMS. O.K. Chung, M.D. Shogren and Y. Pomeranz, U.S. Grain Marketing Research Laboratory, 1515 College Ave., Manhattan, KS 66502.

Recent trends in usage of fat in the breadmaking industries include significant replacement of lard and shortening by pumpable vegetable oils in combination with surfactants as dough strengtheners and/or conditioners. At the Hard Wheat Quality Laboratory of the U.S. Grain Marketing Research Laboratory, shortening (3% flour weight) has been used for several decades as one of the ingredients in the evaluation of baking quality of wheats in plant breeding programs. This study was to investigate the use of combinations of oils and surfactants in evaluating wheats in plant breeding programs. Cold pressed soy oil and sunflower oil at the 2% (flour weight) level, each with 0.2% sodium stearoyl-2-lactylate (SSL) or diacetyl tartaric acid esters of monoglycerides and diglycerides (DATE), replaced the conventional 3% shortening in formulations. The study was conducted on 18 wheat flours (protein content from 10.5 to 16.9%) that varied in mixing time from 1 5/8 to 6 7/8 min and loaf volume (100 g flour) from 710 to 1295 cc when 3% shortening was used. The 2% oil plus 0.2% surfactant combinations yielded comparable mixing times. Sunflower or soy oil with SSL or DATE produced bread with loaf volumes greater than those with 3% shortening except for one variety (Pro Brand 835) grown in Belleville, KS. In addition, 1% oil plus 0.3% surfactant combinations also were tested: except for the Pro Brand 835 variety, this combination yielded loaf volumes that were equal to, or slightly greater than, those with 3% shortening. Thus, either 2% oil plus 0.2% surfactant or 1% oil plus 0.3% surfactant can be used instead of 3% shortening in evaluating wheats in plant breeding programs. Differentiation and ranking of breadmaking potential of the wheat varieties by both formulations were similar. Replacement of shortening by oil plus surfactant combinations has the advantages of easier handling and of employing in test baking formulations that are comparable to those used in the baking industry.

2

SOME FACTORS RELATING FATS TO BREAD FIRMING. Joseph G. Ponte Jr., Kansas State University, Dept. of Grain Science, 210 Shellenberger Hall, Manhattan, KS 66506.

White pan bread remains the most important single bakery food produced in the U.S. One of the commercially significant quality attributes of this product is the softness of the crumb. Certainly the usage of fats in bread formulations is primarily related to efforts to inhibit bread firming. Over the years considerable work has been done to identify factors relating fat (and surfactant) usage to bread firming, but much remains unclear. Types of fats used by the baking industry have changed: from a widespread use of lard, the industry has gone to various plastic vegetable shortenings, fluid shortenings and now to oils, in conjunction with certain surfactants. Fat levels in bread have declined in recent years. The present paper will discuss these changes in fat usage to produce white pan bread and will consider relationships among types of fats, bread storage temperature, effects on overall bread properties, and how these influence bread firming.

3

PROPERTIES AND FUNCTIONALITY OF FATS IN SWEET GOODS AND CRACKERS. Clemence Kumah Dartey, Nabisco Brands, Inc., Fair Lawn Technology Center, 2111 Route 208, Fair Lawn, NJ 07410.

Fats are important and play major functions in the formulation and manufacture of many bakery products. In the baking industry, fats are primarily of vegetable oil origin. However, animal fats such as lard, tallow and butter also are used in certain applications. Fats used in baking may be plastic shortenings, fluid shortenings or liquid oils. Surfactants may be added to these fats during processing to extend or modify their functional properties. Many factors are considered in selection of shortening types for various baked goods. These include the physical and chemical properties of fat, their inherent functional characteristics, type of baked products, price and handling qualities of fat. The nutritional or health benefits also are increasingly taken into consideration. A general overview is presented on the physical, chemical and functional properties of fats or shortenings used in production of various baked goods. Fat requirements, their selection criteria and the characteristics of baked goods are discussed.

4

FUNCTIONAL EFFECTS OF DOUGH CONDITIONERS ON CHANGING OIL REQUIREMENTS IN BAKING INDUSTRY. John L. Van Haften, R.J. Tenney and V.D. Berry, C.J. Patterson Company, 3947 Broadway, Kansas City, MO 64111.

Shortening systems used in the wholesale bread baking industry have changed in the past decade from predominantly animal based products to liquid, vegetable oil based products. Shortening use levels also have trended downward. This paper examines the role of surfactants in this transition. The effect on bread quality, volume response and firming for the most commonly used dough strengtheners and softeners is compared in various shortening systems. Relative effects at different levels also are compared. Also examined are recent trends in compositional changes of bread softeners. Relative effects of monoglycerides from partially hydrogenated base oils versus fully hydrogenated oils are compared.

5

FATS AND SURFACTANTS USED IN THE EUROPEAN BAKING INDUSTRY. T. Jónsson and N. Krog, Grindsted Products A/S, Edwin Rahrs Vej 38, DK-8220 Brabrand, Denmark.

Types of bread in the European countries vary considerably from country to country, and the single varieties of yeast-raised bakery goods within countries, i.e. Germany, exceed 200 types. The majority of the daily bread contains no fat at all. Exceptions are white pan bread produced by the Chorleywood process which contains 0.7-1% fat and the so-called toast-bread (sandwich bread) which may contain up to 5% lard or shortening. Certain bun varieties, tea breads, danish pastry and specialty goods like brioche, croissants, stollen or panetone contain high levels of fats (20-66% based on flour weight) mainly in the form of butter or margarine. Fat containing cakes or cookies are predominantly made with margarine or butter rather than shortenings, which are used only in the United Kingdom. Surfactants as dough strengtheners or crumb softeners are used in white pan bread in all countries except France, Italy and Switzerland, where surfactants are permitted only in specialty breads, rolls and buns. For production of specialty breads, rolls or buns it is common practice in the European countries to use so-called improvers (Backmittel) which are well balanced compositions of surfactants, oxidation/reduction agents, enzymes, yeast food and other minor ingredients. Diacetyl tartaric acid esters of monoglycerides (DATEM) are the most important dough conditioners, while stearoyl-lactylates have a limited use in Europe. Monoglycerides are used widely as crumb softeners and antistaling agents in wheat bread. Distilled monoglycerides, lactic acid esters of monoglycerides, polyglycerol esters or propylene glycol esters are used in chemically leavened cakes in the form of powdered cake improvers.

6

FATS AND OILS: NUTRITIONAL DIMENSIONS. J. Edward Hunter, Procter & Gamble Co., 6071 Center Hill Rd., Cincinnati, OH 45224.

This talk will cover nutritional issues of current interest in relation to dietary fats and oils. I will review first two areas of current controversy with respect to dietary fat, namely, how fat may be associated with the development of heart disease and cancer. There is still debate among health authorities as to what, if any, specific dietary recommendations are appropriate for healthy people. Second, I will discuss two recently published studies which add new perspective to the proposed relationship between dietary fat and heart disease or cancer. A study by Mattson and Grundy has suggested that oleic acid may be as useful as linoleic acid in a cholesterol-lowering diet. Work by Kritchevsky et al. has indicated that total calories consumed may be of greater importance than total fat consumed in affecting the ability of a diet to promote tumor development. Finally, despite recommendations by several health advisory organizations that we decrease fat consumption for health reasons, disappearance and sales data indicate that our total consumption of fat actually has not decreased during the past 20 years. The decreasing age-adjusted mortality rate for heart disease and the relatively constant or slightly declining mortality rates for breast cancer and colon cancer during this period cannot be explained satisfactorily by changes in total fat consumption alone.

SESSION B High Performance Liquid Chromatography in the Analysis of Lipids I Monday morning

7

FRACTIONATION AND ANALYSIS OF LIPID CLASSES, FATS, AND OILS BY HPLC VIA A FLAME IONIZATION DETECTOR. O.S. Privett, W.L. Erdahl and F.C. Phillips, Hormel Institute, University of Minnesota, 801 16th Ave. N.E., Austin, MN 55912.

The triglyceride species composition of a number of common animal fats, vegetable and fish oils was determined by reversed-phase octadecylsilane chromatography by means of the flame ionization detector developed in this laboratory. Identification of triglycerides was made by determining all species that theoretically could be present in the sample and their order of elution by a computer program based on fatty acid composition and calculated, as well as experimental, theoretical carbon numbers (TCN). The peaks in the chromatogram are identified by matching them with TCN values relative to their abundance and from known species as markers. Quantitative analysis was made on the basis of the proportionality of peak areas. The general techniques were applied to the preparation of widely different fractions of triglycerides as well as the lipid classes, which also were determined quantitatively, with columns of different sizes and packings in experiments on the translation of analytical separations to a preparative scale.

8

HPLC OF TRIGLYCERIDES USING GRADIENT ELUTION. B. Hersloef, G. Kindmark and C. Thoerngren, KabiVitrum AB, P.O. Box 12170, S-102 24 Stockholm, Sweden.

So far most HPLC separations of triglycerides described in the literature have been based on isocratic elution. It is obvious that in many cases gradient elution would facilitate the procedure and allow different vegetable oils to be analyzed by a defined one-step method. This has been very difficult due to the absence of a commercially available universal detector for this purpose. However, the use of a so-called mass detector made it possible for us to investigate gradient elution systems and compare with isocratic systems. The selectivity of different solvent systems will be discussed together with possible advantages of ternary mixtures. The influence of stationary phases from different sources on the separation have been investigated and also will be discussed.

9

REVERSE PHASE HPLC OF PHOSPHOLIPIDS. N. Sotirhos, B. Hersloef and C. Thoerngren, KabiVitrum AB, P.O. Box 12170, S-102 24 Stockholm, Sweden.

The analysis of fatty acyl components in phospholipids is a tedious, time consuming process. Hydrolytic degradation followed by gas chromatographic analysis provides information about total fatty acid distribution. Selective partial hydrolysis with phospholipases determined the fatty acid distribution at positions sn-1 and sn-2 of the phospholipid molecule. However, information about fatty acid pairing in individual molecular species is not obtained. A high performance liquid chromatographic (HPLC) method was developed to analyze molecular species of phospholipids. The separation was obtained by isocratic elution on a reverse phase C-18 column, and a mobile phase consisting of methanol, acetonitrile and water. UV absorbance at 214 nm was used to detect the phospholipids. A light scattering mass detector also was utilized to obtain quantitative information. The separation of molecular species of several phospholipids such as phosphatidylcholine and phosphatidylethanolamine from eggs and soybeans will be discussed. The proposed method is sensitive enough to permit analysis of phospholipids from many biological sources.

10

HPLC OF TRIGLYCERIDES SYSTEM AND SOLVENT EFFECTS. E.G. Perkins and David Hendren, Burnsides Research Laboratory, University of Illinois, 1208 W. Pennsylvania Ave., Urbana, IL 61801.

The separation of natural triglyceride mixtures by high performance liquid chromatography is well established. Common systems include either adsorption or reverse phase system employing octadecyl bonded small particle size packed columns and either isocratic or gradient elution with organic mobile phases. It is now possible to separate critical pairs of triglycerides on efficient bonded phase columns and obtain a profile of the triglyceride composition of a natural fat within 30 min. However, for efficient use of instrument time it is useful to carry out such separations in even shorter times, and if possible with simpler isocratic solvent systems. This would be an advantage since there would be no equilibration of the column required as in the case of gradient work. In the present studies attention was given to system details such as flow rates, sample size, concentration of injected sample and size of displacement loop used in the injector. Solvent effects concern resolution and analysis time. Furthermore, the solubility of more saturated triglycerides, such as those containing palmitic and stearic acid, must be considered in the choice of mobile phases. Lack of solubility of certain triglycerides in mobile phases can interfere with resolution and recovery of components as well as contribution to column and system deterioration. Many combinations of simple solvents such as acetone, and varying amounts of acetonitrile, methylene chloride or ethyl acetate yield adequate resolution of critical pairs of triglycerides. However, in these studies, acetone used alone as a mobile phase was found to be as effective as previously reported systems in producing adequate separation of natural triglyceride mixtures for quantitation and 'profiling' purposes. Furthermore, the analysis time has been reduced from approximately 30 min to 12 min.

11

HIGH PERFORMANCE GEL PERMEATION CHROMATOGRAPHY OF METHYL ESTERS, MONO-, DI- AND TRIGLYCERIDES MIXTURES. Constantina N. Christopoulou and E.G. Perkins, 205 Burnsides Research Laboratory, University of Illinois, 1208 W. Pennsylvania Ave., Urbana, IL 61801.

Separations of natural and synthetic mixtures of methyl esters, mono-, di- and triglycerides have been obtained by high performance liquid chromatography in both the normal and reverse phase mode. In general, silica as well as octadecyl, amino and cyano bonded columns and non-aqueous gradient elution systems with special types of detectors have been employed. Since the development of high resolution gel permeation chromatographic columns the separation of low molecular weight compounds with relatively small differences of molecular weight has been made possible in a simple and rapid way. In the present study, the application of high resolution gel permeation chromatography to the separation of methyl esters, mono-, di- and triglycerides is reported. Analysis was carried out on 5 μm particle diameter cross-linked styrene-divinylbenzene columns with either tetrahydrofuran or toluene as eluting solvent. Refractometry was the mode of detection. The elution pattern of methyl esters, mono-, di- and triglycerides was determined and base-

line separations of standard mixtures were obtained. Finally the method was applied for the separation of commercial mixtures of mono-, di- and triglycerides as well as those resulting from the lipolysis of various oils by the use of pancreatic lipase.

SESSION C Jojoba
Monday morning

12

THE STATUS OF JOJOBA AS A COMMERCIAL CROP. C.A. Whittaker, The Jojoba Growers Association, 3420 E. Shea Blvd. #125, Phoenix, AZ 85028.

The structure and properties of jojoba oil, a liquid wax ester, and its derivatives have suggested potential uses as an industrial material particularly in the lubricant, cosmetic and pharmaceutical industries. In addition, recent research indicates that jojoba oil may be useful as a low calorie food for human and animal consumption. A very small market of 100 to 200 tons per year of jojoba oil has developed in recent years, primarily among small cosmetic manufacturers, based upon the limited supply available from harvesting native stands of jojoba in the Sonoran Desert. In the period 1978-1984, approximately 40,000 acres of jojoba have been established and are beginning to produce yields on a commercial scale. Harvesting equipment designed for use on grapes, blueberries and raspberries is being used on jojoba while new harvesters are being developed. Several small mechanical extraction facilities process jojoba seed from both native and domesticated crops. The availability of jojoba oil is expected to increase significantly and the cost of production to decrease as existing plantations mature and solvent extraction facilities become available. Advances in agronomy and the selection of higher yielding cultivars will further increase the availability of oil and decrease the cost of production in the long term. Significant research has been completed on the basic chemistry of jojoba oil and its derivatives. However, more product formulation and testing will likely be required before jojoba products can be manufactured and marketed on a large scale.

13

DETERMINATION OF ACID VALUE, PEROXIDE VALUE, COLOR AND PURITY OF JOJOBA OIL. Ralph L. Price, Department of Nutrition and Food Science, University of Arizona, 309 Agricultural Sciences Building, Tucson, AZ 85721 and Hal C. Purcell, The Jojoba Growers Association, Phoenix, AZ.

The AOCS Official Methods CD 3A-63 and 28.022-28.023 for acid value and peroxide value, respectively, the Gardner method for color, and a thin-layer chromatographic method for purity of jojoba oil were collaboratively studied by 10 laboratories. The eight samples analyzed consisted of pressed jojoba of varying sources which had been spiked with 0-5% corn oil. Four analyses of each sample were performed by each method. This collaborative study tested in-laboratory and between-laboratory variation of analyses of jojoba using methods previously accepted for vegetable oils.

14

ORGANIC REACTIONS OF JOJOBA OIL: I. TRANSESTERIFICATION; II. PYROLYSIS. P.S. Landis, Chemistry Department, Glassboro State College, Glassboro, NH 08028.

Esterification of jojoba-derived fatty acids or transesterification of jojoba oil directly with neopentyl polyols including pentaerythritol, neopentyl glycol and trimethylolpropane provides esters with high viscosities and excellent lubricity characteristics. Catalytic pyrolysis of jojoba over a zeolite catalyst produces olefins boiling in the gasoline range. Slow thermal pyrolysis at 425 C under nitrogen or pyrolysis over non-acidic alumina produces polyunsaturated hydrocarbons and acids typical of a concerted beta elimination reaction. Gas chromatography combined with high molecular weight mass spectrometry provides a useful, rapid technique for identifying component mixtures in jojoba oil, the ester mixtures and the pyrolysis products.

15

EFFECTS OF FEEDING JOJOBA WAXES TO RATS. A 90-DAY STUDY. R. Stalder, M. Marchesini and A. Bexter, Nestec Ltd., Research Department, CH-1800 Vevey, Switzerland.

The liquid waxes of jojoba *(Simmondsia sinensis)* are a mixture of esters of long-chain fatty acids and long-chain primary fatty alcohols. Jojoba waxes are only partially digested in the gastrointestinal tract. Therefore, there is some interest in their use as a low-calorie fat in human nutrition. The aim of the present feeding study was to evaluate the biological effects of jojoba waxes when fed to rats over a 3-mo period, incorporated into a basal diet at 2.5, 5.0 and 10.0% (w/w) levels. In female rats, body weight gain was lowered by the waxes, while increased transaminase and alkaline phosphatase activities were found in the plasma of both sexes at 4 and 13 weeks of the experiment. These effects can be attributed to the waxes themselves and not to the presence of impurities such as nitrogen-containing substances in the oil. No pathological observations were found in the livers of the experimental animals.

16

MITOCHONDRIAL Ca^{2+}-TRANSPORT KINETICS IN LIVERS OF RATS FED JOJOBA WAXES. R. Guidoux and K. Anantharaman, Nestec Ltd., Research Department, CH-1800 Vevey, Switzerland.

Changes in the fatty acid composition of dietary lipids are known to modify the structure of membrane phospholipids, which in turn may influence membrane transport processes. We investigated the influence of dietary jojoba *(Simmondsia sinensis)* waxes on Ca^{2+} fluxes, into and from liver mitochondria. Three groups of young, male rats were fed 2.5, 5 or 10% (w/w) jojoba waxes, mixed in a standard laboratory diet. Two groups of control animals received the same standard diet, either alone or supplemented with a mixture of 5% paraffin oil and 5% corn oil. Livers from 3 to 4 rats of each group were removed after 7-13 weeks of feeding. Mitochondria were prepared and incubated (about 1 mg protein/ml) at 25 C in a medium containing 120 mM KCl, 20 mM HEPES (pH, 7.4), 10 μM Ca-EDTA, 5 mM succinate, 2 μM rotenone, 2 mM Pi, 4 mM $MgCl_2$, 3 mM ATP and 25 μM oligomycin. Rates of Ca^{2+} efflux from mitochondria (through the ruthenium red-insensitive pathway) and Ca^{2+} influx into mitochondria were evaluated at different and steady extramitochondrial pCa values (from 4.6 to 5.8), which were measured with a Ca^{2+}-selective electrode. Similar data were obtained in animals from the two control groups, with Ca^{2+} efflux rates ranging from 0.9 to 2.0 μg-ion/g protein min, and maximal rates of Ca^{2+} influx ranging from 540 to 690 μg-ion g/protein min. Measurements in livers from jojoba-fed rats did not differ detectably from control values. Rates of Ca^{2+}-transport were, thus, unrelated to either the duration of jojoba feeding or the level of jojoba waxes in the diet.

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COSMETICS BASED ON JOJOBA OIL: EMULSION CHARACTERISTICS. H. Libby, S. Libby, R.L. Realina and F. Tayag, Libby Laboratories Inc., 1700 Sixth St., Berkeley, CA 94710.

Initial study on methodical evaluation of the physical characteristics of jojoba oil in cosmetic products indicates very favorable oxidative stability compared to mineral oil and high oleic triglycerides. The objective of the continued study is a comparison of jojoba oil again with mineral oil and triglycerides as the non-polar phase in various emulsion systems. Formulations including 7.5%, 15% and 30% non-polar phase were evaluated. Data is presented to compare relative emulsion stability and aesthetic characteristics. A general conclusion of this study indicates a valid rational basis for use of jojoba oil in cosmetic emulsion systems with particular reference to physical parameters.

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NATURE OF THE STORED FAT IN RATS FED JOJOBA WAXES. K. Anantharaman, P. Reinhardt, Nestec Ltd., Research Department, CH-1800 Vevey, Switzerland.

Abstract not available at press time.

Meetings

SESSION D Specialty Lipids and Their Biofunctionality Monday morning

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MEDIUM CHAIN TRIGLYCERIDES AND STRUCTURED LIPIDS. Vigen K. Babayan, Harvard Medical School, 194 Pilgrim Rd., Boston, MA 02215.

Diet and nutrition have taken on a great deal more meaning in recent years as data on health and well being are evaluated. The treatment of the critically ill and means of alleviating their problems is being associated with nutritional support. Medium chain triglycerides and other structured lipids prepared from caprylic and capric acids and a good source of polyunsaturated fatty acids such as linoleic acid are showing promise as the next generation of specialty lipids. Not only are such structured lipids promising in the treatment of the critically ill patient, but they are indicated to be the preferred lipids in our diet for good health and nutrition. The rationale for such structured lipids is discussed and reviewed to set the stage for the speakers who will follow with specific areas of application.

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ABSORPTION OF SAFFLOWER OIL AND STRUCTURED LIPID PREPARATIONS IN PATIENTS WITH CYSTIC FIBROSIS. Van S. Hubbard, Westwood Building, Room 3A18B, National Institutes of Health, Bethesda, MD 20205, and Mary C. McKenna, Department of Pediatrics, University of Maryland School of Medicine, 655 W. Baltimore St., Baltimore, MD 21201.

Patients with cystic fibrosis (CF) and pancreatic insufficiency have changes in the fatty acid composition of their blood and tissue lipids consistent with early essential fatty acid deficiency, despite regular pancreatic enzyme supplementation. These patients usually have decreased linoleic acid, and increased oleic, palmitoleic and eicosatrienoic (20:3ω9) acids relative to normal values. The decrease in plasma and tissue linoleic acid levels generally has been attributed to the malabsorption associated with pancreatic insufficiency observed in 85-90% of CF patients. Decreased linoleic acid levels also could be due, in part, to a restriction of dietary fat intake or an increased metabolic requirement for linoleic acid in CF patients with inadequate caloric intake. Decreased caloric intake coupled with increased caloric needs in CF patients could lead to oxidation of linoleic acid to meet immediate energy needs and consequently decrease the linoleic acid available to be utilized as essential fatty acids –for elongation to arachidonic acid, prostaglandin biosynthesis, incorporation into membrane phospholipids, and other essential fatty acids functions. Thus, a dietary supplement which is calorically dense provides additional essential fatty acids and is well tolerated would be expected to be useful in the CF population. As part of a study to find such a dietary supplement as well as to determine the relative role of malabsorption as the cause of the fatty acid alterations, the increase in plasma linoleic acid was determined after ingestion of various lipid supplements. CF patients with documented pancreatic insufficiency and normal control subjects were given each of four different lipid supplements on separate days (a minimum of 3 days apart). The supplements were commercial safflower oil, Microlipid®, Captex 810B® and Captex 810D®. The linoleic acid contained in these supplements expressed as % total fatty acids present was 74%, 74%, 25% and 40% respectively. The Captex oils are experimental randomly esterified synthetic triglycerides. Fasting subjects consumed 36 g of lipid in a milkshake containing 15 g of protein and 45 g of carbohydrate. Plasma samples obtained at 0, 2, 4, 6 and 8 hr after the meal showed that CF patients absorbed linoleic acid from all of the lipid preparations tested when administered with their regular dose of pancreatic enzyme supplement. The mean maximal increase in % plasma linoleic acid in CF patients was not different from controls after ingestion of safflower oil, Microlipid and Captex 810B. With Captex 810D, the CF patients had a significantly higher increase in % plasma linoleic acid than controls, 6.75% versus 2.27% respectively at 2 hr ($p<0.02$), and 11.10% versus 4.65% at 8 hr ($p<0.01$). The CF patients absorbed the Captex products faster than controls, suggesting that the medium chain length fatty acids in these structured lipids facilitated their utilization by CF patients. Lingual lipase, an enzyme secreted from the Ebner's glands at the base of the tongue, might be responsible for this latter observation. Lingual lipase acts preferentially on shorter chain triglycerides such as those present in the Captex products. Lingual lipase activity has been found to persist in the upper small intestine of CF patients, possibly because of the lower pH and decreased bile acid concentrations. Our results indicate that malabsorption alone cannot account for the inadequate or marginal essential fatty acid status of CF patients, and that long term consumption of supplemental linoleic acid in addition to adequate caloric intake should improve the linoleic acid status of most, if not all, CF patients. Randomly esterified synthetic triglycerides containing a large amount of linoleic acid seem to be well tolerated and effective supplements of both essential fatty acids and calories for CF patients, especially since the presence of shorter chain length fatty acids appears to enhance their utilization in this population of subjects.

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ICOSANOID SYNTHESIS BY PLATELETS OF CHILDREN WITH CHOLESTATIC DISEASE. Olivier Amédée-Manesme, l'Hopital de Bicêtre, Department de Pédiatrie, Hepatologie, 78 Rue du General-Leclerc, 94270 Bicêtre, France, and Jacqueline Dupont, Iowa State University, Department of Food and Nutrition, 107 MacKay Hall, Ames, IA 50011.

The possibility of malabsorption of triglycerides contained in infant formulas of children with cholestasis suggests a deficiency of essential fatty acids and therefore probable effects on icosanoid metabolism. Children with either biliary atresia (BA) or syndromatic paucity of interlobular bile ducts (PIBD) were evaluated as to synthesis of prostaglandins (PG), E_2, $PGF_{2\alpha}$ and thromboxane (TX) by whole blood incubated at 37 C for 10 min. The children with BA appear to have excess icosanoid synthesis, and those with PIBD are severely deficient in icosanoid synthesis. The icosanoid data will be correlated with linoleate and arachidonate content of serum to determine whether the abnormalities are associated with essential fatty acid deficiency or a defect in regulation of icosanoid synthesis. Supplementation of the infant formula with Captex 810 will be evaluated.

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INFLUENCE OF MEDIUM CHAIN TRIGLYCERIDES ON RAT MAMMARY TUMOR DEVELOPMENT. Leonard A. Cohen, Naylor Dana Institute for Disease Prevention, 1 Dana Rd., Valhalla, NY 10595, and Diane Ordway Thompson, State University of N.Y., College at Purchase, Purchase, NY 10577.

It is well established that high-fat (HF) diets containing long-chain unsaturated fatty acids (FA) stimulate the development of experimental mammary tumors. In this study, we tested the effects of HF diets rich in medium chain triglycerides (MCT) on the development of the N-nitrosomethylurea (NMU)-induced rat mammary tumor. In addition, serum lipids and the FA profiles of both serum and tumor lipids were assessed. Thirty animals/group were initiated with NMU on day 50 of age and fed diets containing 23% corn oil (wt/wt); 5% corn oil, and 6% corn oil + 18% MCT, for a period of 200 days. Total tumor incidence was 87% in the HF-corn group, 66% in the LF-corn group ($p>0.06$) and 60% in the HF-MCT group ($p<0.03$). The mean latent period for the three groups was 90, 120 and 120 days, respectively. Median serum cholesterol levels were 81, 109 and 107 (mgs/100 ml) respectively ($p<0.001$); serum triglycerides were similar in all three groups. Serum FA analysis revealed a difference in C18:2 levels: 29% of total FA in the HF-corn group were C18:2, compared to 13% of total FA in the other two groups. The ratio of serum C18:2/C20:4 was 1.2, 0.45 and 0.46 in the HF-corn, LF-corn and HF-MCT groups, respectively. Tumor phospholipid (PL) FA profiles closely paralleled those found in serum: C18:2 made up 11% of total FA in the HF-corn group and 4 and 5% in the LF-corn and HF-MCT groups. The ratio of C18:2/C20:4 was 0.65, 0.17 and 0.29, respectively, for the three groups. These results indicate that a HF diet containing 18% MCT exerts tumor growth effects similar to that of a LF diet. The similarity of serum FA profiles in the LF-corn and HF-MCT groups supports the idea that the major part of dietary MCFA is rapidly absorbed and oxidized and little is incorporated into serum lipoprotein or cell membrane. Analysis of tumor PL C18:2/20:4 ratios suggests that animals fed

HF-corn diets convert proportionately less C18:2 to C20:4 than animals fed HF-MCT or LF-corn diets. This may be due to inhibition of FA desaturases by high corn oil intake.

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MEDIUM CHAIN TRIGLYCERIDE AND STRUCTURED LIPIDS AS UNIQUE NON-ENERGY SOURCES IN HYPERALIMENTATION. Edward A. Mascioli, Vigen K. Babayan and George L. Blackburn, New England Deaconess Hospital, Nutrition/Metabolism Laboratory, 194 Pilgrim Rd., Boston, MA 02215.

Current clinical intravenous lipid emulsions, derived from safflower and soybean oils, serve as a source of linoleic acid and a non-glucose nitrogen-sparing fuel. Their administration is not without risks, however. Slow clearance from the blood, clearance not equalling oxidation, small proportions oxidized within several hours, and a high proportion stored limit their usefulness. Other potentially very significant problems are impairment of immune function through reticulo-endothelial system blockage and reduced phagocytic chemotaxis. Medium chain triglyceride (MCT) emulsions have been investigated recently in animals and humans. Unlike long chain triglycerides (LCT), a high proportion of MCT are rapidly oxidized and poorly stored, when infused, serving as a more effective energy source. Structured lipids, randomly re-esterified triglycerides from a mixture of hydrolyzed medium and long chain triglycerides, have been studied in animals. Recent experiments in burned rats given total parenteral nutrition (TPN) consisting of amino acids, glucose and either LCT, MCT or structure lipids have shown a nitrogen-sparing effect of MCT and better albumin levels and hepatic protein synthesis with structured lipids. In a burned guinea pig TPN model where a radiolabelled bacteremia was induced, the distribution of the bacteria taken up into organs varied depending on the lipid infused. Only LCT allowed more bacteria to be trapped in the lungs as opposed to in the liver. Human infusions of MCT, which we have performed recently, demonstrate their safety and utility as a readily oxidizable fuel. These studies serve as background for further clinical work to delineate the proper role for these lipids in hyperalimentation.

SESSION E Productivity Management Monday morning

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PRODUCTIVITY MANAGEMENT IN THE OIL, FAT AND CHEMICAL DERIVATIVE PROCESSING PLANTS. Peter Kalustian, Peter Kalustian Associates, Inc., 239 Reserve St., Boonton, NJ 07005.

Improved productivity and resultant lower plant operating costs are necessary and should be the significant goals for all operating managers. Such objectives can be within reach whether in an old, outmoded plant or a modern, highly automated and efficient physical plant. There should be in existence updated and written process and service manuals. These should be available not only to managers and supervisors but especially to all operating personnel. Training sessions are necessary for supervisors and operators. All process operations should be monitored by the operator maintaining appropriate log sheets. By such information, management should be able to ascertain production rates, yields, output, chemical and supply use, service and utility use, and other information. This information can then be made available to the operator. There also should be accurate department labor and operating costs issued monthly by the accounting department. Such information also should be available to the specific department operator. Such information should include all utility, service and maintenance departments where labor utilization is of greater significance. Specific examples will be presented.

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IMPROVING PERSONAL PERFORMANCE AND MANAGEMENT PRODUCTIVITY. Robert H. Rushowy, P/S & Associates, 10 Saint Mary St., Toronto, Ontario, Canada M4Y 1P9.

Most of us in our private lives find, as consumers, a notion of instant gratification attached to goods and services which are available. Much of this "learned" behavior is carried with us to our places of work and results in a measure of anticipation or expectation of instant or immediate solutions to the problems of management. Scientific and mathematical models generally have served us well in projecting and analyzing natural phenomena. However, they may in fact hinder the development of social skills. Management productivity is extremely difficult to measure directly because so much of it is non-quantifiable. However, marked improvement in both real and perceived performance can result through stress reduction, effective group cooperation and competition and the establishment of appropriate standards. Prescriptive solutions may not succeed because of the inappropriate use of rational models to describe non-rational or emotion-laden situations. This paper will focus on pragmatic elements of professional management as it relates to the technically oriented manager.

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PRODUCTIVITY MANAGEMENT IN VEGETABLE OIL REFINERIES. Norman J. Smallwood, A.E. Staley Mfg. Co., 1940 E. Hull Ave., Box 3071, Des Moines, IA 50316.

On-line computer process control offers a significant opportunity for productivity improvement in vegetable oil refineries. Compared to traditional manual operation, computer operated plants can be at least three times more productive. The long-term productivity, yield and quality improvements realized from totally computerized operations are sufficient to justify the substantial up-front investment if effectively executed. The critical elements in realizing the productivity potential are computer hardware, computer software and the plant operating team. Computer hardware options are numerous and must be evaluated carefully to make the optimal choice. Technical expertise which includes both computer control systems and vegetable oil processing unit operations is vital in selecting the hardware. Software must be custom developed for each process by plant and requires a major commitment of resources: technical expertise, time and money. Computer controlled processes do not operate unless everything is right: control devices, computer software and computer hardware. Thus, startup on or conversion to computer process control is demanding. Commitment, discipline, patience and perseverance are essential personal and organizational qualities necessary to succeed. Finally, the plant operating team must be up to the task of understanding, utilizing, maintaining and improving the computer control scheme. Development of a participative, high performance, team oriented people system is the key to assuring the success of the venture.

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MANAGEMENT SYSTEMS FOR CREATIVE PRODUCT DEVELOPMENT. Martin E. Ginn, IIT School of Business Administration, Illinois Institute of Technology, IIT Center, Chicago, IL 60616.

A consideration of various routes for creative product development has suggested that ambidextrous methods of management are most appropriate. This conceptual development has led to an examination of how group versus individual processing impacts on the individual engaged in creative product development. Based on certain scenarios and field experiences, supported by the literature, it is postulated that participation of individuals in innovating groups can have positive as well as negative impacts. Thus, creativity may be facilitated or impeded by certain "tensions" depending on the quality and duration of group involvement. The analysis suggests that management approaches should permit certain contingencies to inject variety into experiences of individuals responsible for new product development. A flexible style of management allows for stimulation within groups as well as the satisfaction of self-actualization needs of individuals engaged in the creative process.

SESSION F Neurochemical Aspects of Lipid Metabolism Monday morning

Abstracts not available at press time.

Meetings

SESSION G Thermal-Oxidative Effects on Lipids Monday afternoon

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AN HPLC METHOD FOR ANALYZING HIGH MOLECULAR WEIGHT COMPOUNDS FORMED IN HEATED OILS. Pamela J. White and Yen-Chin Wang, Iowa State University, 111 MacKay Hall, Ames, IA 50011.

A simple and rapid method, based on size exclusion high performance liquid chromatography (HPLC), was developed for measuring high molecular weight (MW) compounds formed during the heating of oil. Formation of the high MW compounds is believed to be a reliable indicator of heat abuse in oils. The HPLC method employs two μ-spherogel size-exclusion columns (500 and 1000 A) in a series, to separate the oxidized compounds. Methylene chloride is used to dissolve the oil samples before injection and as the HPLC mobile phase. Compounds are detected at a wavelength of 233 nm, using a variable wavelength detector. The higher MW compounds elute first. The method was examined in the following study. Two sources of soybean oil were heated under laboratory conditions at 182 ± 2 C for eight 7-hr days. Samples were taken periodically and tested using the HPLC method described. Oil samples from two commercial deep-fat frying operations were similarly tested. In all cases size, number and apparent MW of the compounds formed increased with increasing frying time. The HPLC procedure was compared to a method involving separation of polar and nonpolar components in a used frying fat by means of column chromatography on silica gel. Both methods supply a quantitative measure of heat abuse in oils and are simple. However, the HPLC procedure provides additional information about the molecular weight distribution of the polar components that are formed.

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DIMER ISOLATION AND CHARACTERIZATION IN THERMALLY OXIDIZED FATS. Constantina N. Christopoulou and E.G. Perkins, University of Illinois, Department of Food Science, 1208 W. Pennsylvania Ave., Urbana, IL 61801.

The isolation and structure elucidation of the dimeric products formed during thermal oxidation was studied. Safflower oil (~20% oleic, 70% linoleic) was subjected to thermal oxidation at 200 C for 24 hr in the presence of air (0.10 ml/min/g). Chromatographic methods (TLC, HPSEC, GLC) were developed in order to follow the formation of dimer and its quantitation. The resulting material after saponification and esterification was fractionated by gel permeation chromatography on a Bio-Beads S-X_3 system with THF as eluting solvent. Subsequently separation of dimers to polar and non-polar dimeric fractions was performed by the use of TLC, GLC and HPLC of reverse and normal phase. Both polar and non-polar dimeric fractions were furthermore fractionated into the major dimeric compounds by HPLC. Preparative isolation of the best separated components was performed, and the collected fractions were subjected to analysis by GC-MS, IR or UV, NMR as well as chemical analysis to aid in their characterization. Finally, mechanisms for the formation of the dimers were proposed, and correlations between thermal and oxidative reactions in regard to dimer formation were explained.

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COMPARISON OF CYCLIC FATTY ACID MONOMERS (CFAM) FORMED DURING THE HEAT TREATMENT OF VEGETABLE OILS. J.L. Sebedio and J. Prevost, I.N.R.A., Station de Recherches sur la Qualité des Aliments de l'Homme, 17 Rue Sully, BV 1540, 21034 Dijon Cedex, France, and O. Morin, I.T.E.R.G., Pessac, France.

The structures of the CFAM were studied using both vegetable oils heated under conditions which are normally used during the frying process and oils which were heated at high temperatures. In the second case, temperatures as high as 275 C were used in order to obtain large quantities of CFAM. This paper will describe only the results obtained for the second method. A sunflower and a linseed oil were heated under nitrogen at 275 C for 12 hr. The resulting heated oils which include some monomeric, dimeric and polymeric triglycerides were saponified, and the fatty acids were esterified by a solution of H_2SO_4 in methanol. The total methyl esters were fractionated into a "polar" and a "non-polar" fraction using a silicagel column and a mixture of petroleum-ether: diethyl ether (95:5) as the solvent system. The non-polar fraction which contained the straight chain fatty acid methyl esters (saturates, mono- and polyunsaturated acids, and their geometrical isomers) and some CFAM were submitted to the action of urea in methanol. The urea adduct fraction contained the straight chain fatty acid methyl esters while the non urea adduct fraction contained the CFAM. These CFAM were further fractionated using the formation of their methoxybromomercuric adducts. An aliquot of each fraction was hydrogenated on PtO_2. The structures of the isolated CFAM were determined using mass spectrometry and ozonolysis in BF_3-MeOH.

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IDENTIFICATION IN LIVER LIPIDS OF RATS FED A HIGH LINOLENIC ACID HEATED OIL OF A 20:5 FATTY ACID HAVING A *TRANS* ETHYLENIC BOND. A. Picconeaux, A. Grandgirard and J.L. Sebedio, I.N.R.A., Station de Recherches sur la Qualité des Aliments de l'Homme, 17 Rue Sully, BV 1540, 21034 Dijon Cedex, France.

Weanling rats were fed for 8 weeks (10% in the diet) with a linseed oil that had been heated at 275 C for 12 hr. Total lipids of rat livers were extracted and esterified by a solution of BF_3-MeOH. A 20:5 geometrical isomer was isolated by a combination of high performance liquid chromatography on a reversed phase column and $AgNO_3$ thin layer chromatography (TLC). This isolated 20:5 fatty acid was submitted to the action of hydrazine in ethanol. This reaction resulted in the formation of a complex mixture of saturate, monoenes, dienes, trienes, tetraenes and pentaene. The monoenes which represent the position and the geometry of the ethylenic bonds in the parent molecule (20:5) were isolated using the methoxybromomercuric adducts fractionation. This separation is taking place only according to the degree of unsaturation of the fatty acid. The isolated monoenes were further fractionated in three bands by $AgNO_3$-TLC. The lower band (Rf 0.23) showed two peaks on a Silar-10C column (ECL 20.46 and 20.58), the middle band (Rf 0.32) two peaks (ECL 20.66 and 20.84) while only one peak (ECL 20.70) was detected in the higher band (Rf 0.43). Each band was then submitted to oxidative ozonolysis in BF_3-MeOH, and the resulting components (monomethylester and dimethylester) were analyzed by gas liquid chromatography on a Silar-10C column and identified by comparison with authentic standards. The two peaks in the band of Rf 0.23 have their ethylenic bonds located in the $\Delta 5$ and $\Delta 8$ positions, the peak in the band of Rf 0.32 in $\Delta 11$ and $\Delta 14$ and the peak in the band of RF 0.43 in $\Delta 17$. The ELC value on Silar-10C and the migration on $AgNO_3$-TLC showed that this $\Delta 17$ monoene is of *trans* configuration. From this data, it can be deduced that the parent molecule is 20:5 $\Delta 5c$, 8c, 11c, 14c, 17t. This component could be a metabolite of 18:3 $\Delta 9c$, 12c, 15t which previously was identified in this heated linseed oil.

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ENZYMATIC HYDROLYSIS OF THERMALLY OXIDIZED CANOLA OIL. J.C. Alexander and H. Yoshida, Department of Nutrition, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

The in vitro hydrolysis of thermally oxidized canola oil by pancreatic lipase was studied. Commerically refined oil was heated at 180 C for 50, 70 and 100 hr with aeration. Monomers, dimers, trimers and polymers derived from the acylglycerols were isolated from the oxidized oils by silica gel column chromatography. After analyses for chemical properties, the oils and individual fractions were exposed to a time-course lipase hydrolysis procedure for a period of 30 min. The heated oils showed progressively less hydrolysis with increased heating time. Among the fractions, even after 100 hr of heating, the acylglycerol monomers were hydrolyzed well, but the hydrolysis of the dimers was half that of the fresh fat, and that for the trimers was less than one quarter. The polymers were hydrolyzed slightly only in the first 5 min. Changes in the composition of the fat, due to formation of oxidized and polymerized products, resulted in reductions in enzymatic hydrolysis. Therefore, thermally oxidized dietary fats could be expected to provide less available energy.

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THERMAL OXIDATION OF BUTTERFAT AND BUTTERFAT FRACTIONS IN COMPARISON TO SELECTED VEGETABLE OILS. D.B. Kupranycz, M.A. (Vic) Amer and B.E. Baker, MacDonald College, McGill University, Dept. Agr. Chem. and Physics, Box 223, Ste. Anne de Bellevue, Quebec H9X 1C0, Canada.

Although butterfat is an important fat used in food preparation, little is known about the thermal oxidative behavior of butterfat and its non-volatile decomposition products. To evaluate the thermal oxidative behavior of butterfat in comparison to selected vegetable oils, 100-g lots of winter and summer butterfat, butterfat fractions (29 and 19 C), canola oil, soybean oil, sunflower seed oil and corn oil were heated at 185 C in the presence of air (30 ml/min) for 8 and 16 hr. Results of gel permeation chromatography of the intact heated fat and oil samples and their methyl esters showed that butterfat triglycerides, like vegetable oil triglycerides, undergo both inter- and intramolecular polymerization reactions, but at a significantly reduced level. For example, canola oil contained 14.813% of dimeric triglycerides and a total of 19.907% trimeric and higher oligomeric triglycerides after 16 hr of heating compared to 12.812% and 9.118% respectively for the reference winter butterfat. Differences among butterfat samples were less pronounced, however, after 8 hr of thermal oxidation, both the solid and liquid butterfat fractions exhibited some stability toward intermolecular polymerization compared to the reference butterfat. After 16 hr of thermal oxidation, the solid fraction at 29 C exhibited the highest stability, while the liquid fraction at 29 C exhibited the lowest stability among the butterfat fractions. The ratio of trimeric and higher oligomeric triglycerides to dimeric triglycerides increased with increasing degree of unsaturation of the fat or oil and with increased time of heating. Similar trends were observed with regard to the degree of intramolecular polymerization. The relationships between triglyceride structure and polymerization of fats and oils are elucidated.

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RETARDATION OF RANCIDITY IN DEEP-FRIED INSTANT NOODLE (RAMYON). Kwang Lae Rho and Paul A. Seib, Kansas State University, Grain Science and Industry, Schellenberger Hall, Manhattan, KS 66502; Okkyung Kim Chung, USDA, USGMRL, and K.S.U., and Do Sup Chung, Kansas State University, AG Engineering.

Three methods of extending the shelf-life of instant fried noodles were examined: 1) addition of 2000 ppm antioxidant (BHA, TBHQ, Poly-A) to the frying oil; 2) coating the inner surface of the polyethylene package with TBHQ equivalent to 200, 500 and 1000 ppm based on the oil in the fried noodle, and 3) addition of a mixture of TBHQ (200 ppm) and EDTA (200 and 500 ppm) to the frying oil. The determination of storage stability was conducted by accelerated aging at 63 C followed by organoleptic determination of the onset of rancidity. When the antioxidants were added to the frying oil, BHA and Poly-A approximately doubled while TBHQ tripled shelf-life of ramyon. Surface application of TBHQ (200 ppm) extended the shelf-life of ramyon twice as long compared to an equal amount of TBHQ in the frying oil. Water activity affected the oxidative stability of lipids in ramyon; rancid flavors developed slowest at a_w 0.2-0.4. Hexanal content in ramyon was found to be a good indicator of development of oxidative rancidity. Organoleptic evaluation showed the flavor of ramyon was objectionable when it contained a hexanal concentration of greater than 3.5 ppm based on the noodle weight. The relative effectiveness of antioxidants in preventing off-flavor development could be determined by the rate of hexanal released in the stored ramyon.

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THE RE-PROCESSING AND RE-USE OF SPENT RESTAURANT GREASE AND THE ENVIRONMENTAL CONTROL PROCEDURES NECESSARY. B.F. Osborne, West Coast Reduction Ltd., 105 N. Commercial Drive, Vancouver, B.C., Canada V5L 4V7.

The re-processing of spent restaurant grease is discussed, including collection, handling and treatment and purification. The blending of this material with other by-products such as an acid oil from a tallow soapstock acidulation process is described, and the overall quality factors necessary for the re-use of this material as a high grade animal feed additive are discussed. The processing of restaurant grease, soapstock, etc. in a modern by-products recycling plant results in unique environmental problems in terms of wastewater and air pollution. Strategies for handling the wastewater are described, the basic techniques used being chemical treatment followed by dissolved air flotation. Overall air pollution control philosophies are discussed, the equipment utilized being air scrubbers with a variety of scrubbing media—water, acid, sodium hypochlorite and bacteria.

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A TECHNIQUE FOR MONITORING THE QUALITY OF USED FRYING OILS. Pei-fen Wu and W.W. Nawar, University of Massachusetts, Department of Food Science and Nutrition, Amherst, MA 01003.

The objective of this work was to develop a practical method for monitoring the quality of oils during the frying process. A special effort was made to find a technique which would not be affected by dilution, since replenishment with fresh oil to varying degrees is a frequent necessity. Nine analytical methods, i.e. measurements of viscosity, polymers, change in dielectric constant, polar compounds, dimers, free fatty acids, smoke point, carbonyls and cyclic monomers, as well as certain combinations of these measurements were evaluated. Since each single method was influenced by replenishment with fresh oil, combinations of two methods were studied in an attempt to produce a single value unaffected by dilution. The ratio polymer/FOS (polymers according to Peled's technique of methylation and extraction, and change in dielectric constant by food oil sensor readings) proved to be not only adequate for monitoring the quality of the used oil, but also was affected minimally by replenishment.

SESSION H High Performance Liquid Chromatography in the Analysis of Lipids II Monday afternoon

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USE OF THE LIGHT SCATTERING DETECTOR IN ANALYSIS OF LIPIDS. A. Prevot and J.L. Perrin, Institut des Corps Gras, Rue Monge, Parc Industriel, 33600 Pessac, France, and A. Stolywho and G. Guiochon, Ecole Polytechnique, Laboratoire de chimie analytique physique, Route de Saclay, 91128 Palaiseau, France.

It is well known that HPLC of complex mixtures like lipids or fat derivatives remains difficult for lack of a suitable detector. In this laboratory-made Light-Scattering-Detector, the column effluent is nebulized in a stream of warm gas in which the solvent vaporizes. The particles of non-volatile solutes scatter the light of a laser beam. Part of the scattered light is collected and fed to a photomultiplier. This detector is particularly suitable for microbore column work, with its required low flow rate (200-300 µl/min) and small dead volume (0.1-0.2 µl). The detector can be used with any solvent, solvent mixture of mobile phase gradient without any baseline drift, as long as all components of the solvent are volatile. The response parameters studied included CO_2 flow rate and voltage of photomultiplier. The operating principle, quantitative properties and the wide possibilities of application of the light scattering detector in the analysis of fats and related products will be discussed. Examples include analysis of triglycerides with a gradient $CH_3CH/CHCl_3$ and analysis of polar components in heated oils.

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HPLC OF FATTY NITROGEN DERIVATIVES OR FROM ON THE HOOF TO ON THE ROAD. Gerald Szajer and Linda Yodual, Akzo Chemie America, 8401 W. 47th St., McCook, IL 60525.

HPLC has gained extensive use in the analysis of fatty nitrogen derivatives. It provides not only separations based on chain length distribution but also chemical functionality. This proves quite useful in following conversions of one type of nitrogen derivative to another. One of the most widely used asphalt emulsifying agents contains a diamine derived from tallow. The reaction sequence is ACID → AMIDE → NITRILE → AMINE → DIAMINE. High pressure liquid chromatography can be used at every step in this sequence.

This paper will present examples of the utility of HPLC in such analyses as carbon length distribution, percent product in the final formulation, and determination of ancillary compounds.

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IDENTIFICATION OF FATTY ACID ESTERS OF CHLOROPROPANEDIOL IN MILK FATS BY LC/MS. J. Cerbulis, O.W. Parks, and H.M. Farrell Jr., Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, PA 19118, and A. Kuksis, L. Marai and J.J. Myher, University of Toronto, Toronto, Canada.

Fatty acid esters of 3-chloropropanediol have been isolated in small amounts from the neutral lipid fraction of goat's milk as a fast moving band by TLC and their general nature established by mass spectrometry. In an effort to discover the origin of the fatty acid pool of these esters, we have compared the acyl chain pairing in the molecular species of the chloropropanediol esters and in the total goat milk fat. For this purpose we employed capillary GLC and reversed phase HPLC in 30-90% gradient of propionitrile in acetonitrile, along with both positive and negative ion mass spectrometry. The spectra obtained in the positive CI had [MH-RCOOH] ions as the base peaks. The molecular weights were confirmed by the weak MH^+ ions. Spectra obtained in the negative CI mode exhibited ions at m/z [M-1], [M+26] and [M+35]. The latter ions represent addition of CN^- and Cl^- ions, respectively. The isotope cluster of the ions at m/z [M+26] and [M+35] are consistent with the presence of one and two chlorine atoms, respectively. The molecular species containing C_{10}-C_{18} fatty acids were identified and quantitated via the appropriate characteristic ions. The composition derived by LC/MS was compared to that expected on the basis of fatty acid composition of the original esters and of the products of pancreatic lipase hydrolysis. The data show that the molecular species of the chloropropanediol diesters are similar to those of the long-chain rac-1,2-diacylglycerols derived from goat milk triacylglycerols. It is suggested that both triacylglycerols and 3-chloropropanediol diesters are derived from the same pool of fatty acid CoA esters. This conclusion is consistent with the racemic nature of the 3-chloropropanediol diesters demonstrated elsewhere.

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A NEW FID FOR THE LC OF OILS, ETC. Jack B. Dixon, Tracor Instruments Austin, Inc., 6500 Tracor Lane, Austin, TX 78725-2100.

Vegetable oils as well as animal oils and fats are a major raw material in industry today. Tracor Instruments has developed a Flame Ionization Detector (FID) for liquid chromatography (LC) which is very useful for the analysis of these raw materials as well as products made from them. The detector which will be described operates by removing the volatile LC solvent from the solutes of interest before detection, thus making the LC/FID more applicable to the analysis and characterization of free fatty acids, long chain alcohols, mono-, di- and triglycerides than the standard UV absorbance and refractive index detectors for LC. The operation of the detector will be illustrated with chromatograms for the characterization of palm and other vegetable oils, the analysis of free fatty acids and alcohols and the analysis of products made from these materials. The LC/FID is becoming the preferred detector for these analyses, due to its ease of operation, sensitivity and linear response.

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OVERPRESSURE LAYER CHROMATOGRAPHY (OPLC). John M. Newman, Newman-Howells Associates Ltd., Wolvesey Palace, Winchester, Hants., SO23 9NB, United Kingdom.

Although essentially a planar chromatography technique, OPLC is more characteristic of HPLC even though the sorbent format used is a classical HPTLC foil. The fundamental application of an 'overpressure' to exclude atmospheric vapor from a planar layer is the key to the distinction between OPLC and current practice. OPLC combined the features of TLC, HPTLC and HPLC and introduces advances in instrumentation design and operation, surpassing the original planar methods, above all in speed and high efficiency of separation. The advantages of this technique are: (1) Absence of a vapor phase above the sorbent layer, combined with a controlled forced-flow mobile phase, substantially increases HPTLC migration distances; (2) The speed of development (5 times faster than HPTLC), apart from dramatically improving separation efficiency, considerably enhances resolution by radically reducing spot diffusion; (3) The ability to variably force the migration of eluents in chromatographic systems of poor wettability removes the limitation in the selection of solvents; (4) Volume of eluent used per 20 × 20 cm plate or foil is less than 5 ml; (5) Temperature at the surface of the sorbent can be fixed and controlled by the use of an external temperature-regulated water bath; (6) Continuous sample development is easily performed, allowing components of slow-mobility to be resolved over longer separation path lengths. Since the solvent front velocity is constant, R_f values can be calculated accurately; (7) The quadratic relationship between migration distance and time does not exist in OPLC. The linear law of time, therefore, offers advantages when substances with small differences in selectivity or complex mixtures with a large number of compounds are to be separated, and (8) Because of the similar operation conditions existing in HPLC and OPLC techniques, OPLC is infinitely more suited to facsimile eluent 'profiling' for HPLC analyses than conventional TLC.

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RECENT APPLICATIONS OF THE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO OILS AND FATS ANALYSIS. Vijai K.S. Shukla, Research Laboratories, Aarhus Oliefabrik A/S, P.O. Box 50, DK 8100 Aarhus C, Denmark.

The technique of high performance liquid chromatography (HPLC) has grown enormously during the past decade and is now accepted as a major tool for the analysis of oils and fats. In the present work very high resolutions of triglycerides in various natural oils, cocoa butter and several exotic fats using columns packed with 3-μm alkyl bonded phase particles will be demonstrated. The application of the technique for the structural elucidation of the seed fat of Pentaclethra will be described. The feasibility of the method for the quick evaluation of the cocoa butter equivalents in cocoa butter will be shown.

SESSION I Surfactants and Detergents I: Performance, Evaluation and Analysis
Monday afternoon

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ANIONIC VS NONIONIC SURFACTANTS IN HARD-SURFACE CLEANING—DIFFERENCES IN SOLID SOIL REMOVAL MECHANISMS. Michael F. Cox, Vista Chemical Company, P.O. Box 500, Ponca City, OK 74602.

Anionic and nonionic surfactants vary significantly in their ability to promote wetting, soil solubilization and surfactant penetration of soil, and in their ability to decrease soil adhesion. Differences in these parameters determine the mechanisms by which surfactants remove solid, organic soils. Their relative performance depends on both soil and substrate, since different soil substrate combinations vary in the surfactant functions they require for effective soil removal. Correlations are made between soil type/substrate type and performance, and between performance and surfactant composition.

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NONIONIC SURFACTANTS CONTAINING PROPYLENE OXIDE. Carter G. Naylor, Texaco Chemical Company, P.O. Box 15730, Austin, TX 78761.

The physical and surface active properties of ethoxylate are altered by the inclusion of propylene oxide (PO) groups. The distribution of PO within the ethoxylate chain can be as important as the relative amounts of PO and EO. The influence of PO on surfactant properties was assessed for several alcohol ethoxylate and nonylphenol ethoxylates. Pour points and gelling tendencies improved at some expense of detergency and wetting power. Hydrocarbon solubility and middle phase behavior also were investigated.

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A LAUNDRY PROFILE: PRODUCTS, PROBLEMS AND SATIS-

FACTIONS. LoErna Palmer Simpson and Linda Medlen Heaton, University of Kentucky, Lexington, KY.

A satisfactory laundry system involves a balance between thermal input (laundry temperatures), mechanical input (agitation, length of cycle) and chemical input (detergent and laundry aids). What is the current laundry profile of American consumers in the mid-1980's? In order to determine this, a 4-page, forced-answer, written questionnaire was administered to over 1100 Kentuckians between October 1983 and March 1984. Among the topics studied were relationships between laundry product categories and laundering problems encountered, laundering temperatures used, and satisfaction with cleaning level achieved. Although warm wash temperature was "usually" used by 72%, respondents did not use only one wash temperature exclusively. Cold, warm and hot temperatures were "sometimes" selected by nearly equal numbers. Stains not completely removed were cited as a problem by 63% of the participants, followed by static cling identified by 43%. Satisfaction with the cleaning level, however, was expressed by 96% of the respondents. Forty-five per cent indicated they were not selecting the same washing product each time. Product selection was influenced more by brand name than by price.

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CHROMATOGRAPHIC DETERMINATION OF THE COMPOSITION OF NONIONIC SURFACTANTS DERIVED FROM FATTY ACIDS. C.N. Wang, L. Yodual and L. Metcalfe, Akzo Chemie America, 8401 W. 47th St, McCook, IL 60525.

Nonionic surfactants based on fatty acids are used widely in many industries. Many of these materials are used in food applications. The determination of the total composition of these surfactants can be a difficult analytical problem. A rapid method for determining the total composition of these nonionic surfactants, such as ethoxylated and propoxylated fatty acids, the polyol esters and sorbitan esters, has been developed. The fatty acid portion is analyzed by the gas chromatography of the methyl esters. The methyl esters are formed rapidly by tetramethyl ammonium hydroxide catalyzed esterification. The usual fatty acid esterification procedures for these particular compounds are slow and difficult to use. Size exclusion chromatography (SEC) is used to characterize the compounds. Characteristic peaks or a series of peaks are obtained that will identify the nonionic compound. We feel this approach is as good as any previously reported.

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ROLE OF Na-POLYACTYLATE IN CARBONATE-BUILT DETERGENTS. M.K. Nagarajan, BF Goodrich (Chemical Group), Technical Center, P.O. Box 122, Avon Lake, OH 44012.

It is known that sodium carbonate can function as a detergent-builder by removing the calcium and magnesium ions from hard water in the form of precipitated calcium-magnesium carbonates. However, these insoluble inorganic carbonates tend to accumulate on washed fabrics and washing equipment parts, thereby resulting in undesirable fabric-encrustation or scaling. Terg-o-meter wash experimental results are presented in this paper which suggest that it is possible to significantly reduce these insoluble carbonate deposits, through incorporation of appropriate levels of sodium polyacrylate as anti-encrustation additive in no-phosphase carbonate-built detergent compositions. The likely mechanism for this calcium-magnesium carbonate anti-precipitation activity of sodium polyacrylate is also arrived at through suitable precipitation-kinetic studies under simulated carbonate-built detergent wash conditions.

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"DESTRUCTIBLE" SURFACTANT-BASED VESICLES FOR CONTROLLED DELIVERY. Timothy G. Golich and David A. Jaeger, Department of Chemistry, University of Wyoming, Laramie, WY 82071.

Two double-chain, "destructible" (cleavable) surfactants have been prepared for the formulation of vesicles for controlled release of entrapped compounds such as drugs. "Destructible" surfactants are stable under certain conditions but are labile under others with respect to cleavage to nonsurfactant compounds. One compound is stable in neutral and basic media but hydrolyzes in acidic media, and the other is stable at 25 C over the pH range 3-11 but hydrolyzes at lower and higher values. Vesicles formed from these compounds have been characterized by gel filtration chromatography, ^{1}H NMR spectroscopy, and differential scanning calorimetry, and have been shown to entrap and release compounds.

SESSION J Specialty Lipids and Their Biofunctionality II
Monday afternoon

55

MEDIUM CHAIN TRIGLYCERIDES IN EARLY LIFE. Sami A. Hashim, Department of Medicine, St. Luke's-Roosevelt Hospital Center and Institute of Human Nutrition, Columbia University, New York, NY 10025.

In man and other mammals, medium chain fatty acids (MCFA) are constituents of breast milk triglycerides. When administered in pure form, medium chain triglycerides (MCT) are easily digested and absorbed under conditions that are adverse to the digestion and absorption of long chain triglycerides (LCT). Moreover, MCT derived MCFA in the lumen and mucosa of the intestine are transported via the poral vein into the liver, where they are extensively oxidized. Thus, those MCFA reaching the liver do not appear as moieties of lipid esters of lipoproteins. Also, less than 3% of ingested MCT are transported as chylomicrons. In view of the efficiency of their absorption and mode of transport, MCT have been used in the treatment of a variety of malabsorption syndromes, including the long chain fat malabsorption encountered in premature infants. Evidence suggests that there occurs a two-fold increase in MCFA in breast milk of mothers at premature delivery, and that the premature infant, when breast fed by its mother, is receiving 17% of total fat as MCFA. By increasing the proportion of dietary MCFA from MCT in the diet of premature infants, it may be possible to circumvent the diminished ability of the premature infant to absorb LCT. Premature infants are born with very little adipose tissue. For example, a premature infant weighing 1500 g harbors only 3% of body weight as fat. As LCT digestion and absorption improve post-natally, there is rapid accumulation of fat, associated with enhanced cellularity of the adipose tissue. Experiments in rats were conducted to test the hypothesis that MCT may be less of a stimulus to adipose tissue proliferation and hypertrophy than LCT. Groups of weanling rats were pair-fed diets of varying levels of MCT or LCT up to 65% of dietary energy, for periods up to 28 weeks. The MCT fed animals had smaller fat depots and adipocyte size, but not cell number, than the LCT fed controls. At weaning, the feeding of MCT may influence adipose tissue mass through the development of smaller adipocytes.

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THE EFFECT OF MEDIUM AND LONG CHAIN TRIGLYCERIDES ON HUMAN ADIPOSE TISSUE METABOLISM. David P. Katz and Jerome L. Knittle, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029.

Lipid emulsions are used as sources of calories or for preventing or ameliorating essential fatty acid (EFA) deficiency in the malnourished patient. It has been suggested that their effect may be more extensive and mediated through the prostaglandin (PG) pathway. Studies in our laboratory, using human adipose tissue (AT), suggest that the nature of the fat calories delivered, i.e., chain length and degree of unsaturation, has an effect on PG metabolism. AT's diverse functions, which are sensitive to hormonal, nervous and nutritional manipulations, include the turnover of dietary fatty acids, de novo fatty acid and PG synthesis. Our studies have assessed the effect of intravenous administration of long chain triglyceride (LCT)-containing emulsions on AT metabolism. AT was obtained before and after 2 and 4 weeks of central hyperalimentation. The results demonstrate that intravenous fat rapidly alters the distribution of EFA found in AT as well as the production of PG. Similar studies have been performed in vitro to assess the effect of varied lipid emulsions on AT production. Physical mixes of medium chain triglyceride (MCT) and/or LCT were used in incubation studies with fat from subjects undergoing elective surgery. These studies demonstrate that the relative ratio of MCT/LCT has a significant effect on

the quantitative and qualitative production of PG by AT. These findings suggest that lipid emulsions could be designed to elicit a desired pharmacological activity mediated through the PG pathway.

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ADAPTIVE CHANGES IN AGING–ROLE OF CHOLESTEROL. Hans Kaunitz, College of Physicians and Surgeons of Columbia University, 630 West 168th St., New York, NY 10032.

The theory is widely accepted that aging processes are caused by DNA damage, leading to deterioration of normal functions. The question of whether some of these metabolic changes are adaptive mechanisms capable of delaying senescence is rarely discussed. Yet some of the enzymatic and immunological alterations associated with aging may fit into this category. Arteriosclerosis is a human aging process which is accompanied by a significant correlation between elevated serum cholesterol and frequent complications (myocardial infarctions, etc.). However, correlations cannot be used for etiological considerations unless otherwise supported. Actually, life expectancy has increased during our century despite higher fat and cholesterol intake. Recent feeding studies in humans do not support the "lipid theory." On the other hand, the cholesterol content of the early atheroma is the same as that of the normal tissue. Later a granuloma-like tissue with high cholesterol content develops, probably having protective properties. Cholesterol has a stabilizing effect on the DNA helix and probably increases the resistance of tissues to damage. Thus cholesterol seems to be part of the adaptive processes counteracting aging.

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BIOLOGICAL ACTIVITIES AND METABOLISM OF AN ANTIHYPERTENSIVE ACETYLATED ETHER-LINKED PHOSPHOLIPID (PLATELET ACTIVATING FACTOR, PAF). Fred Snyder, Merle L. Blank, Ten-ching Lee and Boyd Malone, Medical and Health Sciences Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37831.

In 1979 a novel class of ether-linked phospholipids (alkylacetylglycerophosphocholines) was described that possesses profound hypotensive (0.1 μg/kg in rats) and platelet-aggregating (10^{-11} M) activities; such lipids also appear to play an important role in inflammatory, allergic and anaphylactic responses and in the stimulation of cellular calcium influx. The term "platelet activating factor" or "PAF" refers to this general group of phospholipid mediators identified as 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines (GPC). Maximum biological activity requires an alkyl moiety, an acetate or propionate group, and a phosphocholine substituent at the *sn*-1, *sn*-2 and *sn*-3 positions of the phospholipid, respectively. Our results with alveolar macrophages (rats), platelets (rabbits), neutrophils and eosinophils (humans), and various rat tissues have established the enzymatic pathways of synthesis and catabolism for the bioactive phospholipids. The cellular precursor of PAF, 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine, is converted to PAF via reactions catalyzed by a phospholipase A_2 and an acetyl-CoA-dependent acetyltransferase. An alternate biosynthetic route for PAF involves alkylacetylglycerols and a cholinephosphotransferase. PAF is inactivated by an acetylhydrolase; the product, 1-alkyl-2-lyso-GPC is rapidly reacylated with a long chain acyl moiety (predominantly 20:4). Acetylhydrolase activity is also present in plasma; it appears to differ only slightly from the intracellular form. The significantly higher activities of acetylhydrolase in the plasma of hypertensive mammals hint that this enzyme might be involved in the pathogenesis of hypertension.

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ROLE OF FATTY ACIDS ON INTERCELLULAR COMMUNICATION. Charles F. Aylsworth, Department of Anatomy, James E. Trosko, Pediatric and Human Development, and Jon J. Kabara, Biomechanics, Michigan State University, 514 E. Fee Hall, East Lansing, MI 48824.

Although the tumor-enhancing effects of high levels of dietary fat (especially polyunsaturated fat) have been well established, the mechanisms involved in their stimulatory effects remain to be clarified. The loss of contact inhibition (in vitro) is a hallmark of many cancerous cells. Also, many known tumor promoters inhibit intercellular communication. We have previously reported that long chain unsaturated fatty acids inhibit metabolic cooperation, whereas long chain saturated fatty acids do not. In the present study we have extended these observations and examined the influence of medium and short chain fatty acids on metabolic cooperation between 6-thioguanine-sensitive ($6TG^S$) and -resistant ($6TG^r$) Chinese hamster cells. Hypoxanthine guanine phosphoribosyl transferase deficient ($6TG^r$) cells (100) were co-cultured with 4×10^5 wild type ($6TG^S$) cells in medium containing 6-thioguanine (10mg/ml) and in the presence or absence of non-cytotoxic concentrations of fatty acids (10.0mg/ml). Treatment, with per cent recovery in brackets, was: vehicle control [27.1], oleic acid (18:1) [67.6], undecylenic acid (11:1) [40.7], sorboc acid (6:2) [28.4] and hexanoic acid (6:0) [28.9]. These and other results suggest an association between chain length and unsaturation (i.e. longer chain [18:1] was more effective than [11:1]) in inhibiting metabolic cooperation. However, differences in the abilities of unsaturated and saturated fatty acids to effect metabolic cooperation was not seen in short chain fatty acids (6:2 vs 6:0). Structure-function relationships found for fatty acids on metabolic cooperation are similar but not identical to the toxic effects of fatty acids on bacteria and other microorganisms.

SESSION K Lipid Metabolism in Disease Monday afternoon

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DIETARY PROTEIN AND ATHEROSCLEROSIS. David Kritchevsky, Shirley A. Tepper and David M. Klurfeld, The Wistar Institute, 3601 Spruce St., Philadelphia, PA 19104.

The first purely nutritional studies of atherosclerosis were performed by Ignatowski in 1908-9. His hypothesis was that a toxic metabolite of animal protein was atherogenic. Interest in the role of cholesterol in atherosclerosis eclipsed work on protein for several decades. The first direct comparison of animal (casein) and vegetable (soy) protein was carried out by Meeker and Kesten in 1940. They found casein to be more atherogenic for rabbits. Carroll (1975) tested a number of animal and vegetable proteins for their effects on cholesterolemia in rabbits. There was a wide range of effects within each group. The least cholesterolemic animal protein (egg white) had an effect only slightly more severe than the most cholesterolemic vegetable protein (wheat gluten). Our hypothesis concerning cholesterolemic effects of protein has concentrated on the lysine/arginine (L/A) ratio (low in vegetable protein and high in animal protein). We tested the effects of protein on cholesterolemia and atherosclerosis in rabbits using a cholesterol-free diet which is hyperlipoproteinemic and atherogenic in this species. In three experiments, we found that casein was 85% more cholesterolemic and 144% more atherogenic than soy protein. Addition of arginine to casein did not affect cholesterolemia but reduced atherosclerosis by 24%. Addition of lysine to soy protein increased serum cholesterol levels by 45% and atherosclerosis by 64%. Comparison of three proteins whose lysine content was similar but whose L/A ratio ranged from 1.44 (fish protein) to 1.89 (casein) to 2.44 (whole milk protein) showed a significant ($p<0.05$) correlation between L/A and atherosclerosis. Huff and Carroll showed that cholesterol turnover was slower in rabbits fed casein than in those fed soy protein. We have found that body pools of cholesterol in rabbits fed soy plus lysine are 62% higher than in rabbits fed soy, and turnover time is increased by 83%. These differences are reflected in patterns of fecal steroids.

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DIETARY PROTEIN EFFECTS ON GALLSTONE FORMATION IN HAMSTERS. David M. Klurfeld and David Kritchevsky, The Wistar Institute, 3601 Spruce St., Philadelphia, PA 19104.

In 1952 Dam and Christensen introduced a diet which caused gallstones in hamsters. The diet contained 74.3% sucrose, 20% casein, 5% mineral mix, 0.5% vitamin mix and 0.2% choline chloride. Dam and others tested many variations of this diet but did not address directly the questions of protein source. In view of the hypocholesterolemic effect of vegetable protein in a number of animal species, we tested the influence of protein type on the lithogenicity of this diet. The first study involved comparison of casein

and soy protein isolate. In four studies (of 45, 45, 70 and 100 day duration, respectively) hamsters fed casein exhibited a 58% incidence of gallstones and those fed soy isolate showed an incidence of 14%. When mixtures of the two proteins were fed, a 3:1 casein:soy mixture elicited an incidence of 38% gallstones, a 1:1 mixture an incidence of 23% and a 1:3 mixture an incidence of 15%. We have offered the hypothesis that the lysine/arginine (L/A) ratio of a protein affects its influence on plasma cholesterol levels. The L/A of casein is about 2.0 and that of soy protein 0.9. Addition of arginine to casein inhibited lithogenicity by 31% (125% more lithogenic than soy isolate); addition of lysine to soy protein enhanced lithogenicity by 350% (38% more lithogenic than casein). Analyses of bile suggest that the major influence of protein is mediated at the level of biliary cholesterol.

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INABILITY OF SKIN ENZYME PREPARATIONS TO BIOSYNTHESIZE ARACHIDONIC ACID FROM LINOLEIC ACID. Robert Chapkin and Vincent A. Ziboh, Department of Dermatology, TB 192, University of California, Davis, School of Medicine, Davis, CA 95616.

The lack of any information as to the origin of epidermal arachidonic acid, an important precursor of eicosanoids in the epidermis, prompted us to determine in vitro whether or not microsomal preparations from rat and guinea pig epidermis possess Δ6 and Δ5 desaturate activities. The incubations were performed in parallel with microsomal preparations from liver of these animals where activities for these enzymes have been reported previously. The conversions of radioactive fatty acids were determined after methylation and separation of the ^{14}C-fatty acid methyl esters by argentation thin layer chromatography. Data from these studies demonstrated that Δ^5 desaturase activity is markedly lower in guinea pig liver than in rat liver. Interestingly, preparations from rat and guinea pig epidermis at all concentrations tested lacked the capacity to transform either linoleic acid into gammalinolenic acid or dihomogammalinoleic acid into arachidonic acid. This observation implies that arachidonic acid that is present in the epidermal phospholipids is biosynthesized elsewhere endogenously and transported to the epidermis for esterification into the phospholipids. The site of this biosynthesis is presumably the liver, and the mode of transport to the epidermis remains to be determined. These studies indicate arachidonic acid per se as an essential fatty acid for the epidermis.

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ISOPRENE: BIOSYNTHESIS AND ROLE IN POLYISOPRENOID METABOLISM. Evan S. Deneris, University of California, Los Angeles, CA.

Work has continued during the past year concerning the origin of breath isoprene. It has been shown in vitro that DL-Mevalonate is converted to isoprene in a system requiring ATP and the cytosolic fraction of rat liver. Furthermore, the biosynthesis appears to be partially non-enzymic and is explained in the following way: Mevalonate is enzymatically converted to the soluble intermediate of sterol biosynthesis, two of these being the C^5 units, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP). As the two C^5 units are formed, a non-enzymatic decomposition to isoprene occurs and proceeds as long as there is an adequate pool of the C^5 units. Picomolar amounts of isoprene are found in a one-hr breath collection from nursing pups. Similar amounts of isoprene are found in the in vitro system. Inhibitors of IPP/DMAPP isomerase and chemical model studies have pointed to DMAPP as the predominant immediate precursor of isoprene. Furthermore, the chemical model studies have shown that the rate of this reaction is acid catalyzed, suggesting a carbonium ion mechanism. The chemical model studies have led me to further hypothesize that isoprene formation is linked to the trans-methyl glutaconate shunt pathway. The evidence gathered so far suggests that isoprene is formed as a consequence of a variable flux through a C^5 pool resulting in the generation of more C^5 units than required by the cell for condensation to geranyl pyrophosphate. It also has been hypothesized that isoprene once formed in the cell can serve as a substrate for oxidative reactions in the endoplasmic reticulum. Evidence supporting this has been obtained using a post-mitochondrial liver homogenate incubated with a pentane/isoprene standard and an NADPH-regenerating system in a closed vessel. This raises the possibility of additional unknown products of mevalonate metabolism.

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STUDIES ON THE METABOLISM OF MALONDIALDEHYDE. H.H. Draper, L.G. McGirr, M. Hadley and L. Polensek, Department of Nutrition, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Following oral administration of [1,3-^{14}C] malondialdehyde (MDA) to rats, 9-17% of the radioactivity was recovered in the urine compared to 2-3% after [1,2-^{14}C] acetate administration. Fractionation of rat urine by ion exchange and HPLC revealed the presence of at least four compounds which yielded MDA on acid hydrolysis. MDA was measured by an HPLC method as the thiobarbituric acid (TBA) derivative. No free MDA was detected. Oral incubation with serum albumin which had been reacted with MDA led to excretion of a major MDA-containing compound. This metabolite was shown to be identical to a compound excreted after administration of a synthetic lysine-MDA adduct. NMR and mass spectroscopy indicated that this compound was the N-acetyl derivative of the lysine-MDA adduct, i.e., N-α-acetyl-ε-(2-propenal) lysine. Its identity was confirmed by synthesis. Excretion of this metabolite was markedly increased by feeding a diet containing cod liver oil. Although it appears to be mainly of dietary origin, its presence in the urine of rats fed a stock diet or a diet containing hydrogenated fat, as well as in fasting urine, indicates that it also is formed as a product of lipid peroxidation in vivo. Increases in urinary MDA occurred as a result of vitamin E deficiency, IP administration of iron nitrilotriacetate and enrichment of the tissues with fatty acids from fish oil. The elevated excretion by vitamin E-deficient rats was eliminated by feeding 0.1% DPPD, a biologically active antioxidant, but not by feeding 0.1% BHA, a non-biologically active antioxidant. These results show that urinary MDA is responsive to MDA consumed in the diet and formed in the tissues. MDA excretion may be a useful index of the effect of various compounds on in vivo lipid peroxidation.

SESSION L Use of Computers in Process Technology Monday afternoon

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SCHEDULING AND ANALYSIS OF PROCESS EVENTS. Gary B. Hirsch and Michael M. Blumenthal, Gantt Systems, Inc., 495 Main St., Metuchen, NJ 08840.

Computers are useful tools for planning and analyzing different kinds of process events. The design, installation and monitoring/control of processes can involve the original design and sequencing of operations, the installation of the process, and the gathering and reporting of process data. Most of this work can be accomplished with simple software running on a personal computer. The theory, and practice, of computerization of a frying process are shown by actual example.

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THE EMERGING ROLE OF COMPUTERS IN THE PROCESS PLANT. John E. Blanchard, The Foxboro Company (D.875), Rte. 140, Foxborough, MA 02035.

Technological, social and economic realities are resulting in the use of computers in all facets of a manufacturing plant. These uses extend from many types of microprocessor-based controllers on the plant floor to professional computers in the manager's office. This paper will highlight some recent innovative applications and discuss methods of successfully integrating computer systems to achieve improved control and plant management. Technologies of today often influence plant operating procedure. Therefore, consideration should be given to potential conflicts with proven management methodologies and constraints on delegating responsibility to various levels of plant personnel and equipment. How management selects and integrates this technology into their unique operating environment will determine the economic benefits they can achieve.

Meetings

67

THE USE OF ROBOTICS IN CHEMICAL ENVIRONMENTS. James N. Little, Richard K. Brown and Jesse Hoffmann, Zymarck Corporation, Zymarck Center, Hopkinton, MA 01748.

A new technology, robotics, already being used in other fields, is slated to have a major impact in automating operations and procedures performed in chemistry laboratories during the eighties. An introduction to laboratory robotics, which combines the technologies of chemistry, analytical instrumentation, computers and robotics, will be presented. Laboratory automation, once limited to computerized data reduction, now includes sample handling and sample preparation, wet chemistry procedures, laboratory process control and instrumental analysis. Laboratory robots, utilizing programmable computers, can easily be reprogrammed to do a variety of laboratory procedures and thus, do not require a large quantity of identical, repetitive operations to justify the investment in capital and time. Examples will be given in automated sample preparation for chromatography, spectroscopy and other laboratory techniques.

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CONTINUOUS BATCH CONTROL WITH COMPUTERS IN EDIBLE OIL PROCESSING. Patrick H. Bush, Philip A. Bollheimer and J. Stephen Harris, PSI Process Systems, Inc., 4466 Elvis Presley Blvd., Memphis, TN 38116.

Programmable logic controllers and computers have been seeking their way into the edible oil industry for several years. The use of these electronic devices has allowed tighter control of operating parameters with increased production while freeing workers to do other tasks. This paper addressed computer methods of process control in the edible oil industry, where continuous and batch processes are coupled to achieve a steady product output. With computers, accurate oil flow control and ratio control of caustic and bleaching clay minimize waste and increase filter life. Coupling the filter flow setpoint to the refinery feed flow while maintaining acceptable tank levels prevents the continuous refining process from leading or lagging the continuous bleaching process. Continuous caustic dilution and addition can be accomplished in exact dry weight ratios regardless of density or oil flow rate. Hydrogenation and blending batch processes historically have required the operator to perform many tasks in preparation for a batch. Computers allow repetitive batches to be run through memory resident recipes that automatically perform ingredient additions and reaction control.

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USE OF COMPUTERIZED DATA ACQUISITION IN MONITORING AND CONTROLLING TECHNICAL PROCESSES. Andrew Reinhardt, Keithley/DAS, 349 Congress St., Boston, MA 02210.

This seminar will discuss procedures and applications for an automated data acquisition and control using the Keithley/DAS Series 500 System with the IBM Personal Computer. The Series 500 is representative of a new class of data management systems now coming into widespread use in research institution. By using personal computers, inexpensive modular hardware and sophisticated software, these systems have transformed the previously complicated and expensive task of data acquisition and real-time control into a routine procedure, thus allowing the experimenter to concentrate on data and results, rather than on interfacing and programming. A wide variety of inputs and outputs are supported directly by the system, including analog input (Volts DC, Thermocouples, RTD's Strain Gages, 4-20mA), analog output (VDC, mA), digital input and output, high-speed pulse counting and solid-state relay switching. The companion software system, Soft 500, supports high-speed data I/O graphics, statistics and a foreground/background multitasking environment. The seminar will introduce examples of current control applications for the Series 500, as well as an overview of the system's overall capabilities and architecture. A question and answer session follows the presentation.

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HIGH SPEED-HIGH TECH–PACKAGING. Franklin P. Khym, Technical Consultant, Vegetable Oil Industry, 11716 Whisper Dew, San Antonio, TX 78230.

High speed wrapping and packaging of margarine and other extrudable products using state of art hot melt system, electronic controls and micro processor monitoring of all functions.

SESSION M Utilization of Vegetable Protein in Foods Monday afternoon

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UTILIZATION OF VEGETABLE PROTEINS IN BAKERY PRODUCTS. William J. Hoover, American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502.

The emphasis in this paper will be on the functional properties of vegetable proteins and their application in bakery foods. In the United States, the use of vegetable proteins has been for nutritional improvement or for specific quality improvement of the finished food. Elsewhere, the emphasis has been an attempt to utilize a local crop by using vegetable proteins to replace wheat flour in bakery product formulations. The availability, economics and technology involved in these efforts will be presented.

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FERMENTED AND UNFERMENTED PROTEIN FOODS FROM LEGUMES. C.W. Hesseltine, Northern Regional Research Center, ARS, USDA, 1815 N. University St., Peoria, IL 61604.

Before recorded time, fermentation was used already as a means to improve legumes as food. This technology was developed almost exclusively in Asia, especially in India, Indonesia, Indochina, China, Korea and Japan. The major legume crops used were soybeans and, to a lesser degree, peanuts. The soybean products we know the most about are miso (bean paste), shoyu (soy sauce), natto, hamanatto, sufu (Chinese cheese) and tempeh. Ontjom is made from peanuts. The two principal nonfermented products are soy milk and tofu. Both are made from soybeans, with the latter being a coagulation of soybean milk to make a soft curd or a harder, cheeselike product. In the preparation of all these foods, several steps are always used including soaking, cooking and the discarding of the cooking water. Using fermentation in making soy foods: (1) destroys some of the undesirable soybean properties; (2) makes it possible to preserve the product without refrigeration; (3) makes the soybean more digestible; (4) enhances flavors; (5) produces desirable nutrients not present in the original product, such as vitamin B_{12}; (6) may drastically change the color and the physical state of the substrate, and (7) may reduce the energy required to produce the food. The following foods are now being produced in the U.S. for the non-Oriental market: tempeh, miso, shoyu and the nonfermented product tofu. Currently, about 24,300 metric tons of the latter product are produced in the U.S. Each of the above foods will be described and the manner of manufacture given.

73

UTILIZATION OF VEGETABLE PROTEINS IN INFANT FORMULAS. W.A.B. Thomson, Ross Laboratories, 625 Cleveland Ave., Columbus, OH 43216.

Infant formulas containing vegetable protein are used successfully for the nutritional management of infants who encounter difficulty when fed milk or milk-based formulas. The vegetable protein must provide the required nutrition while indigenous anti-nutritional factors are reduced and controlled to acceptable levels. The selection and processing of vegetable proteins to yield infant formulas which meet regulatory and nutritional requirements will be discussed.

74

DEVELOPMENT OF IMITATION CHEESES FROM PLANT PROTEINS. Khee Choon Rhee, Texas A&M University, Food Protein Research and Devlopment Center, F.M. Box 183, College Station, TX 77843-2476.

Cheese is an important, nutritious food in the human diet. However, due to rapidly increasing prices of dairy products, it is gradually being priced out of the reach of lower income groups, including

retired people on fixed incomes. Cheese analogs are believed to be a potential solution to this economic problem. By substituting lower-priced vegetable sources for higher-priced milk-derived protein and fat ingredients, cheese analogs would extend the supply of some varieties of cheese and lower costs to consumers. Successful marketing and consumer acceptance of imitation cheese products eventally will be determined by their functional characteristics such as texture, melting behavior, flavor and color, as well as the cost and availability of raw ingredients and final products. To develop and improve the quality of plant protein-based analog cheese, effects of plant proteins such as soybean, peanuts, sunflowerseed and cottonseed and their modifications on the textural and melting properties of analog cheeses have been studied. The results thus far obtained indicate that acceptable analog cheese can be produced from properly manufactured peanut and soy proteins. This paper discusses some of the approaches, protein manufacturing and modification methods, formulations and quality evaluation methods used in the manufacturing of selected imitation cheese products.

75

THE FUNCTIONALITY OF OILSEED PROTEINS AND THEIR PERFORMANCE IN BAKED PRODUCTS. Sharon L. Melton, University of Tennessee, Department of Food Technology and Science, P.O. Box 1071, Knoxville, TN 37901.

Oilseed proteins are added to baked products (1) to increase the amount and/or improve the quality of their protein; (2) to improve quality characteristics such as texture, and (3) to replace more expensive food proteins. Addition of any oilseed protein in excess of 5 to 10% for nutritional purposes or replacement of a more expensive protein such as milk or egg proteins usually has deleterious effects on baked product quality. These effects can be lessened and even overcome in many cases by altered production methods, by addition of special ingredients, or by chemical and/or enzymatic modifications of the oilseed proteins. Many of these alterations and modifications resulting in commercially available oilseed protein products have not been reported. Recently, however, some studies concerning the effect of different production techniques and modifications on the functionality of oilseed proteins have been reported. Prediction of protein behavior in food systems on the basis of functional characteristics alone, however, is difficult. The most frequently used functional characteristic for selection of an oilseed protein for specific food use is its solubility. Of equal importance in baked products such as chiffon desserts and leavened products is the foaming behavior of the proteins under a variety of conditions, or in cakes, the ability of proteins to aid in formation and stabilization of emulsions. Performance of selected oilseed proteins (some of which may be modified) in baked products will be reviewed in relation to their functional characteristics and, possibly, their chemical and physical properties.

SESSION N Fats and Oils Processing (General) I Tuesday morning

76

PHOSPHORUS COMPOUNDS IN REFINED PALM OIL. W.L. Siew and Augustine S.H. Ong, Palm Oil Research Institute of Malaysia, P.O. Box 10620, Kuala Lumpur, Malaysia, and J.C. Allen, North East Wales Institute, United Kingdom.

The phosphorus compounds present in refined palm oil were studied. They consist of inorganic phosphates and an organic phosphate complex. The organic complex was extracted from laboratory refined palm oil using methanol and purified using column and thin layer chromatography techniques. Similar compounds were found in commercial refined oils. Identification of the organic phosphate using thin layer chromatography and various staining reagents showed that these compounds were formed during the deodorization process and were not present in the crude oils. NMR and IR spectroscopic studies showed the presence of glyceridic bonds. Possibilities pointed to the phosphorylation of monoglycerides and diglycerides with residual phosphoric acids during the deodorization process.

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RATES OF ADSORPTION OF SOY OIL PHOSPHOLIPIDS ON SILICA AND THE EFFECT OF SILICA CONCENTRATION. Helen G. Brown and Harry E. Snyder, University of Arkansas, Department of Food Science, Route 11, Fayetteville, AR 72701.

Previous studies have shown that soy oil phospholipids can be irreversibly adsorbed on silica from a hexane:isopropanol (99:1) miscella. This adsorption results in a Freundlich isotherm. These studies on soy oil phospholipid have been extended to measure rates and extent of adsorption of phospholipids which depend on the starting concentration of phospholipids. We found that the extent of adsorption also depended on the concentration of silica. With increasing concentration of silica, more total phospholipid is adsorbed, but less is adsorbed per gram of silica. To take advantage of the more complete adsorption on silica at high silica concentration, beds of silica were prepared and crude soy oil miscellas passed over the beds. Conditions for adsorption and desorption of soy oil phospholipids from these silica beds will be described.

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PILOT PLANT STUDIES ON EXTRACTING COTTONSEED WITH METHYLENE CHLORIDE. L.A. Johnson, J.T. Farnsworth, N.Z. Hassanen and E.W. Lusas, Food Protein R&D Center, Texas A&M University, College Station, TX 77843-2476.

The practical feasibility of using methylene chloride to simultaneously extract oil, aflatoxin and gossypol from cottonseed was demonstrated in a 56-hr trial using a pilot plant-scale continuous extractor. Nine different treatments, varying in residence time, solvent:flake ratio and flake preparation method were evaluated. Residual oil contents of the meal were lower than those typically observed with hexane extraction under like conditions. Aflatoxin content was greatly reduced, making possible the salvage of cottonseed meal that otherwise would exceed current levels of restriction. Because gossypol also is extracted, it may be possible to produce cottonseed meals that are well suited for use in poultry and swine feeds. Meal extracted with methylene chloride desolvented easily, and residual solvent levels were less than 30 ppm. The oil was refined and bleached to acceptable quality, and no residual aflatoxin was observed in the alkali refined oil.

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FACTORS INFLUENCING SOYBEAN OIL EXTRACTION RATES. Kurt L. Wiese and Harry E. Snyder, Department of Food Science, University of Arkansas, Route 11, Fayetteville, AR 72701.

Soy oil is extracted from flakes at a continually decreasing rate. The reason for this pattern is not well understood but is probably due to time for diffusion of the solvent into and out of the flakes or to intact tissue in the flake that solvent doesn't readily penetrate. We initiated experiments on this problem using electron microscopy to study the extraction. Transmission electron micrographs failed to distinguish between lipid in unextracted flakes and lipid remaining in partially or fully extracted flakes. Experiments with the various fixation, staining and dehydration procedures will be discussed, but these have failed to improve differentiation between extracted and non-extracted tissue. It is well known that soy protein binds lipid in aqueous systems. We investigated possible lipid binding by proteins in hexane by equilibrating miscella with defatted flakes. These experiments gave no evidence for lipid binding by proteins in hexane miscellas. Experiments with intact soy cotyledon tissue show very little hexane penetration at room temperature but considerable penetration at 50-60 C. Data on extraction rates with flakes and with different mesh flours will be presented and discussed in relation to controlling factors for soy oil extraction.

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EXAMINATION OF THE SELF-DEGUMMING PROPERTIES OF PHOSPHOLIPIDS IN SOYBEAN OIL. J.E. Ragan, Ralston Purina Company, Protein Technologies R&D, 4RN, Checkerboard Square, St. Louis, MO 63164, and A.P. Handel, Drexel University.

Freshly extracted crude soybean oil (ca. 12 liters) was obtained from a commercial soybean oil refinery and divided into ca. 360 g aliquots. The aliquots were stored at 24 C and 5 C for varying lengths

of time up to two weeks and analyzed for total phosphorus. The concentrations of phosphatidylethanolamine (PE), phosphatidic acid (PA), phosphatidylinositol (PI) and phosphatidylcholine (PC) were determined by phosphorus analysis following separation by HPLC. Fractions from the aliquots were degummed and total and individual phosphorus contents were determined as described above. Total phosphorus decreased ca. 41% in crude oil stored at 24 C and ca. 34% in crude oil stored at 5 C over two weeks. PE and PC precipitated more readily than PI and PA. Total phosphorus remaining in degummed oil was least after 12 hr storage of the crude oil and increased as the storage period became longer. The PE, PI and PA concentrations showed similar trends in degummed oil. However, the PC concentration was least at 0 hr and increased thereafter. These results will be discussed in terms of phospholipid-phospholipid interactions and possible interactions between phospholipids and other compounds present in the oil.

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SEQUENTIAL EXTRACTION PROCESS FOR EXTRACTING OIL AND AFLATOXINS FROM COTTONSEED. N.Z. Hassanen, L.A. Johnson, J.T. Farnsworth and E.W. Lusas, Texas A&M University, Food Protein R & D Center, College Station, TX 77843-2476.

A new process was developed for extracting oil and aflatoxin from cottonseed using 91% isopropanol. This new sequential extraction technique involves extracting only oil in the first step and reclaiming the solvent by chill separation. No net extraction of aflatoxin was observed in the first step, because it remained in solution with the reclaimed solvent. Aflatoxin was extracted in the second step by extracting with fresh 91% isopropanol and reclaiming the solvent by evaporation. The oil content was reduced to less than 2% in the first step and less than 0.5% in the second. Aflatoxin was reduced from about 200 ppb to 20 ppb. This sequential extraction technique eliminates the need to use two different solvents and separate extractors, a technique that has been used to achieve similar extraction efficiency.

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DETERMINATION OF PHOSPHORUS IN SOYBEAN OIL USING NEPHELOMETRY. Roger D. Sinram, A.E. Staley Manufacturing Co., 2200 E. Eldorado St., 63 Building, Decatur, IL 62521.

Colorimetric phosphorus analyses are tedious and time consuming. An alternative procedure using nephelometry has been developed that is much less demanding analytically to measure phosphorus content of soybean oil. The nephelometric method utilizes the relationship between phosphorus level due to phosphatides in soybean oil and turbidity. Phosphorus analyses were performed on over 50 samples of crude, degummed, refined and bleached soybean oils employing the standard colorimetric approach (AOCS Official Method CA 12-55) and the nephelometric method using a Hach Model 18900 ratio turbidimeter. The resulting phosphorus versus nephelos data graphed a nearly linear relationship (correlation coefficient of 0.91) for phosphorus levels ranging from 0 to 170 ppm. It was necessary to modify the procedure slightly by first diluting the sample with lower phosphorus oil (Staley EDSOY) to accomodate analyses of crude soybean oil with phosphorus content in the 400 to 500 ppm range. This also gave a nearly linear correlation when phosphorus was plotted against nephelos (correlation coefficient of 0.96). The nephelometric procedure has been used for three years at A.E. Staley Mfg. Co.'s Des Moines Oil Refinery, for effective process control of degummed, caustic refined and bleached soybean oil. Nephelometric phosphorus results could be known within 10 min of sampling, while several hours were required using the conventional analysis.

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THE FUNCTION OF BLEACHING EARTHS IN THE PROCESSING OF SOYBEAN OIL. David B. Shaw, R.S. Taylor, M.J. Sidebottom and M.G. Ball, Laporte Inorganics, P.O. Box 2, Moorfield Rd., Widnes, Cheshire, WA8 OJU Great Britain.

The use of acid activated montmorillonites in the refining of soybean oil is a widely accepted process. In this paper the current state of knowledge from published sources is appraised. In our own work, it is shown how by modification of physical and chemical properties materials with specific performance characteristics may be produced. The impact such changes have upon the quality of fully refined oil is demonstrated. Examination of surface area, pore size distribution and surface composition of bleaching earths is described. This information is then used to achieve the optimum performance by modification of a particular clay. The sorption process occurring during triglyceride bleaching is complex. Consideration is given to the mechanisms by which trace components of the oil such as pigments, phosphatides and soaps are removed. Other clays have been considered as possible alternatives to acid activated montmorillonites, but are shown to fall short in performance. It is demonstrated how changes in bleaching earth properties and oil processing conditions affect soybean oil color, composition and oxidative stability.

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THE POTENTIAL OF RECOVERING VITAMIN E FROM OIL PALM INDUSTRY. A. Gapor, A. Kato, T. Kawada and H. Watanabe, Palm Oil Research Institute of Malaysia, P.O. Box 10620, Kuala Lumpur, Malaysia.

Vitamin E, which is naturally present in crude palm oil, is concentrated in palm fatty acid distillate (PFAD) during the deodorization step in physical refining. The potential of PFAD as a source of vitamin E will be discussed. The level of vitamin E in oil palm leaf also was found to be high and therefore identified as another source of vitamin E. Methods for the isolation of vitamin E will be discussed.

85

UPDATE ON EDIBLE BLEACHING. W. Zschau, Süd Chemie, Postfach 20 22 40, 8000 Munich 2, West Germany.

Bleaching clay has been used in the refining of edible fats and oils for more than a century. It was used as a tool for color improvement mainly. Since the early 1970s its task has changed considerably, however. The importance of other parameters grew, as the understanding of the process in fats and oils improved. Nowadays bleaching clay is mainly used to reduce the amount of gums, soaps, oxidation products, certain colorbodies as chlorophyll and heavy metals, for instance nickel, in the postbleaching. Data will be given for canola, palm and soy oil and tallow.

SESSION O Flavor Chemistry of Fats and Oils I
Tuesday morning

86

FLAVOR CHEMISTRY OF FRIED CHICKEN. Chi-Tang Ho and Stephen S. Chang, Rutgers, The State University, Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, New Brunswick, NJ 08903.

The volatiles with a genuine flavor of the original fried chicken were isolated from 150 lbs. of fried chicken. A total of 134 compounds were identified in the volatiles of fried chicken. The volatile flavor compounds that contribute to fried chicken flavor may be formed from constituents of the chicken during the frying process; thermal and oxidative decomposition of the frying fat itself, and the interaction of decomposition products from the chicken and the frying fat. The compound classes identified in the volatiles of fried chicken included hydrocarbons, alcohols, aldehydes, ketones, acids, esters, pyrazines, pyridines, oxazoles, thiazoles and trithiolanes. Four trithiolanes, 3,5-dimethyl-1,2,4-trithiolane, 3,5-diisobutyl-1,2,4-trithiolane, 3-methyl-5-butyl-1,2,4-trithiolane and 3-methyl-5-pentyl-1,2,4-trithiolane identified in fried chicken flavor possess roasted and pork rind-like aromas and could be important contributors to chicken flavor. They probably are formed through the interaction of aldehydes and hydrogen sulfide. Aldehydes are formed either through lipid oxidation or Strecker degradation. The identification of alkyloxazoles and alkylthiazoles with long-chain alkyl group substituted at the C-2 position of the oxazole or thiazole ring suggested that they may form through interaction between frying fat or its decomposition products with amino acids.

Meetings

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EFFECTS OF MINOR COMPONENTS ON THE FLAVOR STABILITY OF SOYBEAN OIL. Behroze S. Mistry and David B. Min, Ohio State University, 122 Vivian Hall-2121 Fyffe Rd., Columbus, OH 43210.

High quality soybean oil was passed through a silicic acid column to isolate minor components. The minor compounds retained on the chromatographic column were separated into 12 subfractions by a combination of step-wise gradient elutions and low temperature solvent fractional crystallization. The effects of these subfractions on the flavor stability of soybean oil indicated that these components were prooxidants. These fractions were characterized by combination of different spectrometries and gas chromatography. The effects of minor components on the flavor stability of soybean oil showed that the decrease in flavor stability could be correlated to the increase in polarity of the eluting solvents and to the increase in hydroxyl groups of the minor components. These minor components seemingly were formed during processing and storage of soybean oil. The strong prooxidant component in soybean oil was positively identified as SN-1-monolinolein by a combination of infrared, mass and nuclear magnetic resonance spectrometries. The flavor stability of soybean oil was greatly improved by the removal of minor components, espeically monolinolein by silicic acid chromatography.

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FRYING ODORS: SENSORIAL AND PHYSICOCHEMICAL APPROACHES. A. Prevot, S. DesBordes, O. Morin and F. Mordret, Institut des Corps Gras, Rue Monge, Parc Industriel, 33600 Pessac, France.

An analytical method based on sensory perception has been used to examine odors developed when frying potatoes under domestic conditions. The panel was invited to attribute a global mark for quality and also to describe the type and intensity of odor detected. The data collected allowed us to follow by means of curves and histograms the sensory development, frying after frying. Significant and reproducible results were obtained for peanut, soybean, rapeseed and sunflower oils. It was then possible to point out correlations between types of odor and the frying conditions, or the preceding technological process. Results from odor trapping experiments on microscale frying followed by GLC/MS also will be presented.

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FLAVOR COMPONENTS FROM BUTTEROIL. COMPARISON WITH COMMON FRYING OILS. M. Bradley and W.W. Nawar, University of Massachusetts, Department of Food Science and Nutrition, Amherst, MA 01003.

In a continuing effort to study the effects of processing conditions on the thermal stability of edible oils, the volatiles produced in milk fat by heating at frying temperatures were examined qualitatively and quantitatively. The fat was heated for various periods with and without the frying of food and the volatiles collected by high-vacuum cold-finger distillation, fractionated into polar and nonpolar fractions, and analyzed by capillary GC/MS. Several common frying oils, treated under identical conditions, were similarly examined. These included tallow and soybean, corn, rapeseed, safflower and olive oils. The relationships between the composition of milk fat, its volatile pattern and its unique stability and flavor characteristics, as well as differences from other oils, will be discussed.

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FLAVOR CHEMISTRY OF FATS AND OILS–PAST, PRESENT AND FUTURE. Sherman S. Lin, Anderson Clayton Foods, 3333 N. Central Expressway, Richardson, TX 75080.

Flavor and flavor stability of oils always has been one of the major concerns of the oil processing industry and the sector of the food industry which uses oil as an ingredient. Flavor chemistry of oils has been studied for a long time, but the major surge of activity did not come until after gas chromatography was invented in the early 1950's. With the advancement of instrumentation, a great number of reports on the isolation, separation and identification of oil flavors were published. The advancement of oil flavor chemistry in the 1960's and early 1970's was very rapid. The oil processing industry has been successful in utilizing the chemical knowledge accumulated in those years to produce better oil products for consumers and institutional customers. However, the progress in recent years has been relatively slow. In this presentation, the author will try to examine what has been achieved in the past, what is in the present status, and what more can be done in the future on flavor chemistry of oils. The presentation is not a review but rather a sharing of personal viewpoints on the subject as a start for the symposium.

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COMPARISON OF GAS CHROMATOGRAPHIC METHODS FOR VOLATILE LIPID OXIDATION COMPOUNDS IN SOYBEAN OIL. E.N. Frankel, Edward Selke, Janet M. Snyder and Kathleen Warner, Northern Regional Research Center, ARS-USDA, 1815 N. University St., Peoria, IL 61604.

Much attention has been given recently to the correlation between volatile analyses by gas chromatography (GC) and flavor evaluation of vegetable oils. However, not much is known on how flavor problems can be related to the nature, origin or significance of volatile compounds. To develop new knowledge on preventing or eliminating the formation of undesirable flavors in foods containing polyunsaturated lipids, we investigated the quantities of selected volatile lipid oxidation products present in samples of known processing and storage histories. The volatiles in soybean oil oxidized under different conditions were examined by three capillary GC methods: (a) Direct injection (8 min heating–180 C), (b) Headspace (20 min heating–180 C, 1 min pressurization), (c) Dynamic headspace and trap (5-15 min purging, heating 60 to 180 C, desorbing from porous polymer trap at 170 C). Fused silica columns were used with bonded mixed dimethyldiphenyl siloxane phases. At peroxide values between 2 and 10, the major volatile products found by the three methods were pentane, hexanal, 2-heptenal, 2,4-heptadienal and 2,4-decadienal. Additional volatiles detected by headspace GC included acrolein, pentene, propanal, 1-penten-3-ol, pentanal, 2-hexenal, pentyl furan, 2-octenal, nonanal and decatrienal isomers. Analyses by the dynamic headspace and trap method on samples heated to 60 C showed significantly less pentane, propanal, acrolein, 2-heptanal and 2,4-decadienal. These volatiles are thermal decomposition products detected in significant amounts only when samples are heated above 100 C. Therefore, compositions of the volatiles obtained by GC methods requiring sampling temperatures exceeding 100 C reflect major amounts of breakdown products of precursors of oxidation, rather than volatiles present in samples at the time of flavor evaluation. A judicious use of several GC techniques is necessary to evaluate volatiles both at time of tasting and volatiles generated from lipid oxidation precursors.

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FLAVOR CHEMISTRY OF OLIVE OIL. Enzo Fedeli, Stazione Sperimentale per le Industrie, Degli Oli e Grassi (SSOG) Milano, Italy.

The social and economic importance of Olea Europea in ancient times is well documented in the classical literature of the Mediterranean Basin. Even today olive oil is one of the most important fats in the diet of a large segment of the Mediterranean population. Most of the oil of high quality is consumed without refining and retains a characteristic flavor very much appreciated by the consumer. The technology of olive oil processing, very conservative in the past, is now in evolution and can influence the quality of the oil in relation to flavor properties as well as the keeping properties of the oil, which is strongly related to the aroma. The presence of bitter constituents is also dependent on processing parameters. The chemical structures of the bitter components present in olive oil have been elucidated in our institute and will be presented together with the structures of phenolic components which affect the flavor of the oil and its keeping properties. Recovery of aroma components from by-products and from low quality olive oils is an additional point which will be considered. The possibility of applying this technology on an industrial laboratory and/or pilot plant scale will be discussed. Most of the research on chemical constituents has been done using HPLC as an analytical and preparative tool. GLC-MS was used to assign the chemical structures along with other chemical tools. The methods utilized will be described briefly.

Meetings

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DYNAMIC HEADSPACE GAS CHROMATOGRAPHY/MASS SPECTROSCOPY TECHNIQUE FOR DETERMINING VOLATILES IN VEGETABLE OILS. David M. Wyatt, Eastman Chemical Products, P.O. Box 431, Kingsport, TN 37662.

A dynamic purge and trap technique has been developed to analyze volatiles formed in ambient stored soybean oil. The work was performed on a Unacon 810B headspace concentrator equipped with capillary column and flame detector. This instrument is interfaced to a HP 5992B benchtop mass spectrometer. This arrangement allows simultaneous quantitative and qualitative analysis. One gram of vegetable oil is purged with nitrogen under mild heat. The volatiles are quantitatively trapped on a Tenax/Ambersorb/graphite trap. This trap can be stored for subsequent analysis or analyzed immediately by thermally desorbing and backflushing the volatiles onto the Unacon. Using nonanen as internal standard, volatiles were monitored in a freshly deodorized soybean oil stored at room temperature and light for 67 days. Analysis indicated an increase of total volatiles from 1.8 ppm on day 0 to 8.2 ppm during this time. Peroxide values ranged from 0.0 to 18.8 during this interval. Specific volatiles identified by mass spectroscopy were pentane, hexanal and 2-heptanal. Response factors for the internal standardization technique varied ±5% during the study.

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QUANTIFICATION OF CARBONYLS PRODUCED DURING DECOMPOSITION OF FATTY ACID HYDROPEROXIDES. B. Saidia and Earl G. Hammond, Iowa State University, Department of Food Technology, Ames, IA 50011.

Linoleic acid-13-hydroperoxide produced by soybean lipoxygenase I oxidation was dissolved in dodecane and decomposed under vacuum to investigate the effect of various decomposition conditions on the yield of carbonyl compounds. The carbonyls were isolated as 2,4,6-trichlorophenylhydrazones and quantified by gas chromatography. Molar yields of carbonyl were 1-5% of the hydroperoxide decomposed. At 40 to 55 C only hexanal was identified among the products. Other products were at best an order of magnitude less than hexanal. At 80 and 160 C considerable quantities of 2,4-decadienal also were produced. The presence of tocopherol, Cu^{++} and Fe^{++} accelerated peroxide decomposition and resulted in lower yields of hexanal. BHA accelerated peroxide decomposition but resulted in higher yields of hexanal.

SESSION P Surfactants and Detergents II—Surfactant Interaction Tuesday morning

95

A REVIEW OF MOLECULAR INTERACTION AND SYNERGISM IN BINARY MIXTURES OF SURFACTANTS. M. Rosen, Brooklyn College, City University of New York, Brooklyn, NY.

Abstract not available at press time.

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INTERACTIONS BETWEEN LINEAR ALKYLBENZENE SULFONATE AND WATER HARDNESS IONS. I. EFFECT OF WATER HARDNESS ON SURFACTANT SOLUBILITY. K. Lee Matheson and Michael F. Cox, Vista Chemical Company, P.O. Box 500, Ponca City, OK 74602.

The relationships between surfactant concentration and calcium ion (water hardness) concentration for three different molecular weight linear alkylbenzene sulfonates are presented in the form of precipitation boundary diagrams. These diagrams describe solubility and micellization behavior of LAS in the presence of calcium. Implications for detergency performance are discussed.

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INTERACTIONS BETWEEN LINEAR ALKYLBENZENE SULFONATE AND WATER HARDNESS IONS. II. REDUCING HARDNESS SENSITIVITY BY THE ADDITION OF MICELLE PROMOTION AGENTS. Michael F. Cox and K. Lee Matheson, Vista Chemical Company, P.O. Box 500, Ponca City, OK 74602.

Agents that promote micellization of linear alkylbenzene sulfonates (e.g. cosurfactants, inorganic salts) also improve hard water detergency by reducing Ca^{++} ion sensitivity. A model is proposed which incorporates micellization behavior and counterion binding. Correlations are made between water hardness sensitivity and detergency performance.

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INTERACTIONS BETWEEN LINEAR ALKYLBENZENE SULFONATE AND WATER HARDNESS IONS. III. SOLUBILIZATION AND PERFORMANCE CHARACTERISTICS OF $Ca(LAS)_2$. Dewey L. Smith, Michael F. Cox and K. Lee Matheson, Vista Chemical Company, P.O. Box 500, Ponca City, OK 74602.

The bulk solubility of $Ca(LAS)_2$ is shown to be affected by the presence of micelle promotion agents (e.g., high-mole EO nonionic surfactants). These agents increase the apparent solubility constant of $Ca(LAS)_2$ and help solubilize it under conditions where precipitation normally would occur. Possible mechanisms for solubization are discussed. Correlations are presented of $Ca(LAS)_2$ solubility and detergency performance.

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SALINITY TOLERANCE ENHANCEMENT IN ANIONIC SURFACTANT SOLUTIONS BY ADDITION OF NONIONIC SURFACTANTS. Kevin L. Stellner and John F. Scamehorn, University of Oklahoma, School of Chemical Engineering and Materials Science, Norman, OK 73019.

The addition of small concentrations of nonionic surfactants to anionic surfactants can increase the tolerance of the aqueous system to salinity and hardness. This decreased tendency to precipitate in these mixed surfactant systems is due to the formation of nonideal mixed micelles. Mixed anionic/nonionic micelles show large negative deviations from ideality; i.e., the mixed micelles tend to form more easily than pure component micelles. As a result, the anionic surfactant monomer concentration in equilibrium with the mixed micelles is lower than that in equilibrium with pure anionic surfactant micelles. Therefore, it requires a higher concentration of cations to exceed the solubility product of the precipitate formed between the cation and the monomeric surfactant anion. In this study, the model surfactant system of sodium dodecylsulfate and a nonphenol polyethoxylate was studied. The monomer-micelle equilibrium and precipitation phase boundaries were measured for various surfactant compositions with NaCl as the added electrolyte. Increased salinity tolerance due to addition of nonionic surfactant is examined in the context of mixed micelle nonidealities.

SESSION Q Chemistry and Biochemistry Cholesterol Oxidation Tuesday morning

100

CHLORINATED HYDROCARBON MEDIATED CHOLESTEROL DEGRADATION. Johan E. van Lier and Réjean Langlois, MRC Group in the Radiation Sciences, University of Sherbrooke Medical Center, Sherbrooke, Quebec, Canada J1H 5N4.

The hepatotoxicity of halogenated hydrocarbons is believed to result from their interaction with the drug-metabolizing cytochrome P-450 systems, which provide electrons for their reduction to halogen anions and alkyl radicals. Although the latter radicals may react directly with cell substituents, the main injurious oxidative species are thought to include alkylperoxy radicals formed upon reaction of the alkyl radicals with molecular oxygen. Formation of these free radicals may initiate radical chain reactions, including lipid peroxidation, which result in biological damage. The most reactive peroxy radical of this type is the trichloromethyl peroxy radical derived from carbon tetrachloride. The $Cl_3C\cdot$ radical is conveniently formed by γ-irradiation of aqueous alcohol solutions containing traces of CCl_4. In this system, the normal radiolysis products of water are scavenged to yield $Cl_3C\cdot$ as the sole reactive species which,

in the presence of O_2, is readily converted to Cl_3COO. Under air-saturated conditions, cholesterol is degraded rapidly in this model system to yield a 2:1 mixture of the 5,6α- and 5,6β-epoxy derivatives of cholesterol. Prolonged irradiation results in epoxide opening by radiolytically formed HCl to give the corresponding chlorohydrin derivatives. Formation of the epoxides may proceed through an intramolecular cyclization reaction of intermediate Cl_3COO-cholesterol adducts at the 6α- or 6β-position of cholesterol. The role of these oxidized sterols with known mutagenic properties in producing tissue injury after exposure to halogenated hydrocarbons is suggested.

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CHOLESTEROL OZONIZATION IN NONPARTICIPATING SOLVENT. Krzysztof Jaworski and Leland Smith, Division of Biochemistry, University of Texas Medical Branch, Galveston, TX 77550.

Previously we have addressed the question whether ozone initiates cholesterol autoxidation in water or alcohols and demonstrated that 5,6-epidioxy-5,6-diol or 6-alkoxy-5,6-epidioxy-5-ols secosterols are sole initial products, with no autoxidation occurring. We now have extended this interest to nonparticipating solvent CCl_4. Cholesterol ozonization products in CCl_4 never have been properly described. Ozonization at room temperature gives four initially formed peroxides more mobile than cholesterol recognized as oligomeric products by ^1H and ^{13}C NMR spectra. More polar products form at longer times. The major early peroxidic product isolated by high performance liquid chromatography gave an acetate upon treatment with acetic anhydride/pyridine. Reduction with Zn/acetic acid gave cholesterol and 3β-hydroxy-5-oxo-5,6-secocholestan-6-al. No products of radical autoxidation of cholesterol were detected, thus demonstrating that ozone did not initiate autoxidation in CCl_4. Ozonization of cholesterol 3β-acetate gave two more polar ozonides resolved only by repeated recycling of their mixture by high performance liquid chromatography. These two products appear to be stereoisomeric secondary (1,2,4-trioxolane) ozonides 5,6α-epidioxy-5α-6-oxa-B-homocholestan-3β-ol 3β-acetate and 5,6β-epidioxy-5β-6-oxa-B-homocholestan-3β-ol 3β-acetate, prepared pure and fully characterized for the first time.

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AUTOXIDATION OF SITOSTEROL IN LIPID SYSTEMS OF DIFFERENT UNSATURATION DEGREE. N. Vl. Yanishlieva-Maslarova and E.M. Marinova, Institute of Organic Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bl. 11, kv. Geo Milev, Bulgaria.

The autoxidation of sitosterol has been studied in triglycerides of different degrees of unsaturation: tristearin (I) and the triglycerides of lard (II) and sunflower oil (III). Under equal oxidation conditions, the increase in unsaturation degree of the lipid substrate leads to an increase in the percentage of changed sitosterol. For instance, if 5% sitosterol are oxidized in I, II and III at 120 C for 2 hr, the percentage of changed sitosterol will be 17.7, 37.4 and 43.6%, respectively. On the other hand, the higher the unsaturation degree of a lipid system, the weaker the oxygen attack on sitosterol compared to that on the triglyceride molecules. Under the above oxidation conditions (120 C, 2 hr, 5% sitosterol), the ratio between the amounts of changed sitosterol and changed triglycerides in I, II and III is 3.7, 1.8 and 0.7, respectively. The main components of the changed sitosterol are found to be: in III, stigmasta-3,5-dien 1, stigmasta-3,5-dien-7-on 2, stigmast-4-en-3-on 3, stigmast-4-en-3,6-dion 4, a mixture of 5α, 6α- and 5β, 6β-epoxy-sitosterol 5, 3β-hydroxy-stigmast-5-en-7-one 6, stigmast-5-en-3β,7α-diol 7, stigmast-5-en-3β,7β-diol 8, an epimeric mixture of 7-hydroxy-5,6-epoxy-sitosterols 9, 5α-stigmasta-3β,5,6β-triol 10; in II, 1, 2, 3, 4, 5, 6, 7, 8; in I, 1, 2, 4, 5, 6, 7, 8. The results show that in the saturated system (tristearin), sitosterol initiates the chains, whereas during the oxidation of the triglycerides of lard and sunflower oil it retards the process to a small extent. The ratio between the concentrations of the oxidized sitosterol products having a higher polarity and those with a lower polarity than that of sitosterol is independent of the unsaturation degree of the lipid medium and has a value of 2-3.

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SOME ASPECTS OF THE ANALYSIS OF MINOR OXYGENATED STEROLS IN SERUM AND IN SERUM LIPOPROTEIN FRACTIONS. Charles J.W. Brooks and W. John Cole, Chemistry Department, University of Glasgow, Glasgow G12 8QQ, Scotland (U.K.), and John MacLachlan and T.D. Veitch Lawrie, Department of Medical Cardiology, University of Glasgow, Glasgow G3 12FR.

There are two major problems in the analysis of sterols that occur at low concentrations in serum, or in other biological material rich in cholesterol. The first is the need largely to remove cholesterol and its esters, so as to concentrate the minor sterols. The second is the artifactual formation of steroids, especially oxygenated sterols derived from cholesterol. Our work is based on extraction of serum, or of serum lipoprotein fractions, with solvents; saponification; chromatographic isolation of minor sterol fractions; derivative formation, and gas chromatography-mass spectrometry (GC-MS). Among sterols that are not formed as artifacts during analysis, 24ξ-hydroxy- and 26(25ξ)-hydroxycholesterol occurred in most of the serum samples studied, in amounts of 25-350 ppm with respect to cholesterol. In a typical analysis, 26-hydroxycholesterol was found in serum (310 ng/ml) and in the derived VLDL, LDL and HDL fractions (120, 65 and 100 ng/ml, respectively). In some sera, 4β-hydroxycholesterol was detected in significant amounts (ca. 20-600 ng/ml). In the case of 7α-hydroxycholesterol, as much as 50 ppm can arise by autoxidation of cholesterol during analysis. However, the raised concentrations (200-300 ppm: 600-900 ng/ml) of this sterol in sera of patients treated with "cholestyramine" are satisfactorily measurable. Special interest attaches to the α- and β-oxides of cholesterol because of their known and potential bioreactivity. The oxides can be produced rapidly from cholesterol in amounts of ca. 50 ppm, even under conditions designed to limit autoxidation; consequently, their concentrations in biological material may be overestimated. In our view, the determination of the oxides is likely to be best achieved by indirect means, based upon their transformations in situ into distinctive products. Two methods under investigation are: (i) enzyme-catalyzed oxidation to yield principally the respective 6α- and 6β-hydroxy-4-cholestenones, which can be readily separated and determined by GC-MS of suitable derivatives; (ii) acid-catalyzed hydrolytic cleavage, whereby the α-oxide affords 5α-cholestane-3β,5,6β-triol, whereas the β-oxide also yields, depending on the conditions, a variety of other characteristic products.

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DEUTERIUM-LABELED CHOLESTEROL AS AN INTERNAL STANDARD IN THE ANALYSIS OF OXIDIZED STEROLS. Bruce Wasilchuk, P. Feibush, P.W. Le Quesne and P. Vouros, Department of Chemistry, Northeastern University, 360 Huntington Ave., Boston, MA 02115.

One of the major problems encountered in the analysis of food and biological samples for cholesterol oxidation products is the spontaneous oxidation of cholesterol in the course of sample preparation. Artifacts arising from this autoxidation process are a potential source of significant error in the analysis. Accurate determination of their origin is, therefore, of paramount importance in order to avoid misinterpretation of the analytical results. In this work the utilization of deuterium-labeled cholesterol as an internal standard for monitoring procedural oxidation is described. Cholesterol containing nine deuterium atoms on the hydrocarbon side chain was synthesized and further purified by reversed phase high performance liquid chromatography. The details of the synthesis will be presented. A measured quantity of the d_9-cholesterol is initially added to the sample. The labeled and unlabeled substrates are thus subjected to identical oxidation conditions during the analyte isolation process. By monitoring the ratios of deuterated to undeuterated oxides and comparing with those of labeled to unlabeled cholesterol, a direct means of determining procedural oxidation of cholesterol is obtained. High isotopic enrichment of the synthesized internal standard and the nine atomic mass unit difference between the labeled and unlabeled analogs provide a spectral pattern free of interference, which facilitates the quantitation by GC/MS. Moreover, the trimethylsilyl derivatives of the labeled and unlabeled cholesterol and its oxides are resolved from each other using bonded phase fused silica capillary columns and flame ionization detection (FID). It appears that the concept of using the d_9-labeled cholesterol as an internal standard may even provide a useful screening procedure for monitoring the

origin of oxidation products of GD/FID alone. Applications to the analysis of prepared foods rich in cholesterol will be presented.

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QUANTIFICATION OF CHOLESTEROL OXIDATION PRODUCTS IN SOME COMMON FOODS. S. Won Park and Paul B. Addis, University of Minnesota, Department of Food Science and Nutrition, 1334 Eckles Ave., St. Paul, MN 55108.

Studies on oxidation of cholesterol in food require selective and sensitive detection techniques and isolation of trace quantities of sterols as cleanly as possible. Common experimental procedures can be summarized in three steps: (1) extraction of total lipids out of food sample; (2) further concentration of sterol fractions, and (3) final analysis by chromatography. In spite of the complexity of the food matrix, some dairy, egg and other animal products have been reported to contain some oxidation products that frequently were found in simple dispersions of cholesterol. However, in many studies, identification of the isolated compound was made on the basis of retention indices without further confirmation by other methods such as mass spectrometry (MS). Another uncertainty comes from the fact that artifacts may be created during isolation. Cholesta-3,5-dien-7-one is an example of an artifact formed due to dehydration of 7-ketocholesterol in hot alkaline medium. Recently, we developed a rapid quantification method for C-7 oxygenated cholesterol derivatives using HPLC. Sterol fractions selectively eluted through a short silica gel column were separated and quantified by HPLC with UV detection, 7-keto cholesterol was detected up to 70 ppm in dehydrated organ meat products. α-epoxide survived saponification well and was detected by capillary gas chromatography. However, direct injection of oxidized sterols onto GC was not totally satisfactory because of the thermal instability and unsatisfactory resolution of some important secondary diol derivatives. Recently, these problems have been overcome effectively by employing capillary column GC after silylating the sterols. The new method was applied to quantification of oxidized sterols in heated tallow. 7-ketocholesterol was formed preferentially over epimeric epoxides and epimeric 7-hydroxycholesterols. However, there was no detectable amount of triol or 25-hydroxycholesterol. All other findings were further confirmed by MS with direct inlet probe or combined capillary GC-MS.

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INTESTINAL ABSORPTION OF CHOLESTEROL AUTOXIDATION PRODUCTS IN DIETARY FATS. J. Bascoul, N. Domergue and A. Crastes de Paulet, INSERM Unité 58, 60 Rue de Navacelles, 34100 Montpellier, France.

Various analytical methods (GLC, HPLC, TLC-FID) some of which (GLC) had already been established and tested for the study of the autoxidation products of cholesterol and cholesterol esters (1,2,3) were used for the characterization and assay of oxysterols in heated dietary fats (tallow) and in some nutriment used in animal experimentation. The mean cholesterol concentration in the analyzed fats is 1.6 ± 0.25 g/kg before heating. Heating (for 48 hr at 150-180 C) leads to a loss of about 25% of cholesterol, one-half of which is being transformed into oxysterols. By decreasing order of concentration are found: the 3β-5-6α-trihydroxycholesterol (100 ppm), the 7α- and 7β-hydroxycholesterol (50 ppm), the 7-oxocholesta-3-5-diene (30 ppm), the mixture of the two epoxids α and β (20 ppm) and the 3β-hydroxy-7-oxo-cholesta-5-ene (10 ppm). The concentration of the side chain autoxidation products is in the range of 1 ppm. The same oxysterols are present in industrial food used for experimentation. The intestinal absorption of two of these oxysterols, the triol and the Δ5 epoxides, has been studied on the rat model using the method of Zilversmit (4). These two sterols cross the intestinal barrier in the proportion of 90%. They are very rapidly cleared from the peripheral circulation to enter the entero-hepatic cycle, from which they are very slowly excreted in the feces. All these data should invite special attention to these nutritional oxysterols potentially atherogenous for the human as well as for the experimental animal.

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EVIDENCE FOR A HYDROXYSTEROL BINDING PROTEIN (OSBP) IN DIFFERENT CELL LINES: CHARACTERIZATION AND BIOLOGICAL IMPLICATIONS. M.E. Astruc, F. Beseme, R. Defay and A. Crastes de Paulet, INSERM Unité 58, 60 Rue de Navacelles, 34100 Montpellier, France.

Hydroxysterols inhibit hydroxymethylglutaryl coenzyme A reductase activity, DNA synthesis and growth in PHA stimulated human lymphocytes, HTC cells and in synchronized rat fibroblasts when added in G_2M phase. In these different models, a cytosolic binding protein specific for sterol hydroxylated on the side chain was evidenced after cell labeling with [^{3}H]-25-OH-cholesterol or in a cell free system. Physico-chemical properties of the complex were sedimentation coefficient about 8S in hypotonic buffer, Kd = 6×10^{-9} M (HTC cells, rat fibroblasts), apparent M.W. 130,000. The 8S protein was specific for cholesterol derivatives hydroxylated on the side chain: no recognition of (a) cholesterol and its derivatives oxidized on C_7; (b) steroid hormones, or (c) vitamin D_3 and hydroxylated vitamin D_3. The observation of an increase (× 3) of binding sites in G_2 M phase is in agreement with the hypothesis that this protein could be involved in the mechanism of DNA synthesis inhibition induced by hydroxysterols.

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LEVELS OF CHOLESTEROL OXIDATION IN SOME SWEDISH FOODS. L. Appleqvist and J. Nourooz-Zadeh, University of Agricultural Science, Uppsala, Sweden.

Abstract not available at press time.

SESSION R Nutritional and Metabolic Effect of Vegetable Protein Tuesday morning

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EFFECTS OF DIETARY COTTONSEED PROTEIN AND CASEIN ON SERUM AND BILIARY LIPIDS, SERUM AMINO ACIDS AND GALLSTONE FORMATION IN THE HAMSTER. George U. Liepa, Nancy DiMarco and Jane Anderson, Texas Woman's University, Department of Nutrition and Food Sciences, P.O. Box 24134, TWU Station, Denton, TX 76204 and Mary Anne Sullivan, Texas Christian University.

The objective of this study was to determine the effects of dietary cottonseed protein and casein on serum and biliary lipids, serum amino acids and gallstone formation in the hamster. Thirty-four male hamsters (60 ± 5 gm) were fed Purina hamster chow for seven days and were then fed either the "Dam Diet" (contains casein as a protein source) or a similar diet containing cottonseed protein. Both diets contained 20% protein which was added as a protein isolate (90% pure). Animals were maintained on experimental diets for 30 days. Gallstones were found in casein-fed animals more frequently than in cottonseed protein-fed animals. No significant differences were observed between the casein- and cottonseed protein-fed hamsters when concentrations of total serum cholesterol, VLDL-LDL cholesterol, HDL-cholesterol, HDL_2- or HDL_3-cholesterol were compared. Significant differences were shown in serum amino acid profiles in that αamino-n-butyric acid was found in greater amounts in casein-fed animals. When biliary cholesterol was measured in the two groups, significantly greater amounts (<0.05) were present in the bile of casein-fed animals. Casein-fed animals also showed lower levels of bile acids. No significant differences in phospholipids were observed between the two groups. Dietary casein appeared to induce gallstone formation in hamsters by altering the cholesterol/bile acid ratio in biliary fluid.

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DIETARY ANIMAL AND PLANT PROTEINS AND THEIR EFFECTS ON LIPID METABOLISM IN STREPTOZOTOCIN–DIABETIC RATS. Nancy M. DiMarco, Nednapis Chantranuwas, Jessie Ashby and George Liepa, Texas Woman's University, P.O. Box 24134, Denton, TX 76204.

Atherosclerosis is a common complication of diabetes mellitus as well as the leading cause of death among diabetics. Diet has been thought to play an important role in reducing the effects of hyper-

glycemia and hyperlipidemia often associated with atherosclerosis. Relatively little attention has been given to dietary plant proteins and their possible hypocholesterolemic effects in streptozotocin-induced diabetes in the rat. The purpose of this study was to determine the effects of various animal (casein and egg white) and plant (cottonseed and peanut) proteins (contained at the level of 22.5% of basal diet) on lipid metabolism in streptozotocin-induced diabetic rats and normal rats. Compared to normal rats, serum glucose concentration was increased significantly in the diabetic rats. Among diabetic rats, serum glucose concentration was 1.4-fold higher in those fed plant protein diets compared to those fed animal protein diets ($p<0.05$). Concentrations of total serum cholesterol and VLDL-LDL-cholesterol also were increased significantly in diabetic rats compared to normal rats. Among diabetic rats fed plant proteins, concentrations of total serum cholesterol and VLDL-LDL-cholesterol, values were 2-fold and 4-fold higher, respectively, when compared to diabetic rats fed animal proteins ($p<0.05$). Diabetic rats fed plant protein diets showed significantly greater deterioration in carbohydrate, protein and fat metabolism when compared to diabetic rats fed animal proteins.

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ROLE OF DIGESTIBILITY IN THE CHOLESTEROL-LOWERING EFFECT OF DIETARY SOYBEAN PROTEIN. A.C. Beynen, Department of Laboratory Animal Science, University of Utrecht, P.O. Box 80.166, 3508 TD Utrecht, and C.E. West, Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BD Wageningen (The Netherlands).

In several animal species the feeding of semipurified diets containing casein as a protein source produces hypercholesterolemia, whereas with soybean protein low levels of serum cholesterol are maintained. We have speculated that the digestibility of proteins is crucial with respect to their effect on serum cholesterol. In vitro work has shown that proteins, which are not completely digested, bind bile acids. Thus, undigested protein may interfere with the absorption of bile acids, which in turn may result in an enhanced loss of steroids with the feces and consequently in lower levels of serum cholesterol. This idea would imply that soybean protein is less digestible than casein, at least in the small intestine where the absorption of bile acids takes place. Indeed, we found in rabbits that the fecal excretion of neutral and acidic steroids was decreased almost immediately (within one day) after soybean protein was replaced by casein and before the concentration of serum cholesterol was increased. However, the mouth-to-anus digestibility of dry matter and nitrogen in rabbits fed either casein or soybean protein was similar. The digestibility hypothesis was studied further in re-entrant fistulated Yorkshire pigs; the fistula was located at the end of the small intestine, just before the ileal-coecal valve. The pigs were fed cholesterol-enriched (0.2%, w/w) semipurified diets containing either soybean protein (24%) or casein. After 28 days serum total cholesterol was increased significantly (by about 0.8 mmol/l) in the animals fed casein. The increase in serum cholesterol was carried almost exclusively by the light subfraction ($1.019 < \text{density} < 1.040$ g/ml) of the low density lipoproteins. Mouth-to-anus and mouth-to-coecum digestibility of dry matter, ash, nitrogen and crude fat were similar in the casein and soybean-protein fed pigs. The pattern of flow of the intestinal chyme (total wet chyme and total chromium) at the end of the intestine differed between the animals fed casein and soybean protein. Casein feeding caused a more pronounced peak at 3 to 5 hr after feeding, but later during the day the flow was lower than in the animals fed soybean protein. Cumulative passage of wet chyme over a 24-hr period was higher at all time intervals in the pigs fed casein. The concentration of nitrogen and dry matter in chyme was lower in casein-fed pigs. Concentrations of bile acids and neutral steroids in chyme are under investigation presently. At this stage we tentatively suggest that because of the high concentrations of nitrogen and dry matter in the chyme of pigs fed soybean protein when compared to their counterparts fed casein, larger amounts of bile acids are bound to undigested material in the former animals. This may interfere with the mucosal uptake of free bile acids and result in interruption of the enterohepatic cycle of bile acids. Consequently, serum cholesterol levels will be lowered.

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NUTRITIONAL VALUE OF VEGETABLE PROTEINS: AN OVERVIEW. Fred H. Steinke, Ralston Purina Company, 3RS Checkerboard Square, St. Louis, MO 63164.

Vegetable proteins are a major source of protein for most of the world's population. They are an inexpensive source of protein and supply a high percentage of the total amino acids in the diet. Vegetable proteins contain various levels of individual amino acids and generally need to be blended with other proteins to optimize their nutritional value. Most vegetable proteins, for example, contain relatively low levels of the essential amino acid, lysine. Information developed over the last decade has demonstrated that vegetable proteins may possess unique properties which make them useful in the food system for managing several chronic diseases. Their value in reducing blood cholesterol levels and in maintaining vascular integrity is particularly noteworthy. In addition, vegetable proteins are low in fat and contain no cholesterol, which makes them useful for individuals who wish to limit intake of these nutrients. Properly processed vegetable proteins can be used in a wide variety of food products to enhance their nutritional value and acceptability. The versatility of vegetable proteins makes them a valuable supplement to the diet.

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VEGETABLE PROTEINS AND MINERAL UTILIZATION IN HUMANS. C. Miles, Energy and Protein Nutrition Laboratory, Beltsville Human Nutrition Research Center, Building 308, Room 213, BARC-West, USDA, Beltsville, MD 20705.

Vegetable protein diets often are assumed to have deleterious effects on mineral utilization. Factors such as phytic acid level, dietary fiber content or protein source of vegetable protein diets have been implicated as decreasing the utilization by humans of minerals such as iron, zinc and magnesium. However, as will be shown by the data presented, results from human studies designed to assess whether the phytic acid level or fiber content of vegetable protein sources such as cereals and legumes may affect mineral availability, do not always support these implications. The effects of soy proteins on iron and zinc utilization when used as a meat extender also will be discussed briefly, and the effects of protein source level on calcium metabolism summarized.

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THE NUTRITIONAL VALUE OF PROTEIN FROM DIFFERENT VEGETABLE PROTEIN SOURCES. C.E. Bodwell, Energy and Protein Nutrition Laboratory, Beltsville Human Nutrition Research Center, Building 308, Room 214, BARC-West, USDA, Beltsville, MD 20705.

The potential protein nutritional value of any protein can be estimated from its amino acid composition if a suitable amino acid reference pattern is available for comparison or "scoring." The patterns of amino acid requirements of the National Research Council and of FAO/WHO/UNU, which differed previously, are now in reasonably good agreement. Based on these patterns, an assessment of the potential value of selected protein sources which are representative of various classes of vegetable proteins will be presented. For all age groups except infants, most vegetable proteins meet 80 to 100% of the requirement for individual essential amino acids and hence are good sources of high quality protein. This is especially true where mixtures of sources are consumed which result in protein complementation. The significance of factors present in plant protein sources which may decrease the potential of vegetable proteins in meeting human dietary protein needs will be discussed briefly.

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CHANGES IN LIPID CLASSES AND FATTY ACID COMPOSITION IN THE DEVELOPING WINGED BEAN SEEDS. Hun-Teik Khor, Department of Biochemistry, University of Malaya, Kuala Lumpur, Malyasia.

Changes in the lipid classes and fatty acid composition were observed in the developing winged bean *(Psophocarpus tetragonolobus)* seeds. The seed lipid content increased gradually and reached a maximum by about the sixth week after flowering (WAF). In the earlier stages of the developing seeds, there were more polar lipids (glycolipids and phospholipids) than neutral lipids, but as the seeds devel-

oped, neutral lipids gradually accumulated while the polar lipids decreased until the sixth WAF. Thereafter both the levels of neutral and polar lipids remained little changed. Changes in the major saturated and unsaturated fatty acids occurred during the development of the seeds. The levels of palmitic and stearic acids decreased, but that of behenic acid increased, as the seed matured. On the other hand, the levels of oleic acid increased while that of linolenic acid decreased rapidly as the seeds matured. The level of linoleic acid fluctuated during the course of development of the seeds but ended up in a slightly higher level than before. The proportions of fatty acid and diacylglycerol were relatively high in the earlier stages of the developing seeds. As the seeds matured, both the levels of fatty acid and diacylglycerol decreased while that of the triacylglycerol increased steadily. In the mature seeds, 80% to 90% of the seed oil is in the form of triacylglycerol.

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PINTO BEAN HIGH PROTEIN FLOUR: CHARACTERISTICS AND UTILIZATION OF DRY ROASTED AIR-CLASSIFIED FRACTION. Mary E. Zabik and Mark A. Uebersax, Michigan State University, Department of Food Science and Human Nutrition, 139B Food Science Building, East Lansing, MI 48824, and Edward W. Lusas, Food Protein Research and Development Center, Texas A & M University, College Station, TX.

Pinto beans, *Phaeseolus vulgaris*, were dry-roasted in a particle-to-particle heat exchanger and milled to yield a high protein fraction. Proximate analyses showed air-classification doubled the percentage of protein in the high protein fraction as compared to the whole pinto bean flour. The high protein flour had the antinutritional factors substantially decreased, had a high nitrogen solubility index and is light in color. Emulsification and foaming properties of the high protein flour were established. Pinto bean high protein/wheat flour blends were incorporated into quick breads, cookies and cake donuts. Substitution levels of 20 to 30% of the flour were feasible in these systems. Farinographs and extensigraphs of the blends were run to establish the effect on bread flour (BF) and whole wheat flour (WWF) rheology. Water absorption, arrival times and extensibility slightly increased, while force to extend the dough slightly decreased and dough stability remained high. Breads also were prepared using both BF and WWF and blends. Breads baked with WWF blends containing 10% high protein pinto bean flour fraction had a slight decrease in volume, were more tender and maintained excellent sensory characteristics. Breads baked with BF/high protein pinto bean flour fraction blends showed less difference in volume and tenderness.

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PROPER USE OF PROTEIN SOURCES. S.H. Fatemi, Iranian Research Organization of Science and Technology, 118 Felestin St., Tehran, Iran.

Protein deficiency is a serious problem which threatens the lives of many populations in many parts of the world. A deep look into the origin and multiple causes of the problem, however, reveals that a solution is not so complicated as it seems. Unsound political, social and economic conditions lead to unequal distribution of food in many societies. Planning for proper use of protein sources can play an important role. Although animal proteins are formed through the consumption of plant materials by animals, only part of the material is converted to animal protein, and the rest is wasted. This waste, especially in the case of low-income countries, where many people are affected by protein deficiency, is harmful and illogical. Statistics for food consumption in 1983 show, in spite of an approximate 1% decrease in food production, milk production increased by 3 to 4% and that of meat by more than 2%. Such an increase in production and consumption of foods, which basically depends on an enormous amount of plant foods, undoubtedly aggravates the nutritional status of low-income people particularly in less developed countries. Although plant proteins compared to animal ones are usually of lower quality, by proper planning based on utilization of plant products, one can attain satisfactory nutrition. This includes using those foods with good quantity and quality of protein (legumes and oil seeds), consumption of the best proportions of different proteins (fortification effect) and finally applying technology in such a way that makes plant protein a substitute for animal protein.

SESSION S Lipids and Immune Response Tuesday morning

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SIMULTANEOUS ASSESSMENT OF ARACHIDONATE RELEASE AND UTILIZATION BY PG SYNTHASE AND LIPOXYGENASE IN STIMULATED RAT MO. Lisa Marshall and Thomas Hoffman, Center for Drugs and Biologics, FDA, 8800 Rockville Pike, Bethesda, MD 20205, and William Becker, CPC International—Best Foods.

Macrophages (MØ) release prostanoids (PG), leukotrienes (LT) and/or hydroxyeicosanoids (HETE) in response to both phagocytic and soluble stimuli. However, studies examining MØ arachidonic acid metabolism seldom focus on both the PG synthase and lipoxygenase enzyme systems simultaneously. To better understand the mechanism of arachidonic acid release and metabolite formation, fresh or cultured rat MØ were pre-labeled with tritium-labeled arachidonic acid and stimulated with either 12-O-tetradecanoate phorbol acetate (TPA), calcium ionophore A23187, latex particles, or serum-treated zymosan (STZ). The medium was analyzed directly for labeled metabolites, using a modified HPLC procedure and an in-line radioactivity detector calibrated for dpm determination. Unlabeled PGE_2 was analyzed in companion cultures by radioimmunoassay. Rat MØ release labeled arachidonic acid, which is totally converted to prostacyclin (6-keto-PGF1α), thromboxane (TXB_2), PGE_2, LTC_4, 15-HETE and 12-HETE. The profile of autacoids is affected by: (1) duration of culture, (2) length of exposure to label, and (3) differing doses of phagocytic or soluble stimuli. Treatment with PG synthase inhibitors (aspirin and indomethacin) or lipoxygenase inhibitors (nordihydroguairetic acid [NDGA] and FPL 55712) produced concentration-dependent changes in the profile which were not confined to the target enzyme. These findings emphasize the need for evaluating the full autocoid profile when assessing arachidonic acid metabolism of intact cells in vitro.

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DIETARY α-LINOLENIC ACID AND ANTI-TUMOR ACTIVITY IN THE MOUSE. Kevin Fritsche and Patricia V. Johnston, University of Illinois, 1208 W. Pennsylvania Ave., Urbana, IL 61801.

This laboratory and others have demonstrated that the consumption of diets rich in ω-3 acids is associated with reduced prostaglandin (PG) synthesis in immunocompetent cells and tumor cells. Prostaglandins have been implicated as natural feedback inhibitors in many immune responses, particularly PGE_2, which has been shown to reduce the tumoricidal activity of macrophages and natural killer cells. These cells are thought to play an important role in immunosurveillance against neoplastic cells in vivo. Additionally, the administration of the PG synthesis inhibitor, indomethacin, reduced the incidence and rate of growth of several types of transplanted mammary tumor cells, despite indomethacin's slight stimulation of tumor cell division in vitro. Studies were undertaken to investigate the interaction between α-linolenic acid (18:3ω3) and anti-tumor surveillance activity in the mouse. Balb/c mice were fed ad libitum purified diets with 10% by weight corn oil or linseed oil, providing a 1:42 or 2:1 ratio of α-linolenic (18:3ω3) to linoleic acid (18:2ω6), respectively. Pups from mothers on these diets were weaned onto their mothers' diets and 4-5 weeks later used as a source of immunocompetent cells. Peritoneal MØs were harvested and tested for basal and stimulated (i.e. 3 days after i.p. injection of 10^7 vaccinia virus) tumoricidal activity against the simian virus transformed fibroblast cell line, SV-3T3, in a 16-hr ^{51}Cr release assay. Splenocytes were isolated and, after removal of RBC and adherent cells, were tested for basal and stimulated tumoricidal activity. Natural cytotoxic cell activity was determined by the 16-hr ^{51}Cr release assay using SV3T3 as targets, while 4-hr ^{51}Cr release from the NK-sensitive YAC-1 cell line was used to determine natural killer cell activity. Dietary-induced modifications in fatty acid composition and PG synthesis of peritoneal MØ and splenocytes will be documented and serve as the basis to discuss differences in tumoricidal activity between the linseed oil and corn oil fed mice.

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THE EFFECT OF VITAMIN E AND DIETARY FAT TYPE ON THE IMMUNE RESPONSE OF YOUNG AND OLD MICE. Simin Nikbin Meydani, Nutrition Center on Aging at Tufts and Brandeis University, and Alice Shapiro, Mohsen Meydani and Jeffrey B. Blumberg, USDA Human Nutrition Research Center on Aging, 711 Washington St., Boston, MA 02111.

Aging is associated with decline of the immune function. Our previous studies showed that the decreased immune responsiveness of the aged mice is associated with increased PGE2 synthesis by splenocytes, and that tocopherol supplementation stimulates their immune response by inhibiting PGE2 synthesis. Therefore, in the present studies we looked at the effect of short-term (30 days) feeding of different dietary fat types; corn oil (CO), coconut oil (COCO) and fish oil (FO) supplemented with different levels of vitamin E (30, 100, 500 ppm) on mitogenic response of splenocytes from 3- and 24-mo old mice to T- and B-cell mitogens. PGE2 synthesis by splenocytes also was measured. The results indicated that the nature of vitamin E effect depends on the type of dietary fat, age of the animal and the type of mitogen. The old mice fed CO and 30 ppm E had significantly less stimulation index (SI) in response to PHA(5 ± 0.7) (mean ± SE) than young mice fed CO and 30 ppm E (21 ± 9). Supplementing the CO diet with 100 ppm or 500 ppm E increased the response of the aged mice so that there was no significant difference between young mice fed CO and 30 ppm E and old mice fed CO and 100 or 500 ppm E (10 ± 3 and 12 ± 4 respectively). Furthermore, there was no difference in SI to PHA between old mice fed FO or COCO diets, E supplementation (100 or 500 ppm) increased the SI to PHA, but this increase did not reach statistical significance. In the young mice fed CO or COCO diets, E supplementation increased SI to CONA, whereas in the old mice statistically significant increases in SI due to E supplementation was observed only in those fed CO diet. The response to LPS was not significantly affected by E supplementation, although vitamin E tended to increase LPS response in old mice fed CO and decrease it in old mice fed COCO or FO diets. Analysis of PG level in spleen cultures indicated that availability of FA precursors for PG synthesis as affected by age, dietary fat type and tocopherol levels might play an important role in mitogenic response of splenocytes.

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EVIDENCE FOR A PHOSPHOLIPID SPECIFIC PHOSPHOLIPASE IN ALVEOLAR MACROPHAGES. Mark D. Wiederhold, Rush-Presbyterian St. Lukes Medical Center, 1725 W. Harrison, Suite 830, Chicago, IL 60612, and David W. Ou, Department of Pathology, University of Illinois, 820 S. Damen Ave., Chicago, IL 60612.

Major alterations of metabolism occur in macrophages when they are appropriately activated. A large number of immunoregulatory substances are released following phagocytosis and membrane receptor activation. Of these substances, arachidonic acid metabolites such as prostaglandins, thromboxanes, leukotrienes and hydroxy-fatty acids represent a diverse and important family of compounds. A key event in the synthesis of these molecules is the action of phospholipases, which liberate arachidonic acid from membrane phospholipids and make this substrate available for eicosanoid synthetic mechanisms. Activation of phospholipases also results in the release of free fatty acids. How effective phospholipases are at providing free fatty acids is an important method of controlling prostaglandin synthesis. The purpose of this investigation was to see under which conditions, if any, liberation of arachidonic acid is enhanced. We have measured the release of free fatty acids from alveolar macrophages following exposure to various stimulatory agents as an indication of phospholipase activity. Six million alveolar macrophages in tissue culture were treated with 20 μl of 5% washed .80 micron latex particles, 20 μl of 1×10^{10} heat killed *Mycobacterium bovis*/ml, 20 μl of 5 μl/ml Zymosan and 20 μl of 4 μg/ml of carregeenan. Cells were incubated in a serum free DMEM medium containing delipidated bovine serum albumin which acts to trap released fatty acids. Cell-free supernatants were extracted with acidified ether and fatty acids methylated with diazomethane or methanolic HCL. Fatty acids were separated by gas chromatography on 10% DEGS with 80/100 Supelcoport. In the control cells, the release of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and arachidonic acid (20:4) occurred at levels less than .02 μg/ml in the supernatants. Following stimulation with latex, mycobacteria, zymosan and carregeenan, the release of 16:0, 18:0, 18:1 and 18:2 into the supernatant was not significantly different from control values, except for latex, which resulted in the release of .024 μg/ml of 18:1 and .027 μg/ml of 18:2. When arachidonic acid levels were determined, however, all stimulated cells produced a dramatic increase in free arachidonic acid release. The levels of arachidonic acid in the media were as follows: Zymosan .038 μg/ml, carregeenan .065 μg/ml, Mycobacterium .034 μg/ml, and latex .053 μg/ml. These values were all significant ($p < .01$). Except for latex, phagocytosis of particles by alveolar macrophages resulted in the specific release of arachidonic acid. These findings support the existence of an arachidonate containing phospholipid specific phospholipase, which has increased activity during macrophage phagocytic activity. Free arachidonic acid can act as a substrate for eicosanoid synthesis, or may itself act as an immunoregulatory molecule.

SESSION T Fats and Oils Processing (General) II Tuesday afternoon

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PHYSICAL REFINING OF EDIBLE FATS AND OILS: MYTHS AND METHODOLOGIES. Lars H. Wiedermann, American Soybean Association, 541 Orchard Road, 15-01 Liat Towers, Singapore 0923, Republic of Singapore.

Physical refining is not a new oil processing technique, and its practice is widely attempted with varying degrees of success. Success is a subjective term, as are these physical refining practices. The opportunities for physical refining of animal or meat fats and hard and soft vegetable oils will be discussed, not reviewed, in terms of defined or "redefined" methodologies. The myths are that physical refining is usually thought of as the elimination, rather than the replacement, of wet or alkali refining, and that the primary purpose of alkali refining is to remove or reduce free fatty acid content. An extension of the latter, to conclude that deodorization is such a replacement step, belies an understanding of oil quality integrity. The primary purpose for alkali refining vegetable oils is to remove residual phosphatides not previously removed during the degumming step; free fatty acid reduction or elimination occurs only incidentally. Physical refining practices need to be defined in terms of producing a finished oil quality that is at least equal to, not necessarily better than, an alkali refined oil. This does not poase a particular problem for meat fats where the suspended or colloidal proteinaceous materials are usually easily removed in a bleaching step. For hard oils, physical refining is a compromise practice. Alkali refining still produces the best oils; however, physically refined oils are marketably acceptable. For soft oils, resulting from conventionally extracted crude oils, the compromise is too great to be acceptable. The so-called "super-degumming" techniques are of limited usefulness and still represent a non-process. The only real opportunity for the physical refining of soft oils is to alter the phosphatide characteristics of the crude oil, and this is consistent with the old adage: "an ounce of prevention is worth a pound of cure." This change in crude oil phospholipid characteristics will be discussed in terms of soybean processing for extraction as illustrated by the Swift and Alcon patented processes. A hypothesis to explain these significant changes in phosphatide quality and quantity will be proposed based on competitive enzymatic reactions.

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PHYSICAL REFINING OF SOYBEAN OIL: A SHELF-LIFE FLAVOR STABILITY STUDY. Lars H. Wiedermann, American Soybean Association, 541 Orchard Road, 15-01 Liat Towers, Singapore 0923, Republic of Singapore; Yau-Kuen Hung and W.S. Lin, Chia Fha Industry Co., Ltd., Taichung, Taiwan, and Philip Yang, American Soybean Association, Taipei, Taiwan.

The previous paper suggests that physical refining of soft oils, such as soybean oil, is not possible using crude oils resulting from conventional extraction practices because complete, or at least adequate removal of non-hydratable phosphatides in conventional crude oils is not possible without alkali refining. Therefore, in order

for physical refining to be satisfactorily applicable to soybean oil it would be necessary to change the character of the crude oil phospholipids. The Alcon process, a cook treatment of flakes prior to extraction, does effect such a quality change and produces a new generation crude oil (M. Kock et al.). This new technology recently has been commercialized at the new Chia Fha extraction plant in Taichung, Taiwan. The Chia Fha facility has the added flexibilities of both alkali and physical refining and a by-pass of the Alcon cooker. This provides the capability for the ultimate test: comparing the shelf life flavor stabilities of a physically refined oil from new technology crude oil versus that of an alkali refined oil from conventional extraction technology, both originating from the same soybean source. This paper will present the results of shelf-life flavor stability studies conducted by two different laboratories under various light and dark storage conditions. It compares three plant test variables: Alcon extracted oil both physically and alkali refined, and conventionally extracted oil which was alkali refined. The latter represents the test control. Additionally, this paper will report compositional analyses of the crude lecithins from both extraction processes, as well as the corresponding phosphatide profiles of their crude and crude degummed oils. Earlier literature suggests significant increases in phosphatidylcholine content of crude lecithins from the new generation crude oil; however, a complete profile picture of other crude lecithin components has not yet been available.

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PRACTICAL EXPERIENCE WITH THE CONDITIONING OF SOY FLAKES (ALCON). Georg Penk, Lurgi GmbH, Gervinusstrasse 17/19, D-6000 Frankfurt am Main, West Germany.

Two solvent extraction plants for soybeans and physical refineries which are equipped with the Alcon process have been in operation in Taiwan for more than one year. In both of the plants, the physical refining process is applied after the soy crude oil has been water degummed. Information will be given about the economic influences, the energy and consumption figures, and the quality parameters of the final products which are produced in these plants.

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FEASIBILITY STUDY ON THE PHYSICAL REFINING OF RICE BRAN OIL. Lucy Sun Hwang and Ray Kwang Wong, Graduate Institute of Food Science and Technology, National Taiwan University, P.O. Box 23-14, Taipei, Taiwan, R.O.C., and Shaw C. Chang, Bureau of Food Sanitation, Department of Health, Executive Yuan, Taipei, Taiwan, R.O.C.

Crude rice bran oil generally contains a high level of free fatty acids (around 10-20%) due to the high lipase activity in the rice bran. Physical refining thus appears to be a desirable refining process to avoid excessive losses of neutral oil during refining. Crude rice bran oil was first winterized to remove the high melting point substances such as waxes. Different ratios of oil/n-hexane mixtures were tested at 0, −5, and −10 C to find the highest yield of winterized oil which can pass the cold test. It was found that the ratio of 60 to 40 (w/w) of crude rice bran oil to n-hexane stored at −10 C gave the best result. The winterized oil was degummed at 25, 40, 55, 70 and 85 C with phosphoric acid and stirred. Three levels of phosphoric acid, three stirring speeds and three stirring intervals also were tried. The best result which removes the most phospholipids was found to degum at 25 C with 0.2% phosphoric acid and stirred at 500 rpm for one hr. The degummed oil was further bleached with acid clay and/or active carbon. 3% acid clay was found to give a satisfactory result. Among the four temperatures tested, 115 C was found to be the best. An increase in bleaching time would result in more completely bleached oil. The bleached oil was finally steam deodorized at 260-270 C under 1-2 torrs of vacuum for one hr or more, depending on the amount of oil used. The physically refined rice bran oil was found to give satisfactory color (3.6 R and 23 Y by 5¼ in. Lovibond) and free fatty acid content (0.38%). Therefore, it seems feasible to refine the dark colored rice bran crude oil by physical refining.

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THE ADVANTAGES OF DEHULLING IN THE PRODUCTION OF MEADOWFOAM OIL. Robert R. Lowry and Ian J. Tinsley, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331.

In 1983 a commercial firm contracted for the first acreage planted to Meadowfoam *(Limnanthes alba)*. Recent work in our laboratory has shown that there are significant advantages to the processor and end users of Meadowfoam by dehulling prior to oil extraction. A very significant reduction in the intensity of the color and a fourfold reduction in sulfur content of the oil resulted. The crude protein content of the meal was increased from 23 to 36%, while the acid detergent fiber decreased to 13% from a previous 28%. Preliminary results suggest there is no significant change in the thioglucosinolate content relative to the protein content. Physical handling costs also may be reduced as the bulk volume is reduced by approximately two-thirds and the weight by one-half. Several methods of dehulling were examined; the most efficient under our conditions involved brief conditioning in boiling water followed by use of a belt thresher after partial air drying. Commercial belt threshers may eliminate the need for pre-conditioning the seed. Costs of dehulling should be more than offset by the reduced volume required for crushing and extraction and the greater ease of crushing.

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HIGH OLEIC SUNFLOWER. PHYSICAL AND CHEMICAL CHARACTERISTICS. Richard H. Purdy, Richard H. Purdy Inc., 16 Josefa Ct., Novato, CA 94947.

Treatment of normal varieties of sunflower seed with chemical mutagens and development of their progenies have resulted in hybrids bearing oil with oleic acid contents in excess of 80% and linoleic acid contents less than 10%. This paper will compare analytical data representative of commercial seed production in Texas, North Dakota and California and the oil and meal products derived from full scale prepress/solvent extraction operations with their normal sunflower seed counterparts.

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THE EFFECTS OF RELATIVE HUMIDITY AND STORAGE ON CHEMICAL AND MYCOFLORAL CHANGES IN OIL TYPE SUNFLOWERSEED. J.A. Robertson, R.G. Roberts and G.W. Chapman, USDA Agricultural Research Service, SAA, R.B. Russell Research Center, P.O. Box 5677, Athens, GA 30613.

Oil-type hybrid sunflowerseed held at 63%, 83% and 93% relative humidities (RH) at 20 C attained equilibrium moisture contents (mc) of 6.7 ± 0.1%, 9.8 ± 0.1% and 13.4 ± 0.3%, respectively, and were stored under these conditions for up to 60 weeks. (Germinability was inversely related to both storage duration and RH. Initially, the seed contained only field fungi (mainly *Alternaria* spp. and *Phoma* sp.) and 0.5% free fatty acids (FFA). At 6.7% mc, recovery of *Alternaria* spp. and *Phoma* sp. declined slowly over time, storage fungi did not invade the seed, and FFA in extracted oil did not increase even after 60 wk of storage. At 9.8% mc, field fungi died out more rapidly than at 6.7% mc, storage fungi (mainly *Eurotium* and *Aspergillus* spp.) were recovered from the seed after 4 wk, and FFA increased to 2.5% after 40 wk. At 13.4% mc, field fungi died out and storage fungi invaded the seed more rapidly than at 9.8% mc, and FFA increased to 6.3% after 24 wk of storage. After 14 wk storage the fungus recovered most frequently was *Aspergillus versicolor*, followed by *Eurotium rubrum*, *E. repens*, *A. alternata*, *E. amstelodami*, *Penicillium* spp. and *Malbranchea sulphurea*. A total of 32 fungal species in 21 genera were identified from the seed stored at the three mcs.

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FACTORS IN STANDARDIZING NUCLEAR MAGNETIC RESONANCE (NMR) TESTING FOR OIL CONTENT OF SUNFLOWER SEEDS. M.L. Iverson and C.A. Watson, Federal Grain Inspection Service, USDA, Bldg. 221, Richards-Gebaur AFB, Grandview, MO 64030.

Wideline nuclear magnetic resonance (NMR) instruments and

methods have been used for a number of years to measure the oil content of various materials including sunflowerseeds. Several variables are important in maintaining accurate measurements. Variables that have been studied include temperature, moisture content, calibration methods, stability and accuracy of reference standards, and instrument response versus oil content and composition. Accounting for the effects of these variables in an NMR procedure for measuring oil in sunflower seed should provide for uniform and accurate measurements of oil content.

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SPECTRUM OF VARIABILITY OF CHARACTERISTICS AND COMPOSITION OF THE OILS FROM DIFFERENT GENETIC VARIETIES OF LINSEED. Madhu Bajpai, Sandhya Pandey and A.K. Vasishtha, Harcourt Butler Technological Institute, Kanpur-208 002, India.

Spectra of variability of 150 genetic varieties of linseed were studied for oil and protein content in seeds and chemical characteristics and fatty acid composition of the oils. The oil content of the seeds varied from 32.9 to 50%, and protein content 10.5 to 20.5%. A negative correlation was observed between the oil content and protein content in the seeds. The variation in the saturated fatty acids in the oils was insignificant except in one variety, LS-3, in which the palmitic acid content was found to be abnormally high. Maximum variations were observed in the content of linolenic acid, which ranged between 40 and 65.8%. The iodine value of the oil varied from 153.1 to 194.5%.

SESSION U Flavor Chemistry of Fats and Oils II Tuesday afternoon

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EFFECTS OF FAT QUALITY, STORAGE TEMPERATURE AND TIME, AND HEADSPACE OXYGEN CONTENT ON FAT OXIDATION IN MODEL POWDERED FOOD PRODUCTS. J. Damon Manes Jr. and Ralph Knights, Mead Johnson & Company, 2404 Pennsylvania St., Evansville, IN 47721, and Steve G. Sorensen, Bristol Myers International Group.

Variables contributing to the oxidation of vegetable oils incorporated into model powdered food products were studied during storage in designed experiments utilizing response surface methodology. Milk based products containing 26% w/w of a fat blend which had been oxidized artificially to three different levels–low, medium and high–were canned with six different headspace gases ranging in O_2 content from 0% to 20% and stored at 25 C to 45 C for a period of time up to 12 mo. Combinations of the variables were chosen so that a response surface prediction equation, or a quadratic function in each variable and combination, could be calculated for each oxidation measurement. Five different chemical photometric assays (UV-diene, anisidine, peroxide, TBA and Kreis tests) were used to measure the oxidation of fat which had been extracted using a liquid/liquid partition after the products had been reconstituted in water and the protein denatured by addition of alcohol. Statistical analyses and contour plots or computer drawn 3-D representations of the extent of fat oxidation were used in the interpretation of results. The data and the prediction equations can be used to emphasize the importance of the quality of ingredients, the handling and manufacturing procedures, and the packaging of powdered products so that the occurrence of fat oxidation is minimized.

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LIGHT STABILITY OF SOYBEAN OIL TREATED WITH BETA CAROTENE. K. Warner and E.N. Frankel, Northern Regional Research Center, 1815 N. University, St., Peoria, IL 61604.

Beta carotene is recognized as a singlet oxygen quencher in model systems, but little is known of its effect in inhibiting photosensitized oxidation in edible vegetable oils. To study the effect of beta carotene on light stability, soybean oils were treated with 1 to 20 ppm of this pigment. Flavor evaluations indicated that (a) citric acid-treated oils containing 5 to 10 ppm of beta carotene had better flavor scores when exposed to light (800 ft candles) for 8 to 16 hrs than oils containing 0 to 1 ppm of beta carotene, and (b) with non-citric treated oils, only those containing 20 ppm of beta carotene were stable to light. Initial Lovibond tintometer color measurements of soybean oil without beta carotene produced readings of 5, yellow; 0.2, red, where color values for the oil containing 20 ppm of beta carotene were 70, yellow; 9.0, red (5¼" tubes). Capillary gas chromatographic analysis by a direct sampling technique showed that at 5 to 20 ppm levels of beta carotene in the presence of citric acid, total volatiles were decreased significantly when compared to the control or oil with only 1 ppm of beta carotene. In the absence of citric acid, only the oil with 20 ppm beta carotene showed significantly decreased levels of total volatiles. Peroxide values were in good agreement with GC volatile analyses in the early stages of light exposure. Spectrophotometric analyses showed that the concentration of beta carotene decreased 10 to 15% after 24 hr light exposure in citric acid-containing soybean oil. Although light deterioration was not significant at high levels of beta carotene, the use of this additive is limited to levels of 10 ppm or less. In the presence of 15 and 20 ppm beta carotene, some off-flavor due to the additive could be detected by our panel. Color evaluations conducted under normal room light showed that the oils with 15 and 20 ppm of beta carotene were given poor color quality ratings. Results indicate that beta carotene at levels from 5 to 10 ppm is a useful additive to protect soybean oil from light deterioration without affecting its color acceptability.

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FATS, SWEETNESS AND "RICHNESS" IN FROZEN DESSERTS. Mina Knezevich, Gourmet Diet Group, 83-10 Britton Ave., Elmhurst, NY 11373.

The last decade has seen the appearance of ice creams with low overrun, generally called premium, having over 75 calories per ounce. One company even has 20% butterfat, which seems to run contrary to cholesterol and atherosclerosis January 1984 reconfirmations. After two years in a soft serve version, a tofu and soybean isolate hard frozen dessert with corn oil has appeared with a national distribution network, claiming to be lactose and cholesterol free. It has 170-210 calories per 4 ounces. The body, smoothness and mouthfeel of such frozen desserts but in a caloric range of 18-28 calories per ounce was achieved by using aspartame with calo fats and calo technologies, including such ingredients as dextrins, polydextrose, sucrose esters, bulcacia, etc. A number of formulations of milk, yogurt, sherbet and tofu are presented. It is surmised that 1985 will be the last expansion year of the megacaloric (16% fat and up) desserts, which may take 10% of the estimated 830 million gallon market. Calo fats from vegetable oils and calo systems will take their place in the picture. Mixtures of dairy and non-dairy desserts in the same products are predicted. Aspartame and super-aspartame will overtake the sugar sweetner market.

SESSION V Lipids in Food Products Tuesday morning

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LIPID-PROTEIN INTERACTIONS AND FAT CRYSTALLIZATION IN WHIPPABLE EMULSIONS. N.M. Barfod and N. Krog, Grindsted Products A/S, Edwin Rahrsvej 38, DK - 8220 Brabrand, Denmark.

The functions of surfactants and protein in whippable emulsions (toppings) were studied. A series of topping powders were manufactured with varying amounts of these ingredients and tested for whippability and foam texturein relation to the viscosity and the degree of destabilization of the reconstituted emulsions. The destabilization process was followed by measuring (a) the release of adsorbed protein from the surface of the fat globules to the bulk water phase, by centrifugation and protein analysis and (b) a spontaneous fat crystallization process taking place in the coalesced fat phase by p-NMR technique. The results indicated that the fat-adsorbed protein was penetrating the fat globule surface and inhibiting the crystallization of the fat phase in the spray-dried topping powder. When reconstituted in cold water, the adsorbed protein was

released into the water phase, followed by a partial coalescence of fat globules and a crystallization process forming a network of fat crystals which contributed to foam stiffness and stability of whipped topping emulsions. The type of surfactant used and its concentration in the fat phase controlled the release of protein from the fat globule surface and hence the degree of destabilization of the emulsion needed to obtain a good whipped product.

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STEROL COMPOSITION OF CRUDE, REFINED, AND BLENDED FATS AND OILS. M.A (Vic) Amer, D.B. Kupranycz, F.M. Gharavy, and B.E. Baker, Dept. Agr. Chemistry & Physics, Box 223, Macdonald College of McGill University, Ste. Anne de Bellevue, Que. H9X 1CO, Canada.

The lipid material of 192 samples of imitation dairy creams, 20 dairy blend spreads (milkfat plus vegetable oils), crude and refined vegetable fats and oils were saponified and the non-saponifiable fractions were chromatographed. In vegetable lipids, three major sterols identified in this study were campesterol, stigmasterol, and β-sitosterol. Other minor sterols were identified as Δ^5-avenasterol, Δ^7-stigmastenol and Δ^7-avenasterol, which were found in measurable concentration (4.18%) in sunflowerseed oil. Cholesterol was found as a minor sterol in almost all the non-dairy creams ranging from traces to as high as 5.6% of total sterols. Milkfat contained cholesterol as its major sterol (98.86%-98.99%) and a minor sterol identified as Δ^5-avenasterol (0.92%-1.14%), a sterol which was not detected in most vegetable fats and has not been reported previously. The technique developed in this study was used as a means to detect the type and the amount of various fat blends employed to formulate imitation creams and spreads.

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PHYSICAL AND CHEMICAL CHARACTERISTICS OF BUTTERFAT FRACTIONS OBTAINED BY A NATURAL CRYSTALLIZATION TECHNIQUE. M.A (Vic) Amer, D.B. Kupranycz, and B.E. Baker, Dept. Agr. Chemistry & Physics, Box 223, Macdonald College of McGill University, Ste. Anne de Bellevue, Que. H9X 1CO, Canada.

Both summer and winter butterfat were fractionated using a laboratory procedure which was designed to simulate a commercial batch fractionation process. The process is based on a slow controlled cooling of the melted fat to match the thermodynamic reactions of the oil, a short stabilization time at the fractionation temperature, and separation of the crystals from the liquid oil by vacuum filtration over a stainless steel perforated disc. Fractionation temperatures of 29 C, 23 C and 17 C for summer butterfat and 29 C, 26 C, 23 C and 19 C for winter butterfat were used to obtain solid and liquid fractions at each temperature. Replication of the winter butterfat experiment showed good reproducibility of the techniques employed. The fractions are characterized according to their fatty acid composition by capillary column gas liquid chromatography (CC-GLC), triglyceride composition by CC-GLC, melting and crystallization behavior by differential scanning calorimetry (DSC), iodine value (Wijs) and capillary tube melting point.

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DETECTION OF ADULTERATED AND MISBRANDED OLIVE OIL PRODUCTS. D. Firestone and J.L. Summers, Food and Drug Administration, DHHS, 200 C Street SW, Washington, DC 20204, and R.J. Reina and W.S. Adams, Food and Drug Administration, Boston, MA.

The Food and Drug Administration has not established definitions and standards of identity for olive oil products. However, it is recognized that consumers want to know whether an olive oil product has been expressed by physical means from sound olives (virgin olive oil), derived from olive residues (pits and pomace remaining after physical expression of the oil) by solvent extraction and refining (refined olive residue oil), or produced by reaction of low grade or by-product olive oils with glycerol (synthetic or "esterified" oil). In order to determine if the labeling of olive oil products is accurate, the Food and Drug Administration has been examining bulk lots of imported olive oil as well as packaged olive oil products. Samples are examined to determine fatty acid composition, sterol composition, erythrodiol (to detect refined olive residue oils), UV absorption, chlorophyll and fatty acids in the 2-position of the triglycerides (to detect esterified oils). Seven of 13 brands of packaged olive oil were found to contain undeclared esterified oil. Analysis of imported olive oil suggests that considerable quantities of esterified oil are shipped to the United States identified on labeling as olive oil.

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POSSIBILITIES OF GAS CHROMATOGRAPHY ON POLAR CAPILLARY COLUMNS IN THE TRIGLYCERIDE ANALYSIS OF FATS AND OILS, WITH SPECIAL REFERENCE TO CHOCOLATE FATS. E. Geeraert, Research Laboratory, Chocolaterie Callebaut, Jacobs Suchard Ltd., Aalstersestraat 124, B-9380 Lebbeke, Belgium, and P. Sandra, Laboratory of Organic Chemistry, State University of Ghent, Krijgslaan 281 (S 4), B-9000 Ghent, Belgium.

A polar capillary column is used for the direct analysis of triglycerides. The necessary selectivity and high temperature stability (up to 360 C) is reached for the separation of nearly all triglycerides in natural fats and oils. However, no differentiation is obtained for positional isomers. In first instance, the method allows quick fingerprinting of oils and fats. The quantification of the different triglycerides opens new possibilities to study the influence of seed varieties and growing conditions, and thus allows authenticity checks. Applications were specially oriented to chocolate fats. Examples are given for cocoa butters of different origins, butter oil, hazelnut oil, almond oil and cocoa butter equivalents. The study deals with identification and quantification of the components of complex chocolate fat mixtures. Also some common vegetable oils were analyzed as basic reference materials. A solution of the oil is injected directly into the gas chromatograph. A custom built movable on-column injector is used. Typical run times range from 15 to 30 min, depending on the molecular weight range of the triglycerides in the oil.

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CALO (LOW CALORIE) FATS AND OILS AND THEIR FUTURE. Robert S. Aires, Bio Techknowledge, Inc., 49 E. 41 Street, New York, NY 10165.

The diet and health foods business in retail shops accounts for $27 billion or 9% of a $300 billion business. It is growing at the rate of 6% per year in contrast to ca. 1% for the general food business. It already includes 38% of reduced fats products. The future will see a tremendous impetus of calo fats which will be tailored and very frequently bio-tailored to our needs. Ten million people take reducing pills and 40 million are on some sort of a diet, including the 20 calorie % diets for those who have had heart attacks. The 43 calorie % fat and oil consumption cannot expand much further despite its taste aspects. Calo fats formulations and systems are the food industry's answer and should account for $2 billion in 1995 and 8% of the estimated 100 million ton industry in two decades. There are at least 15 different chemical ways and systems for achieving calo fats, and 83 known technologies. Calo fats could also be consumed by the 35 million mild hypertensives, 11 million diabetics and nearly a million who find yearly that they have cancer. A recent brand label has surfaced. Experiences of a Washington D.C. food chain which has been marking the fats and carbohydrate content of foods are reviewed as well as 16% fats for the desserts, tofu desserts and the use of bulking agents such as Glucosan, Polydextrose, Galactogen, etc.

SESSION W Analytical Methods for Protein I Tuesday afternoon

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OPTIONS AND STRATEGY IN GEL ELECTROPHORESIS. B.C. An der Lan, Section of Macromolecular Analysis, Laboratory of Theoretical and Physical Biology, National Institute of Child Health

and Human Development, National Institutes of Health, Bethesda, MD 20205.

As the number of specialized techniques in gel electrophoresis increases, the choice of the optimum separation tool for any particular application becomes increasingly complex. Rationales are presented to avoid arbitrary choices. To resolve single components from other contaminants optimum resolving conditions are sought. This entails optimizing a) the solvent, particularly through choice of a detergent for water insoluble species, b) the pH and ionic strength of the buffer milieu, using either continuous or discontinuous systems, i.e. capable of generating a moving boundary (if species to be separated differ only in net charge, a pH gradient forming system, such as an electrofocusing or Immobiline system, would be chosen), c) the gel matrix, which is dictated by the size range of the species (an optimally resolving gel concentration can be calculated for any two species), d) the length of migration path, which is restricted by time dependent zone diffusion and/or charge isomerism, e) gel thickness, which is restricted by the heat dissipation characteristics of the apparatus and f) detection and quantitation methods. To resolve multicomponent systems, both two-dimensional and multiple-stage methods are available. Necessarily, these operate under average conditions of a/e), and thus cannot be optimal for all components. For wide size ranges average pore sizes or pore gradients are suitable.

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TWO-DIMENSIONAL GEL ELECTROPHORETIC SEPARATION OF PROTEINS. Sheikh M. Basha, Florida A&M University, Peanut Research Laboratory, Division of Agricultural Sciences, Tallahassee, FL 32307.

Unlike one-dimensional gel electrophoresis, two-dimensional gel electrophoresis gives improved resolution of proteins due to its superior resolving power and sensitivity. In this system, proteins are separated according to isoelectric point by isoelectricfocusing (IEF) in the first dimension and according to molecular weight by SDS-gel electrophoresis in the second dimension. Proteins are extracted from the tissue either by sonication or homogenization with a solution containing 9.3 M urea, 5 mM K_2CO_3, 0.5% DTT and 2% nonionic detergent NP-40. IEF is carried out in 4% acrylamide gels in the presence of 9.3 M urea, 2% NP-40 and appropriate ampholine mixture. After focusing for 18 hr the gels are equilibrated in SDS buffer and then subjected to SDS-gel electrophoresis in 10% acrylamide slab gels. Following electrophoresis, proteins are detected by staining the gels with Coomassie Blue or silver stain or by autoradiography. Quantitation of individual protein spots may be performed either by cutting out individual spots, eluting and measuring the dye or by two-dimensional gel scanner and computer processing technique. Two-dimensional gel electrophoresis can be applied to identify genetic variation, determine protein structure, and study the effect of curing, storage and processing conditions on protein structure.

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IMMUNOCHEMISTRY: THEORY AND APPLICATIONS. J.N. Neucere, USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., P.O. Box 19687, New Orleans, LA 70179.

Interactions of antibodies and antigens remain the central theme of modern immunochemistry. The physicochemical heterogeneity of antibodies and the diversity of antigenic determinants have been recognized for over a century; however, present-day concepts regarding the chemical basis for recognition by antibodies were established during the last 25 years. In the early sixties, it was shown that a common structure of immunoglobulins consisted of two light and two heavy peptide chains that are linked by disulfide bridges of cystine. Proteins trigger immune responses that are dictated by specific determinant groups. Sequential determinants involve a linear sequence of several amino acids; conformation-dependent determinants are derived from three-dimensional structures. Thus, immunization with an antigen induces antibody molecules complementary to specific immunogenic groups. Immunochemical analyses of protein have evolved rapidly in the last two decades. These include detection, quantitation, and structural elucidation of proteins and hapten-protein conjugates in relation to toxicology, molecular biology and other related fields. The relevance of modern techniques such as enzyme immunoassays, production and uses of monoclonal antibodies and specific detection following separation procedures will be discussed.

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LIQUID CHROMATOGRAPHY OF PROTEINS: STATUS AND TRENDS. Robert A. Barford, USDA, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.

The reversed-phase chromatography of small proteins and peptides at low pH with mobile phases modified with large proportions of organic solvents has become routine. Retention in these systems correlates well with values predicted from amino acid composition. A model of the sorption of proteins to modified silicas provides information that is extending the utility of HPLC into areas previously tractable only by the slower, more traditional, chromatography. Measured surface tensions of proteins (γ_p) are generally in the 65-70 ergs/cm^2 range, whereas typical surface tensions of column packings (γ_s) may range from 30-50 ergs/cm^2 depending on the structure of the bound groups. Examination of the interrelationships of γ_s, γ_p, and γ_m (mobile phase surface tension) reveals that desorption of protein may be favored without resorting to large proportions of organic modifier by increasing γ_s or reducing γ_m. One approach to accomplish chromatography at nonacidic pH is with buffers containing nonionic surfactant at concentrations above the CMC. Frontal experiments suggest that when polyethoxylated alcohols are added to the mobile phase, protein sorption is mediated through sorbed layer of surfactant. Appropriate increase in γ_s is achieved also through syntheses that produce surfaces that are essentially hydrophilic but lightly loaded with respect to alkyl groups. These concepts for controlling sorption/desorption along with advances in ion exchange technology and in affinity chromatography provide a battery of techniques for faster and more selective protein separations.

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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF CEREAL PROTEINS: METHODS AND APPLICATIONS. Jerold A. Bietz, Northern Regional Research Center, USDA, ARS, MA, 1815 N. University St., Peoria, IL 61604.

The complexity of many protein mixtures, such as those of cereal grains, necessitates improved analytical separation methods. One such method, reversed-phase high-performance liquid chromatography (RP-HPLC) on porous silica-based columns having various hydrophobic bonded phases, has now been applied to proteins of wheat, maize, barley and oats. RP-HPLC, based on hydrophobic interactions between protein surfaces and the support, gives high-resolution separations, thus complementing methods based on size or charge. RP-HPLC is fast, sensitive, reproducible, and easily automated and quantitated. Applications to date include cultivar identification, quality prediction, preparative as well as analytical separations, selection during breeding using marker proteins and demonstration of genetic relationships. Computers may facilitate automated varietal identification, and permit analyses of hybrids and mixtures. RP-HPLC is still being modified to decrease analysis time, improve precision and increase resolution. RP-HPLC is becoming an excellent protein analysis technique to predict, measure, improve and assure good quality of all cereals and cereal-based products.

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MODERN METHODS USED IN SEQUENCING AND SYNTHESIZING PROTEINS. K.J. Wilson and T.G. Geiser, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404.

Modern molecular biology is dependent on the availability of protein structural information. Primary sequence information is used for (a) synthesizing oligonucleotide probes for cloning purposes; (b) preparing synthetic peptides for use as antigens; (c) characterizing processing regions in native and closed products, as well as confirming cloned protein sequences; and (d) producing synthetic peptides, or analogs, for laboratory and clinical purposes. Lately the quantities of material available for obtaining protein sequence data for such uses are often found limiting. Micro-level isolations and characterizations are presently dependent on HPLC and SDS-PAGE

methods. The sequencing or chemical degradation of these polypeptides have required the development of new instruments (gas-phase sequencer) and sample handling techniques. The solid-phase synthesis of peptides has also been improved through the introduction of a microprocessor-controlled, fully automated instrument. The chemistry, designed around highly reactive symmetric anhydrides, and sample transfers involving solvent exchanges, have resulted in consistent high yields of quite pure product. This paper will discuss methodology for micro-scale isolation, sequencing of peptides and proteins, and peptide synthesis.

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AMINO ACID ANALYSIS: AN ASSESSMENT OF CURRENT TECHNIQUES. Robert W. Zumwalt, University of Missouri, Dept. of Biochemistry, Rm. 4, Agriculture Bldg., Columbia, MO 65201.

Ion-exchange, gas liquid and reversed-phase liquid chromatography offer means for instrumental analysis of amino acids. Each presents advantages, capabilities and limitations for identification and quantitation of amino acids. These methods are continuously subject to change and improvement, which has focused on the constant quest for enhanced sensitivity, speed, specificity and simplicity, and the advantages and capabilities of the different procedures can now be clearly understood. This presentation will discuss three instrumental chromatographic methods for amino analysis: IEC, GLC (packed, capillary, and enantiomeric labeling) and RPLC. Factors which produce variance between the actual amino acid composition of proteins and the amino acid analysis data of protein hydrolysates will also be reviewed. Advances in ion-exchange resins have resulted in highly stable, uniform resins which yield highly efficient columns, reducing hydrolysate analysis times to 30 min or less. Detection systems utilizing ninhydrin, fluorescamine, *o*-phthalaldehyde or other reagents have dramatically increased sensitivity. The two most widely used derivatives for GC are the N-TFA n-butyl esters and the N-HFB isobutyl esters. Fused silica bonded phase capillary columns offer high resolution extending to resolution of amino acid enantiomers. Precolumn and post-column derivatization techniques for RPLC have been developed, and fluorescence and ultraviolet detection are used with the various reported derivatives.

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FLOW-INJECTION ANALYSIS-APPLICATIONS TO PROTEIN CHARACTERIZATION AND ANALYSIS. Gary R. Beecher and Joseph T. Vanderslice, U.S. Department of Agriculture, Nutrient Composition Laboratory, Building 161, BARC-East, Beltsville, MD 20705.

Flow-injection analysis (FIA) is the analytical procedure by which sequential discrete samples are inserted into a nonsegmented continuously flowing liquid stream with subsequent detection of the analyte. This procedure was discovered simultaneously in the United States and in Europe during the early 1970s. It takes advantage of laminar flow characteristics of solutions in small bore tubes (<0.8 mm internal diameter). Simple expressions, based on numerical solutions of the diffusion-convection equation, have been derived that describe dispersion of the sample bolus and residence times of the sample in simple flow-injection systems. These equations have been applied to the determination of molecular and kinetic parameters using FIA. A brief overview of FIA and a discussion of laminar flow in small bore tubes will be presented. The application of FIA to a number of specific procedures important in the characterization and analysis of proteins, e.g. titrations, total protein, amino acids, ammonia, enzyme activity and diffusion coefficients will be discussed. The use of theoretical expressions for the future development and applications of FIA will be presented.

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FOURIER TRANSFORM INFRARED SPECTROSCOPY IN PROTEIN CONFORMATION STUDIES. Heino Susi and D. Michael Byler, USDA, ARS, Eastern Regional Res. Ctr., 600 East Mermaid Lane, Philadelphia, PA 19118.

As early as 1950, Ambrose and Elliott discovered that proteins in the alpha-helical conformation have a strong absorption band close to 1650 cm^{-1} whereas those with the extended beta-structure absorb near 1630 cm^{-1}. Subsequently, infrared spectroscopy was developed into a major tool for protein structure analysis, but it remained essentially a qualitative method until the advent of Fourier transform infrared (FTIR) spectroscopy, which permits a signal-to-noise ratio of 1000:1 or better. This, in turn, has led to approaches such as derivative spectroscopy, Fourier self-deconvolution and detailed analysis by curve fitting. By a combination of these procedures it is now possible to analyze quantitatively the conformational structure of dissolved protein molecules in terms of substructures such as the alpha-helix and extended beta-strands, and to detect some specific types of turns. Because deconvolved spectra are used to perform detailed analysis by curve-fitting, modest values of the deconvolution parameters are employed in order to avoid artifacts and distortion of the resultant band areas. The signal-to-noise ratio must be sufficiently high and solvent subtraction carried out with great care. Data were obtained in deuterium oxide solution to avoid interference by the strong absorption of water around 1640 cm^{-1}.

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DETERMINATION OF PROTEIN OIL AND FIBER COMPONENTS IN OILSEEDS BY NEAR INFRARED REFLECTANCE SPECTROSCOPY. Philip C. Williams, J.A. Panford and J.M. deMan, Canadian Grain Commission, 1404-303 Main Street, Winnipeg, Manitoba, Canada R3C 3G9.

Protein oil and fiber components were determined in the ground seeds of eight oil-bearing crops; rape, flax, sunflower, safflower, sesame, palm kernel, groundnuts and soybean. Wavelengths of mathematical treatments were optimized for determination of all constituents by scanning near infrared reflectance spectroscopy. Optimum wavelengths differ between crops demonstrating need for wavelength flexibility in the instrumentation. Spectra are presented for protein and other components isolated from different seed types to illustrate band positions.

SESSION X Lipid Metabolism, General Tuesday afternoon

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CHOLESTEROL METABOLISM AND SERUM LIPOPROTEINS OF RABBITS FED SEMI-PURIFIED DIETS VARYING IN PROTEIN AND CARBOHYDRATE SOURCES. John E. Bauer, University of Florida, Box J-144, JHMHC, Gainesville, FL 32610-0144.

New Zealand white rabbits, matched for size, were pair-fed low-fat, pelleted diets containing either casein-sucrose (CS) and soy-protein-sucrose (SS) or casein-dextrose (CD) and soy-protein-dextrose (SD) for a period of 175 days. Animals fed the casein containing diets rapidly became hypercholesterolemic, whereas atheromatous lesions were absent in all groups. Serum lipid analysis revealed increased amounts of both free and esterified cholesterol in the casein-fed groups although the free cholesterol content as a percentage of the total cholesterol (% FC) remained constant. Serum triglyceride (TG) and phospholipid (PL) concentrations were unchanged for the most part with perhaps a slight increase in serum TG content among the sucrose fed rabbits. Analysis of liver lipids likewise revealed significant increases in both free and esterified cholesterol. The % FC of casein-fed rabbit livers was increased, however, in contrast to the serum data. In addition, a trend toward lower liver TG content among the sucrose-fed rabbits and a lower PL concentration in the CS diet-fed group was noted. Liver HMG-CoA reductase activities were not statistically different in any of the dietary groups studied. Serum lipoproteins were visualized and separated by prestaining the sera with Sudan Black IV before subjecting them to single spin density gradient ultracentrifugation. Lipoprotein fractions, collected on the basis of their appearance in these gradients, were characterized via cholesterol distribution and compositional analysis. For all animals, it was found that greater than 70% of the total serum cholesterol was transported on lipoproteins of hydrated density <1.040 g/ml. Compositionally, these lipoproteins from the casein-fed rabbits were cholesteryl ester enriched and TG poor when compared to their soy protein diet-fed

pairs. No differences in the % FC was observed, however. Lipoprotein fractions with hydrated density >1.040 g/ml showed few compositional differences among all groups, yet the % FC of these fractions with casein feeding was nearly twice those of the soy protein diet-fed animals. Total lipoprotein mass, including both low and high density fractions, increased in both the CS and CD diet-fed animals compared with the soy protein groups. On a relative basis, however, decreases in the high density fraction were observed in the casein-fed rabbits. The use of relative high density to low density lipoprotein ratios calculated either from lipoprotein cholesterol or lipoprotein mass data may still be one of the best indicators in assessing lipoprotein changes due to dietary modification.

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BROMINATED FATTY ACIDS, THEIR OCCURRENCE AND MODE OF ACTION IN MAMMALIAN SYSTEMS. Ian J. Tinsley and Robert R. Lowry, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331.

Brominated oils have been used as food additives for many years. More recently they have found use as flame retardants, and halogenated fatty acids are being investigated as possible agents for myocardial imaging. Fatty degeneration and necrosis of the myocardium was the most striking response in rodents fed brominated oils or brominated fatty acids. In rodents fed brominated oils or brominated fatty acid monoglycerides, liver enlargement is observed and if tetrabromostearate is fed, liver lipid is increased. An increase in the level of γ-linolenic acid in liver lipids of rats fed brominated oils suggests that brominated acids may affect the elongation/desaturation sequence involved in the production of polyunsaturated fatty acids. There is some difference in response depending on whether the rats are exposed to brominated oils or an equivalent mixture of brominated monoglycerides. The brominated acids are incorporated into lipids with dibromostearate showing a particular affinity for heart lipids and with tetrabromostearate, showing a preference for hepatic lipids. Active debromination of these compounds is indicated by increased urinary bromide after exposure. These substituted fatty acids are also subject to β-oxidation with the C_{16} + C_{14} metabolites being detected in tissue lipids. Shorter chain brominated dicarboxylic acids have been detected in urine.

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EFFECTS OF *cis* AND *trans* 18:1 ON LIPOGENESIS IN MICE. Bryan J. Mulvihill, Beth Wilck-Gerow, Brian L. Walker (deceased) and Monique Von Weinder, Department of Nutrition, University of Guelph, Guelph, Ontario N1G 2W1.

Male C57BL/65 lean and ob/ob mice, 9 weeks old, were fed purified diets containing 10% fat for 13 weeks. Fat mixtures of olive oil:sunflower oil (O/S) and hydrogenated soybean oil:sunflower oil (H/S) were used. The O/S diet contained 63% *cis*-18:1 whereas the H/S diet contained 24% *cis* and 35% *trans*-18:1. Three feeding regimes were used, ad lib.-fed lean, pair-fed ob/ob, and ad lib.-fed ob/ob mice. The ^3H_2O incorporation into tissue lipid (dpm/g/hr) was used to measure in vivo lipogenesis in liver and adipose tissues. There was no significant effect of diet on the incorporation of ^3H_2O into liver lipid for the lean or obese strains. However, a significantly greater incorporation of ^3H_2O into liver fat was observed in the ob/ob mice as compared to the lean. Dietary fat had a significant effect on the incorporation of ^3H_2O into adipose tissue with the H/S-fed group having a greater incorporation as compared to the O/S-fed animals. No strain difference was observed for ^3H_2O incorporation into adipose tissue with these diets.

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FATTY ACID COMPOSITIONS OF RAT LIPIDS FOLLOWING INTAKE OF DIETS WITH VARIOUS RATIOS OF *trans* FATTY ACIDS TO LINOLEIC ACID. Carl-Erik Høy and Gunhild Hølmer, Department of Biochemistry and Nutrition, The Technical University of Denmark, Bldg. 224, DK 2800 Lyngby, Denmark.

The effects of partially hydrogenated fish oils containing *cis*- and *trans*-C 20- and C 22-monoenes on the metabolism of polyunsaturated fatty acids were studied in the rat. Five groups of rats were fed diets containing 20 wt % fat. In the dietary fat the total *trans* content was 33%, whereas the ratio between dietary *trans* fatty acids and dietary linoleic acid varied between 17.4 and 2.3. Furthermore, a group fed partially hydrogenated soybean oil with a *trans* to linoleic acid ratio of 4.0 was included to evaluate the possible effect of chain length of dietary *trans* fatty acids. The fatty acid compositions of phosphatidylcholines, phosphatidylethanolamines, cardiolipins, and triglycerides from liver, heart and kidney as well as triglycerides from adipose tissue were determined.

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INFLUENCE OF DIETARY (n-3)-FATTY ACIDS ON THE COMPOSITION OF RAT ORGAN PHOSPHOLIPIDS. Gunhild Hølmer, Svend Kaasgaard and Carl-Erik Høy, Department of Biochemistry and Nutrition, The Technical University of Denmark, Bldg. 224, DK-2800 Lyngby, Denmark.

The significance of (n-3) versus (n-6) fatty acids is currently being discussed although it seems obvious that the optimal dietary ratio is difficult to define. The necessity of the fatty acids of these two families depends not only on the organ, but also on the phospholipid class examined, reflecting the major physiological role and cellular function. The present experiment elucidates the fatty acid distribution in phosphatidylethanolamines, mainly functioning as structural components of membranes, and phosphatidylinositols, furnishing fatty acid precursors for prostanoids and acting as regulators in receptor-mediated enzyme activation, from liver, kidney and testis of rats fed diets with varying amounts of (n-3) and (n-6) fatty acids.

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Withdrawn.

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LIPID AUTOXIDATION IN THE HUMAN RED BLOOD CELL. F.J. Bunick and W.W. Nawar, Department of Food Science and Nutrition, University of Massachusetts, Amherst, MA 01003.

This work is part of a continuing study in which the human red blood cell membrane in the form of freeze-dried ghosts is used to examine autoxidation in an authentic biological membrane. Previously, we reported results showing that the membrane was resistant to oxidation, requiring over 30 days to absorb as much oxygen as the extracted bulk lipids obsorbed in 5 days. Additional studies of this system have been carried out including further volatile and non-volatile product analysis. Membrane and bulk substrates produced the same homologous series of alkanes and alkenes typical of alpha, beta and gamma oxidations and of decarboxylation. The absence of the aldehyde series and the presence of a substituted aromatic amine dominating the membrane volatiles, points to protein involvement in these oxidations by Schiff-base formation with aldehydes and by peptide scission yielding nitrogenous compounds. Furthermore, the observation that ghosts whose native structure was disrupted with solvents oxidized slowly like the intact ghosts supports the view that the major differences observed in the kinetics and chemistry of the membrane and bulk lipid oxidations appear to be attributable more significantly to the proteins of the membrane than to molecular orientation of the lipids.

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MODIFICATION OF MEMBRANE LIPIDS IN VITAMIN E-DEFICIENT RATS WITH AND WITHOUT CCl_4 ADMINISTRATION. Guey-Shuang Wu, Robert A. Stein and James F. Mead, University of California, Los Angeles, Lab of Biomedical & Environmental Sciences, 900 Veteran Ave., Los Angeles, CA 90024.

Following the studies of lipid peroxidation in a series of simple to complex model systems, our effort is now extended to include the study of in vivo tissue peroxidation. Wistar rats were fed a vitamin E-deficient diet for a prolonged period immediately following weaning. Using rats fed a vitamin E-supplemented diet as controls, searches for the possible peroxidation products and for the modification in the composition of membrane constituents in both lung and liver of these animals were carried out. The effects of CCl_4 poisoning on E-deficient rats were also studied. It was found that

the E-deficiency changes the fatty acid compositions of the membranes considerably, with decreased quantities of 18:1 and 18:2 in both lung and liver. In the severely E-deficient animals, the cholesterol content as also found to be lower in the lung, but not in the liver. The CCl_4 administration apparently causes no immediate further modification in either fatty acid composition or cholesterol content. The CCl_4 poisoning, however, increased the formation of peroxidation products, especially from cholesterol. These cholesterol oxidation products obtained from the tissue were very similar in nature to the products from the autoxidation of mixed liposomes–dipalmitoylphosphatidyl choline and cholesterol. To a much smaller extent, some peroxidation products derived from 20:4 were also found from these treated animals.

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POSSIBLE MECHANISM UNDERLYING INTER-INDIVIDUAL VARIABILITY IN THE SERUM CHOLESTEROL RESPONSE TO DIETARY CHOLESTEROL. A.C. Beynen and L.F.M. Van Zutphen, Department of Laboratory Animal Science, University of Utrecht, P.O. Box 80.166, 3508 TD Utrecht, and M.B. Katan, Department of Human Nutrition, Agricultural University, 6703 BC Wageningen, The Netherlands.

Differences in the response of serum cholesterol to dietary cholesterol in rabbits and rats have a genetic basis. In inbred rabbits, a 5-fold greater response was found in two hyperresponsive strains than in two hyporesponsive strains. Similar differences in serum cholesterol response between inbred strains of rats were also observed. Human hypo- and hyperresponders to dietary cholesterol also exist. We have investigated whether differences in responsiveness are caused by differences in endogenous cholesterol synthesis. In rats on a low cholesterol diet, whole body synthesis, measured as fecal excretion minus uptake, was 17 μmol/day in a hyperresponsive strain as opposed to 32 μmol/day in a hyporesponsive strain. In man, synthesis on a low-cholesterol diet, measured as fecal bile acid and steroid output minus cholesterol consumption, was associated negatively with the subsequent response to dietary cholesterol ($r = -0.44$, $n = 32$, $p < 0.05$). Thus hyperresponsive subjects may have less room for down regulation of cholesterol synthesis after cholesterol loading. Suggestive evidence for this thesis came from another experiment, where we measured serum concentrations of lanosterol, a precursor of cholesterol and a possible indicator of cholesterol biosynthetic activity. Serum lanosterol on a low-cholesterol diet was found to be 3- to 4-fold higher in 4 hyporesponders than in 2 hyperresponders. Furthermore, serum lanosterol was found to decrease after inclusion of extra cholesterol in the diet in 4 human hyporesponders but not in 2 hyperresponders.

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COMPARISON OF NUTRITIONAL TRENDS IN TAIWAN VERSUS THE UNITED STATES. Lung-Bin Hau, Graduate Institute of Food Science & Technology, National Taiwan University, Taipei, Taiwan, Republic of China, and W.W. Nawar, Department of Food Science & Nutrition, University of Massachusetts, Amherst, MA 01003.

A comparison is made between Taiwan and United States with respect to dietary trends and general health statistics. The average daily intake of dietary fat in Taiwan is approximately 74 g/person, less than half that in the United States (160 g/person). This accounts for ca. 32% of the caloric intake (43% in the U.S.A.). Although the present trend in Taiwan has been towards a decrease in total caloric intake, the percentage of calories from fat has been increasing. During the last 30 years, energy intake from rice has decreased dramatically, whereas that from legumes, cooking oils and animal foods has significantly increased. Composition of the diet consumed in Taiwanese cities will be contrasted with that of rural areas. Since 1967, vascular lesions affecting the central nervous system constituted the major cause of death in Taiwan; while cancer was the second. As of 1982, the situation has been reversed; cancer became the first, and cerebrovascular disease the second leading cause of death. Statistical data will be given regarding trends in blood pressure and serum lipoproteins, both in the general population and for certain aborigine tribes. The trends observed in health and disease cannot be attributed solely to the changes in dietary fat intake. Several other dietary and non-dietary factors (e.g. total calories, sodium intake, atmospheric pollution, life style, etc.) are involved.

SESSION Y Biological Activities of Oxidized Sterols Tuesday afternoon

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BIOLOGICAL ACTIVITIES OF SOME OXYGENATED STEROLS. Nobuo Ikekawa, Department of Chemistry, Tokyo Institute of Technology, Meguro-ku, Tokyo 152, Japan.

Recent observations of the biological activities of some oxygenated derivatives of cholesterol in our laboratory will be discussed. (I) Inhibition of enzymatic convertion of dihydrolanosterol into cholesterol by oxygenated sterols. Seven oxygenated sterols were tested for their effect on cholesterol biosynthesis from 24,25-dihydrolanosterol by rat hepatic subcellular $10^4 \times g$ supernatant fraction. The sterols (40 μM) exhibited considerable inhibitory effects on the synthesis of cholesterol from [24,25-^{3}H]-24,25-dihydrolanosterol (18 μM). 5-Cholest-8(14)-en-3β-ol-15-one had the greatest effect (64% inhibition). (II) Quick modulation of platelet aggregation by oxygenated sterols in plasma. We have investigated the effect of oxygenated sterols on platelet aggregation induced by thrombin and ADP. All the oxygenated sterols with a hydroxyl group on their side chains enhanced thrombin-induced aggregation at 25 μM. In the case of ADP-induced aggregation, however, only 22S-hydroxycholesterol enhanced the aggregation and 22R-, 24S- and 25-hydroxycholesterols inhibited the aggregation. (III) Lysis of platelets and erythrocytes by the incorporation of 22R-hydroxycholesterol. We have found that 22R-hydroxycholesterol lyses not only platelets but also erythrocytes in dose-dependent manner. Elevated temperature was required for the lysis, probably to redistribute the sterol in the lipid biolayers in the plasma membranes.

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CYTOTOXICITY AND ATHEROGENICITY OF OXIDIZED CHOLESTEROL. C. Bruce Taylor, Albany Medical College of Union University, New Scotland Avenue, Albany, NY 12208, and Shi-Kaung Peng, Department of Pathology, Harbor-UCLA Medical Center, Torrence, CA 90509.

Cholesterol feeding is commonly used for the induction of experimental arteriosclerosis, and ingestion of cholesterol-containing foods has been widely considered a risk factor in human arteriosclerosis. However, it also has been known that cholesterol is unstable and easily oxidized when stored in air at room temperature. The most frequently occurring spontaneous oxidation products of cholesterol, which have been identified in U.S.P. grade cholesterol and a number of commonly consumed foods, are 25-hydroxycholesterol, cholestane-3β, 5α, 6β-triol, 7-ketocholesterol, 7α- and 7β-hydroxycholesterol. Concentrates of these oxidation products of cholesterol, when given by gastric gavage at 250 mg/kg to rabbits, significantly increased the number of dead aortic smooth muscle cells within a period of 24 hr. Similar necrogenic effects have been found in cultured aortic smooth muscle cells, in which 25-hydroxycholesterol and cholestane-3β, 5α, 6β-triol are shown to be most toxic at levels of less than 10 μg/ml in the culture medium. Interestingly, purified cholesterol has no effect on the smooth muscle cells. Using scanning electron microscopy, both compounds, when given intravenously, produced balloon- and crater-like defects on the intimal surface followed by adhesion of platelets and leukocytes and formed microthrombi on arterial walls. Further studies using non-human primates demonstrated 25-hydroxycholesterol was 30% absorbed as measured by the dual-isotope plasma ratio technique; also, a high concentration of its radioactivity in plasma was found in VLDL and LDL and very little in HDL. This finding strongly implicates oxidized cholesterol in atherogenesis.

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COMPARATIVE ATHEROGENIC EFFECTS OF CHOLESTEROL AND CHOLESTEROL OXIDES. N.A. Higley, J.T. Beery and S.L.

Meetings

Taylor, Food Research Institute, University of Wisconsin, 1925 Willow Drive, Madison, WI 53705, and J.W. Porter and J.A. Dziuba, Lipid Metabolism Laboratory, Wm. Middleton V.A. Hospital, Madison, WI 53705.

Research indicating that the autoxidation products of cholesterol are associated with atherogenicity has led to a comparative study of the subchronic effects of feeding cholesterol and cholesterol oxides to rabbits. Three groups of 5 rabbits each were fed semipurified pelletized rabbit diets: (i) unsupplemented control, (ii) supplemented with twice recrystallized cholesterol (greater than 90% cholesterol and no known oxides by HPLC), or (iii) supplemented with a mixture of cholesterol oxides purified by preparative HPLC (0.06% cholesterol by analytical HPLC). At the completion of the 10-week feeding study, the rabbits were killed and the arterial system removed after in situ aortic pressure perfusion-fixation. At the time of death, all of the cholesterol-fed animals had lesions, and the total number of lesions for cholesterol-fed animals was approximately six times greater than the total number of lesions in the oxide-fed animals. Areas were selected for light microscopy to further characterize the lesions. The tissues from cholesterol-fed animals showed the most dramatic changes in collagen and elastin. Calcification also predominated in the lesions of cholesterol-fed animals. The severity of the lesions of the oxide-fed animals as judged by the site of the lesion, the extent of intimal and medial involvement, collagen and elastin changes, and calcification was not as great as the lesions of the cholesterol-fed animals. This study has shown that (i) cholesterol oxides in the concentrations and relative compositions administered here are markedly less atherogenic to rabbits than cholesterol, and (ii) highly purified cholesterol is atherogenic.

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VARIATIONS OF THE MEMBRANEOUS CHOLESTEROL CONTENT ALTER THE CALCIUM INFLUX THROUGH THE CALCIUM-SPECIFIC ENTRY CHANNEL IN HUMAN ERYTHROCYTES. M. Stimpel, L. Neyses, R. Locher, R. Streuli and W. Vetter, University Hospital, Department of Internal Medicine, Medical Policlinic, Rämistrasse 100, CH-8091 Zurich, Switzerland.

It is well-known that a positive correlation exists between hypercholesteranemia and atherosclerosis. The reason for this correlation is as yet unknown and the mechanism whereby cholesterol causes cellular alterations of the vascular tissue is entirely unclear. Recent experiments with calcium entry blockers indicate that these compounds are capable of suppressing atherogenesis in cholesterol-fed rabbits. This suggests that cholesterol may affect the movement of calcium ions across the membrane of endothelial and/or smooth muscle cells. In order to study the influence of the cholesterol content on the calcium entry channel, the human red blood cell was used as a model system. This cell type facilitates calcium flux measurements, since it is free of any intracellular calcium sequestering organelles and lacks a Na/Ca exchanger. Moreover, it has a calcium channel with pharmacological properties very similar to the channel in endothelial and vascular smooth muscle cells. The cholesterol to lecithin ratio (C/L ratio) of the membrane was modified experimentally by incubating the cells (15 hr, 25 C) with liposomes of defined C/L ratios. Subsequently, net 45 Ca^{++}-influx into the cell was measured by inhibiting the Ca-ejecting ATPase with vanadate. Additionally, the use of nitrendipine, a potent calcium channel blocker, allowed the determination of the Ca-influx through the calcium channel. A positive correlation between the 45 Ca^{++}-influx and the molar C/L ratio of the membrane was found over a wide C/L range. A molar C/L ratio of 1.4 in the membrane increased calcium influx by 150% compared to controls (molar C/L ratio = 0.8, calcium influx rate = 100%), while a molar C/L ratio at 0.75 decreased calcium influx by 50%. We conclude that the cholesterol content of the membrane greatly influences the calcium channel and thus plays a pivotal role for the availability of calcium as a second messenger. These findings may provide a link between high plasma cholesterol and the development of atherosclerosis.

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OXIDIZED DERIVATIVES OF CHOLESTEROL AND ITS INTERFERENCE WITH THE CALCIUM CHANNEL. L. Neyses, M. Stimpel, R. Locher, W. Vetter and R. Streuli, University Hospital, Department of Internal Medicine, Medical Policlinic, Rämistrasse 100, CH-8091 Zurich, Switzerland.

Much evidence exists that autoxidation products of cholesterol may be involved in the pathogenesis of atherosclerosis. It has been demonstrated that oxidized sterol compounds (OSC) cause severe damage to the endothelium when injected into rabbits and that they occur in human atherosclerotic plaques. Since calcium has been suggested to play a pathogenetic role in the development of atherosclerosis as well, it seemed tempting to study the influence of OSC on the calcium influx through the calcium entry channel. Because of multiple advantages, the human red blood cell was used as a model system. The calcium-ejecting ATPase was inhibited by vanadate. The cells were loaded with OSC at concentrations between 1.25-25$\times 10^{-5}$ mol/l, i.e. 0.075-1.5 μg OSC/10^7 cells. At 5$\times 10^{-5}$ mol/l, 22-hydroxycholesterol, cholestan 3β, 5α, 6β'-triol, 5α-cholestan, 3β-ol, cholestan 3β 5α-diol, 6-one and 5α-cholestan 3β-ol, 7-one stimulated Ca-influx by 86%, 88%, 40%, 21% and 27%, respectively. In contrast, 25-hydroxycholesterol, 7β-hydroxycholesterol, 20α-hydroxycholestrol and 7-ketocholesterol inhibited by 76%, 55%, 32% and 34%, respectively. Both stimulation and inhibition were dependent on the amount of OSC incorporated into the membrane. The calcium-channel blocker nitrendipine dose-dependently inhibitied the influx up to 70%. More than 90% of the total stimulation or inhibition of calcium influx by OSC was accounted for by stimulation or inhibition of the nitrendipine-inhibitable part of calcium influx. These results demonstrate that oxidized derivatives are able to modulate the calcium channel in human red blood cells in a highly specific manner.

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RELATION OF CHOLESTEROL OXIDATION PRODUCTS TO ATHEROSCLEROSIS. L.H. Krut, Department of Medicine, Baragwanath Hospital & University of Witwatersrand, P.O. Bertsham 2013, Johannesburg, South Africa.

Arterial endothelium is subject to inevitable injury. Plasma low density lipoproteins (LDL) gain access to the subendothelial space at these sites in quantities directly proportional to their concentration in plasma. LDL are precipitated in these areas with disruption of the lipoprotein complexes, leaving free lipid deposited. There are limited mechanisms for clearing this lipid. Triglycerides and phospholipids can be metabolized locally, but cholesterol cannot. Clearance of cholesterol would depend on its solubilization by phospholipid. The proportion of phospholipid to cholesterol in LDL is such that there can never be enough phospholipid to solubilize all the cholesterol, which crystallizes. Crystalline cholesterol is not readily cleared and is sclerogenic. Thus compounds promoting solubility of cholesterol in tissue could have relevance in preventing atherosclerosis. Cholesterol subjected to oxidation yields several compounds which act synergistically with phosphatidylcholine to enhance enormously the solubility of cholesterol in supersaturated solution in a triglyceride oil and also in aqueous medium. When pure cholesterol is implanted subcutaneously in rats, none of the cholesterol is cleared and it evokes a massive sclerotic reaction. If cholesterol is mixed with its oxidation products prior to implantation, all the sterols go into solution and are cleared rapidly and completely, leaving negligible residual sclerosis. The above observations could have relevance to atherogenesis in man. Foods of animal origin left exposed to light and air result in the spontaneous generation of new compounds which also show synergism with phosphatidylcholine in promoting solubility of cholesterol in supersaturated solution in triglycerides. The generation of these compounds is prevented by modern food-handling techniques. It is suggested that modern technology designed to prevent spoilage of foods of animal origin has eliminated from the Western diet compounds which prevent the crystallization of cholesterol in the arterial wall, consequently promoting atherosclerosis.

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THE EFFECT OF CHOLESTEROL OXIDATION PRODUCTS ON MEMBRANE FUNCTIONS. Shi Kaung Peng, Robert J. Morin and Steve Sentovich, Department of Pathology, Harbor-UCLA Medical

Center, 1000 West Carson St., Torrance, CA 90509, and C. Bruce Taylor, Albany Medical College.

Certain oxidation products of cholesterol have been shown to be cytotoxic. If this damage occurs to the arterial cells, these compounds may play an important role in the initiation of atherosclerosis. The viability of cells depends upon the integrity and function of cell membranes. The effects of cholesterol oxidation products on membrane associated functions, including membrane bound enzyme activity, carrier mediated transport and endocytosis were therefore investigated. For the study of membrane bound enzymes, Na^+,K^+-ATPase and 5'-nucleotidase were measured both biochemically and cytochemically by electron microscopy. Cultured aortic smooth muscle cells which were incubated with 10 μg/ml of cholestane-3β,5α,6β-triol (triol) or 25-hydroxycholesterol (25-OH) for 24 to 48 hr showed marked decreases of ATPase and 5'-nucleotidase activity when assessed by electron microscopic cytochemistry. Biochemical assays also showed an inhibitory effect of the sterols on 5'-nucleotidase activity, but decreased ATPase activity only in cells incubated with triol for 48 hr. The discrepancy may be attributable to a different sensitivity between these two assays. For study of carrier mediated transport, radiolabeled 2-deoxy-D-glucose, a glucose analogue which is not metabolized in the cells, was utilized. The uptake of this labeled compound was determined in culture and aortic smooth muscle cells which were preincubated with several cholesterol oxidation products for various time periods. Triol had the most potent and rapid inhibitory effect on hexose transport (36% inhibition in 15 min); the effect was reversible after removal of the sterol from the medium. Hexose transport was only slightly inhibited by 25-OH after more than one hour incubation. To determine the effects on endocytosis, the cultured aortic cells were preincubated with various sterols for 15 min to 24 hr, then incubated with horseradish peroxidase (HRP) at 1 mg/ml for 1 hr before lysis with Triton X-100. The uptake of HRP was measured spectrophotometrically by using a substrate mixture of H_2O_2 and O-dianisidine. Again, triol was most inhibitory and showed this effect after only 15 min of pre-incubation; 25-OH showed no significant effects after a 24-hr pre-incubation. In conclusion, the data suggest that these two most cytotoxic cholesterol oxidation products may have two different cytotoxic mechanisms. The prompt onset of the effect of triol may be attributable to an incorporation of the sterol into the cell membranes. On the other hand, 25-OH, a potent inhibitor of HMG CoA reductase and cholesterol biosynthesis, may have a delayed effect on membrane function by depleting the cholesterol available for membrane synthesis.

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METABOLIC RESPONSES OF THE LAYING HEN TO DIETARY 7-KETOCHOLESTEROL, OXIDIZED CHOLESTEROL AND PURE CHOLESTEROL. Edward C. Naber, Ruben E. Vargas, John B. Allred and Matthew D. Biggert, Ohio State University, Poultry Science Department, 674 W. Lane Ave., Columbus, OH 43201.

Experiments were conducted to study the effect of 7-ketocholesterol (7-k) in the presence or absence of pure cholesterol (PCH) or oxidized cholesterol (OCH) in diets of laying hens on reproductive performance and several parameters of cholesterol metabolism. In the first experiment, cholesterol synthesis and transport was examined by the in ovo incorporation of ^{14}C-acetate into yolk triglycerides and cholesterol. Energy balances also were conducted. In the second experiment, hepatic HMG CoA reductase activity was measured in vitro to evaluate potential cholesterol synthesis. In both experiments reproductive performance and egg yolk cholesterol concentration were measured. Dietary PCH or OCH significantly reduced relative acetate incorporation into yolk cholesterol, while 7-k had no effect on carbon flow from acetate into egg cholesterol. While 7-k alone did not alter total yolk cholesterol concentration, it moderated the effect of PCH or OCH on increasing yolk cholesterol concentration. No consistent effects of dietary sterols on reproductive performance or energy balance were observed. Hepatic HMG CoA reductase activity was suppressed dramatically by feeding PCH or OCH and moderately suppressed by 7-k. In combination with PCH or OCH, 7-k did not further depress enzyme activity. The observation that 7-k alone depressed hepatic HMG CoA reductase activity without changing relative acetate incorporation into yolk cholesterol while limiting cholesterol deposition in egg yolk from PCH or OCH is interpreted to mean that 7-k may stimulate sterol transport and excretion while limiting cholesterol synthesis.

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THE FORMATION AND CYTOTOXIC PROPERTIES OF CHOLESTEROL OXIDES. Alex Sevanian and Andrew Peterson, Institute for Toxicology, University of Southern California, 1985 Zonal Ave., Los Angeles, CA 90033, and James Trosko, Department of Pediatrics and Human Development, Michigan State University.

The oxidation of cholesterol in membranes is known to produce several isolable products. Among the major oxidation products are the 6- and 7-hydroperoxides, the corresponding oxysterols and the diastereomeric 5,6 epoxides. These products can be formed through oxy-radical attack on cholesterol, and we have found them also to reflect the oxidative process in membranes as induced by free-radical reactions. Formation of the epoxides has been demonstrated in liver microsomes incubated with NADPH plus iron, via the "microsomal P-450 dependent lipid peroxidation." Lipid autoxidation in artificial membranes also yields cholesterol epoxides (CE) in appreciable quantities, along with the above named oxidation products. We have reported previously that initiation-type reactions which are dependent on a flux of oxy-radicals and are operationally associated with early stages of lipid peroxidation generate largely the alpha isomer of CE. Propagation-type reactions, characteristic of lipid peroxide-dependent lipid peroxidation, produce largely the beta isomer of CE. The ratio of these diastereomers can be indexed readily to the mode and extent of lipid peroxidation. Analysis of tissues and biological fluids reveals isomeric ratios of CE's typical of a propagation-type reaction mechanism. The occurrence of CE's in biological systems is also influenced by the activity of an apparently specific CE hydrolase which catabolizes both isomers with equal facility. The enzyme experiences end-product inhibition by cholestantriol (CT), inhibiting beta isomer hydration more effectively than the alpha isomer. In addition, 7-oxysterols, and to a lesser extent the 6-oxysterols, are effective inhibitors of CE hydrrolase, again preferentially inhibiting beta isomer hydration. The net result in cells may be to augment the steady state levels of beta over the alpha isomer. Studies with cultured cells show that both isomers can be mutagenic, with the expression of mutagenesis determined by level of epoxide hydrolase activity. Low level mutation is associated with relatively high CE hydrolase activity, but this is accompanied by significant increases in cytotoxicity. The cytotoxicity is attributed to CT as we have observed the order of cytotoxity in V79 cells to be CT ≫ beta CE > alpha CE. We shall present evidence for the role of these compounds in promoting the mutagenic and possibly the carcinogenic process in cells. Thus it appears that expression of genotoxicity can be enhanced under conditions which either inhibit CE hydrolase or reduce the cytotoxicity of these compounds. In this respect, 7-ketocholesterol appears to be a particularly effective potentiator of mutagenesis.

SESSION Z Human Milk Lipids
Tuesday afternoon

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LIPASES IN HUMAN MILK. Margit Hamosh, Georgetown University Medical Center, 3800 Reservoir Road, N.W., Washington, DC 20007.

Human milk contains two lipases: lipoprotein lipase (LPL) and bile salt-stimulated lipase (BSSL). LPL regulates the supply of circulating lipid to the mammary gland for milk fat synthesis, and is first detected in milk after parturition. BSSL, active in neonatal fat digestion, is present in mammary secretion prepartum (70 days before term delivery) and is probably a constant component of mammary secretory cells. BSSL activity is 10- to 50-fold higher (20-100 μmol FFA produced/ml/min with tri-3H-olein substrate) than LPL activity (0.5-5.0 μmol FFA/ml/min) in human milk. The level of both lipases in milk is similar, irrespective of length of pregnancy (26-40 wk). Human milk provides the term and preterm infant not only with nutrients but also with the digestive enzymes (lipase, amylase) needed for their absorption. This talk will discuss the role of LPL and BSSL in milk fat synthesis, digestion in the newborn and storage of banked milk.

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LIPID COMPOSITION OF BREAST MILK FROM MOTHERS OF TERM (T) AND PRETERM (PT) INFANTS. Joel Bitman and D.L. Wood, U.S. Dept. Agriculture, Beltsville, MD 20705, and N.R. Mehta, P. Hamosh, and Margit Hamosh, Georgetown University Medical Center, Washington, DC 20007.

Milk was collected from mothers of 18 very premature (26-30 wk gestation age), 28 premature (31-36 wk) and 6 term (37-40 wk) infants on postpartum day 3 (colostrum) and days 7, 21, 42 and 84. Lipids were analyzed by thin layer (TLC) and gas liquid (GLC) chromatography. Total fat content increased during lactation whereas phospholipids (PL) and cholesterol declined. PL were separated from neutral lipids by chromatography using a silica Sep-Pak cartridge. Milk lipids were separated by TLC into classes: PL, monoglycerides, free fatty acids, cholesterol, 1,2-diglycerides, 1,3-diglycerides, triglycerides and cholesteryl esters. PL were separated by TLC into classes: sphingomyelin (S), phosphatidylcholine (PC), -serine (PS), -inositol (PI), and -ethanolamine (PE). Changes in fatty acid (FA) composition of the milk and within individual PL classes occurred only during the maturation of milk from the secretion of colostrum to mature milk. Mature milk at days 21, 42 and 84 was very constant in FA composition. Medium-chain FA were 10% in colostrum and increased to 18% only in pre-term (PT) milk. Compensatory decreases were observed in PT 18:1. Long-chain polyunsaturated FA were higher in PT than in term (T) milk. PL were extremely constant, being 38, 28, 9, 6, 19 for S, PC, PS, PI, PE, throughout lactation in PT and T milk. During the first 3 wk, S showed an increase in 22:0, 24:0 and 24:1 and a decrease in 16:0. 18:2 increased and 18:1 decreased in phosphoglycerides during the first 3 wk of lactation. The differences in preterm milk may be of nutritional significance for the lipid requirements of the premature infant.

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ABSORPTION OF FATTY ACIDS FROM HUMAN MILK AND FORMULA-FED PRETERM INFANTS. M.T. Clandinin, Departments of Foods & Nutrition, and Medicine, The University of Alberta, Clinical Sciences Building, Edmonton, Alberta T6G 2G3, Canada, and J.E. Chappell, Department of Nutritional Sciences, University of Toronto, 150 College Street, Toronto, Ontario M5S 1A8, Canada.

During the first four neonatal weeks serial, total fatty acid and individual fatty acid balance studies were completed in 35 "healthy," appropriate for gestational age, premature infants. Infants were less than 1,500 g at birth, descriptively similar and, at the time of study, were receiving similar volumes of either preterm mother's milk or formula (SMA_{20}). Total fatty acid and major fatty acid constituents were similar between the two feeding regimes. Total fecal output (g/day) and total fatty acid excretion (g/Kg/day) were higher, whereas subsequent total fat absorption (g/Kg/day) and coefficient of absorption were significantly lower in the formula-fed group when compared to the breast milk-fed infants ($p < 0.001$). Administration of oral calcium supplements in the form of calcium lactate (1.5-2.0 mmol/Kg/day) decreased total fatty acid absorption in both the breast milk-fed group ($p < 0.01$) and formula-fed group ($p < 0.001$). Furthermore, the effect of feeding ($p < 0.0001$) and oral calcium ($p < 0.001$) independently influenced coefficients of absorption for major fatty acids fed (i.e., $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2(6)}$). It was concluded that oral calcium supplements alter significantly the efficiency of lipid absorption in enterally fed preterm infants. Although the main indices for growth were similar between the feeding groups, infants with efficient rates of total fatty acid absorption attained faster rates of gain in weight and skinfold thickness.

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COMPARISON OF MILK TRIGLYCERIDES FROM MICE FED *trans* OR CONTROL FAT DIETS. Beverly B. Teter, Luz M. Neira, Mark Keeney and Joseph Sampugna, Department of Chemistry, University of Maryland, College Park, MD 20742.

Triglycerides (TG) were isolated from milk of C57B1/6J mice or from stomach contents of suckling pups. Mice were reared on one of several isocaloric diets differing in fat content (20 or 40% of calories), *cis*-9-*cis*-12-octadecadienoate (18:2) level (2.5, 6 or 12% of calories) and *trans* fatty acid (t-18:1) content (0, 5.0 or 10% of calories). Control diets were formulated to give compositions which were nearly identical to the appropriate *trans* diet, except that oleic acid replaced the *trans* isomers. The fatty acid composition of milk fat TG was influenced by the composition as well as the level of dietary fat. Compared to low fat diets, high fat diets depressed de novo synthesis of milk fatty acids, as evidenced by lower percentages of short and medium chain fatty acids and higher percentages of 18:2 and t-18:1 in milk TG. Levels of 18:2 and of t-18:1 in milk fat increased as their levels increased in the diet. In general, the fatty acid composition of triglycerides isolated from stomach contents and milk fat were similar, but not identical. In particular, those of stomach contents had higher ratios of 18:2/*trans*-18:1 and 16:0/16:1, compared to milk fat triglycerides. These results suggest that triglycerides in stomach contents are not necessarily representative of milk fat triglycerides.

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TOTAL PHOSPHOLIPID ANALYSIS IN HUMAN MILK WITHOUT ACID DIGESTION. Kenneth E. Hundrieser, R.M. Clark and R.G. Jensen, Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06268.

A colorimetric method for measuring the organic phosphorus in human milk was studied. In the method, a chromogenic reagent was complexed with the phosphorus moiety of intact phospholipids, eliminating the need for acid digestion of the lipids. The procedure was compared to a modified Fiske and Subbarow method for measuring inorganic phosphorus after sulfuric acid digestion of the milk lipids. Human milk samples were assayed and compared to phosphatidylcholine, phosphatidylethanolamine and sphingomyelin standards. Spectral curve absorption maxima prepared from phospholipid standards and lipids from milk were equivalent and occurred between 720 and 740 nm. Calibration curves prepared from the three phospholipid standards were equivalent as measured by the new method. A reproducibility study with 20 total lipid samples was run. The phosphorus measured ranged from 4.7-5.5 μg with a mean ± SD of 5.2 ± 0.2 μg P. The coefficient of variation of the reproducibility study was 3.8%. The recovery of phosphatidylcholines added to 10 mg samples of human milk triacylglycerols as measured by the new method ranged from 99.0 to 102.1%. A least squares regression equation was developed between the new method and the Fiske and Subbarow method after acid digestion. The y intercept, a measure of constant error, was calculated to be 0.13 mg P/ml milk. The slope, a measure of proportional error, was calculated to be 0.09. Random error was estimated by the standard error of the estimate in the y direction, Sy = 0.18 mg P/dl milk and by the correlation coefficient, r = 0.980. We concluded from these results that the new method provides a satisfactory alternative for the determination of organic phosphorus in human milk. It is simple, accurate, and less time is needed than the methods requiring acid digestion.

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ANALYSIS OF TOCOPHEROL IN HUMAN MILK. Carol J. Lammi-Keefe, University of Connecticut, Department of Nutritional Sciences, U-17, Storrs, CT 06268.

Tocopherol in human milk was determined with high performance liquid chromatography (HPLC). This method is rapid and sensitive and permits the separation and quantitation of the closely related tocopherol isomers. HPLC operating conditions were: column, $-NH_2$ (25 cm X 4.6 mm); solvents, n-hexane:2-propanol (98:2); flow rate, 2.0 ml/min; detector, UV, 290 mm. We have characterized the sources of variation when milk is extracted with a modified Folch method (Clark, R.M., et al., 1982, J. Ped. Gastroentero. Nutr. 1:311) and the extract injected onto the column. Duplicate extractions of the milk and five injections per extraction reduce the errors in α and γ tocopherol determinations over one extraction and one injection by approximately 50%. This method has been employed to determine tocopherol levels in breast milk. Between 2 and 16 weeks postpartum, tocopherol content decreased.

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LIPIDS IN HUMAN MILK: A REVIEW OF RECENT RESEARCH.

Meetings

Robert G. Jensen, R.M. Clark, A.M. Ferris and C.J. Lammi-Keefe, Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06268.

Samples of human milk which are to be analyzed for lipids should be extracted immediately, pasteurized or stored at −70 C to prevent lipolysis. The lipid content is best determined by the Creamatocrit method or by extraction followed by gravimetric determination. Modified Folch or dry column extractions are satisfactory. Triacylglycerols account for 98+% of the lipids with ca. 10 mg of cholesterol per 100 ml of milk and a phospholipid content of up to 1% of the total lipids. Methods for these and other analyses will be discussed. Data on the influence of stage-of-lactation, diet, diurnal rhythmn and gestational age on the class and fatty acid composition of human milk lipids will be presented as will information on the fat-soluble vitamins.

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MATERNAL VARIABLES AND THE SECRETION OF LIPIDS IN HUMAN MILK. Cutberto Garza, David L. Hachey, Nancy L. Butte, and Peter D. Klein, Baylor College of Medicine/Dept. Pediatrics, Children's Nutrition Research Center, Medical Towers Bldg., Suite 501, 1709 Dryden, Houston, TX 77030, and Edward Emken, USDA, Northern Regional Research Center, Peoria, IL.

Of the three major energy sources in human milk, the concentration of total lipids shows the most variability between individuals. Forty-five mothers of exclusively breast-fed infants have been studied over the first four months of lactation. These studies probed possible associations between either the caloric content of milk (kcal/g) or the caloric production (kcal/d) and a number of maternal variables including dietary intake, weight, weight change during lactation, body fat content and the change in body fat during lactation. Concurrently, separate studies using stable isotope tracers were conducted in three women to estimate the amount of dietary fatty acids incorporated into human milk lipids and to describe the dynamics of milk triglyceride synthesis. Significant positive associations were found between caloric content and/or production and maternal energy intake and measures of maternal energy stores, but the associations were relatively weak. No differences in the characteristics of women producing milks with the highest and lowest deciles of fat content were detected. However, consistency was observed in the fat content of milk produced by individual women during the four months of observation. A ten-hour delay occurred between the ingestion of a meal containing lipid tracers and the peak secretion of isotopically labeled lipids in milk. In contrast, peak isotopic enrichments in plasma chylomicrons and very low density lipoproteins were observed by four hours, the earliest time that plasma was sampled. Milk from one breast was sampled at designated intervals over 72 hr; 4.87 ± 0.57% of the tracer dose of palmitate-d_2, 6.65 ± 1.88% of the oleate-d_6 dose and 4.65 ± 1.13% of the linoleate-d_4 dose were recovered during this sampling period. These studies demonstrate the feasibility and desirability of measurement of potential interactions between diet, nutrient stores, hormonal responses and the dynamics of lipid transport and synthesis in the same individual.

SESSION AA Trends in Edible Oil Processing and Consumption in Various Parts of the World I Wednesday morning

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SOYBEAN OIL PROCESSING AND UTILIZATION IN EUROPE. Roger Leysen, American Soybean Association, Centre International Rogier, bte 521, 1000 Bruxelles, Belgium.

After reviewing briefly some new technological developments for soy oil (physical refining, superdegummed oil, the ALCON process), most of the paper will concentrate on consumption trends. The market will be examined both from the production capacity and from the consumption angle. As to production capacity, the imbalance between oil and meal consumption will be discussed. The consumption of soy oil in different food products will be reviewed (table and frying oils, margarines, mayonnaises). Competition from oilseeds grown locally will be highlighted.

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THE PALM OIL PROCESSING INDUSTRY IN MALAYSIA. Malcolm MacLellan, Palm Oil Research Institute of Malaysia, P.O. Box 10620, Kuala Lumpur, Malaysia.

The author traces the development of the palm oil refining industry in Malaysia and discusses its current status and likely development.

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TRENDS IN EDIBLE OIL PROCESSING AND UTILIZATION IN CENTRAL AMERICA. Carlos Farner, O.S.T.I., Neumunz Inc., Apartado Postal 2676, Guatemala, Cuidad.

Geographical and statistical data on Central America is included in the introduction. The production of oilseeds for the last 5 years is given as well as an analysis of the agricultural aspects of oilseeds in Central America. In most countries there is a definite decrease in the production of oilseeds and consequently there has been an increase in imports. Actually, only Honduras is self sufficient and has an excess of palm oil, although they import some liquid oils. There is formal palm oil extraction in Honduras and Costa Rica. The other countries have solvent extraction plants and some screw press oil mills, mainly for cottonseed. Refineries and finished products plants are very modern and equipped to manufacture edible oils. The production and utilization of locally produced oleochemicals is only just beginning. Modern and sophisticated laboratory equipment exists only in a few institutions and some universities.

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TRENDS IN EDIBLE OIL PROCESSING AND UTILIZATION IN ASIA. Lars H. Wiedermann, American Soybean Association, 541 Orchard Road, 15-01 Liat Towers, Singapore 0923, Republic of Singapore.

Asia is many countries spread over a broad land mass, representing large populations having varied agricultural traditions and cultural eating habits. Even today this diversity is reflected by their different oil sources, both indigenous and imported, and their varied approaches to fats and oils processing and utilization. The best way to look at Asia is in terms of its component parts and a logical division for our subject is: 1) East Asia, the Japanese influence: Japan, South Korea and Taiwan; 2) Southeast Asia, the ASEAN countries: Thailand, Malaysia, Indonesia, Singapore and the Philippines; 3) the Asian Sub-continent: India, Pakistan and Bangladesh, and 4) the People's Republic of China, mainland China. Since the PRC will be discussed by other speakers, this paper will only be concerned with subject discussions for the first 3 country categories. This presentation will review the subjects of oil sources, utilization and processing practices and trends in terms of the commonalities within and between these categories rather than on a country-by-country basis. Palm and soybean oils are the 2 primary imported oils in these areas. For soybean oil, this is as both an oil and as soybeans for local crushing operations. Groundnut or peanut oil, rapeseed (HEAR) oil, rice bran oil, coconut oil and palm oil in Indonesia and Malaysia represent the main indigenous oil sources. Meat fats are also involved in minor but not insignificant trading. The oil processing practices are as varied as their equipment, ranging from mortar and pestle operations for rapeseed oil to the latest technology for physical refining of soybean oil. Utilization patterns are changing rapidly. Even in areas where change is slower, it is sometimes difficult to recognize the "old family recipe."

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NRRC RESEARCH DEVELOPMENTS OF INTEREST TO EDIBLE PROCESSORS. T.L. Mounts, Northern Regional Research Center, ARS-USDA, 1815 N. University Street, Peoria, IL 61604 USA.

Research at the Northern Regional Research Center is directed to the establishment of new knowledge concerning edible oil quality and stability. A laboratory-scale adiabatic reactor has been constructed for simulation of storage-damage to soybeans. Oil extracted from damaged beans showed increased free fatty acid concentrations,

and decreased phosphorus and linolenic acid contents. Increased levels of non-hydratable phospholipids have been observed in oils from damaged beans. Use of food-grade surfactants as degumming adjunts has been evaluated for improved removal of non-hydratable phospholipids. Although some surfactants showed positive effects in oils from damaged beans, phosphorus levels in the degummed oils remained too high for optimum processing. Techniques for determining ultratrace levels of metals in hydrogenated vegetable oils and fats have been developed and applied to the analysis of commercial products. Results indicate that current post-hydrogenation processing effectively removes residual nickel catalyst from partially hydrogenated soybean oils used for salad/cooking oils and margarines, but removes it less effectively from more highly hydrogenated fats used in plastic shortenings. Control of lipid synthesis during soybean seed development may provide an alternative to post-harvest processing techniques for oil stability. Acyl carrier protein (ACP) was chosen as a representative marker of the fatty acid synthetase pathway. Enzymic and immunochemical assay techniques have been developed to quantitate ACP in soybeans. A study of the various stages of seed development indicated that the ACP activity increase correlated well with the in vivo increase in lipid synthesis. Major control over the increase in lipid synthesis arises from regulation of the levels of the fatty acid biosynthesis proteins. Increased understanding of these biosynthetic mechanisms will enhance the potential to tailor the soybean genetically to yield oil with improved stability.

SESSION BB Analytical Methods for Protein II
Wednesday morning

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LABELED ANTIBODIES AS PROBES FOR HISTOCHEMISTRY AND CYTOCHEMISTRY. Julius W. Dieckert, Texas A&M University, Department of Plant Pathology and Microbiology, and Marilyne C. Dieckert, Texas A&M Research Foundation, College Station, TX.

Polyclonal (PCAbs) and monoclonal antibodies (MAbs) are finding increasing use as probes for identifying specific molecules in biological matrices of diverse kinds. By using appropriate labels for the primary or secondary antibodies, the probes can be recognized by light or electron microscopy. Fluorescent derivatives of the antibodies are commonly made with fluorescein or rhodamine isothiocyanate and used with an epifluorescence microscope. In transmission electron microscopy, antibodies may be tagged with gold spheres, enzymes and ferritin. These and other markers will be discussed. PCAbs and MAbs depend for specificity on one or more antigenic determinants, or epitopes, on the proteins of interest. A certain structural integrity of the epitope must be maintained for the antigen/antibody reaction to occur. Care must be taken in specimen preparation to maintain the structure of the epitope with respect to the antibody activity. Current thinking and methodology to achieve this goal for diverse types of epitopes will be reviewed. Particular attention will be given to fixation and embedding methods. In some instances false, positive or negative results are obtained. Nonspecific adsorption may be a problem with certain embedding media and sample matrices. For example, plastics often absorb proteins and are known to nonspecifically adsorb antibodies. Some cells have specific receptors for the F_c region on immunoglobulins. Negative results are obtained when the labeled antibody cannot reach the target. Recent advances in methods for recognizing and solving these problems will be discussed. Several examples will be given illustrating the effective use of PCAbs and MAbs as histochemical and cytochemical probes.

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THE USE OF ROBOTICS IN AMINO ACID AND PROTEIN ANALYSIS. W. Jeffrey Hurst and Robert A. Martin, Jr., Hershey Foods Corporation, 1025 Reese Avenue, P.O. Box 805, Hershey, PA 17033-0805.

The use of laboratory robotics in the determination of amino acids and proteins will be described. The robot adds the derivatizing reagent to the sample or standard in a serial fashion, mixes the samples and withdraws an aliquot for subsequent HPLC analysis. After it prepares the sample it then injects it on the HPLC. The power/event controller of the robot actuates the gradient start for the final determination. At the termination of the analysis, the robot actuates the gradient reset in preparation for the next analysis. This type of analytical method allows the elimination of some of the kinetic problems associated with this determination. It additionally allows for the unattended accomplishment of this assay.

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HIGH-RESOLUTION NMR EXPERIMENTS FOR SOLID PROTEIN SAMPLES. Gary E. Maciel, Colorado State University, Department of Chemistry, Colorado State University, Ft. Collins, CO 80523.

Advances in high resolution NMR techniques for solids make it possible to examine protein-containing solids directly by NMR, using ^{13}C (or ^{15}N for isotopically enriched samples). The use of high-power ^{1}H decoupling and magic-angle spinning (MAS) for line narrowing of and cross polarization (CP) for sensitivity enhancement provides essentially a routine high-resolution NMR technique for complex organic solids. This kind of technique has been used in the study of protein components in food plants and in biosynthetic studies. In the biosynthetic area there have been studies employing combinations of ^{13}C and ^{15}N experiments (including ^{13}C-^{15}N cross-polarization). Recent applications of 2-dimensional spin exchange experiments, in which nearby nuclei exchange spin states, show promise for studying a variety of geometrical arrangements in solid proteins.

NMR studies of proteins should benefit greatly from further developments of high-resolution NMR techniques capable of providing higher levels of structural detail. These techniques will include a variety of 2-dimensional FT NMR approaches, as well as techniques in which structural distinctions are made on the basis of the coherent evolution of ^{13}C spins under structure-dependent interactions with other nuclei. This talk will examine the present status and prospects for high-resolution NMR techniques for solid proteins.

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OVERVIEW OF RAPID ANALYSIS INSTRUMENTS FOR OILSEEDS AND OIL MILL PRODUCTS. J.P. Wagner, J.T. Farnsworth and L.A. Johnson, Food Protein R&D Center, Texas Engineering Experiment Station, Texas A&M University, Faculty Mail Box 183 College Station, TX 77843.

A state-of-the-art review was carried out to identify instruments and sensors capable of providing rapid analysis of oilseeds, by-products, combustible solvents and control of selected process conditions. The broad-based review covers various laboratory instruments, portable sensors and selected on-line transducers. The operating principles for the various sensors are detailed, potential areas of application are outlined and potential or known problem areas are pointed out. The sensors are further classified according to maintenance requirements, ease of operation and response characteristics. Ca. 15 different sensors (discussed under gas analyzers, humidity, moisture, electrical conductivity, other analyzer types and interface or emulsion level controllers) appear applicable to plant floor or on-line analysis. These analyzers are recommended for additional study. A summary of the important results derived from this review is given as follows: (a) Several types of combustible gas analyzers appear capable of reliably monitoring vapor concentrations from hydrocarbon solvents like hexane, alcohols, ketones, etc. Sensors are available that may be classified as strictly on-line, portable or capable of sampling multiple locations from an environmentally isolated central station using the sampling tube network method. (b) Different types of gas sensors also appear applicable to selected toxic gases likely to be found in the working environment as environmental emissions or in areas that could be low in oxygen, such as sumps, storm drains, etc. (c) Sensors reviewed under other methods, such as moisture, total soluble solids, protein, zeta potential, etc. also have potential in oil mill and product analysis. Some, such as pH probes, have been used as on-line analyzers in other industries for many years, whereas others (for protein, fat moisture, etc.) have only recently been used in industry. Further testing is therefore required before clear recommendations can be made. (d) A brief review of control methods, particularly those known to be non-fouling or non-instrusive, indicates potential applicability of a number of devices. (e) Not much is known about optimum sensor positioning and spacing for processes requiring multiple sensors. In

addition, different levels of vibration, dust, processing aids employed in refining, etc. will require each operating oil mill to determine sensor maintenance requirements. (f) There is need for increased research and development efforts on rapid analysis methods.

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MONITORING PROTEOLYSIS BY OSMOMETRY–A RAPID METHOD. Theodore M. Wong and C.O.L. Boyce, Novo Laboratories, Inc., 59 Danbury Rd., Wilton, CT 06897-0820.

The fast-moving field of biotechnology today is delivering new and more plentiful enzyme preparations. Most experts agree that these enzyme preparations will lead to improved food quality and functional properties, and efficiencies in processing. Proteinase can be used to modify protein to improve functional properties. Furthermore, protein hydrolysates can be used in dietetic feeding for patients who cannot take undigested protein. Functional properties of a modified protein are dependent on how much hydrolysis has occurred. This parameter is referred to as degree of hydrolysis (DH). It is defined as the ratio of the number of peptide bonds cleaved to the total number of peptide bonds of the protein. Osmometry is a simple and rapid method to monitor DH. A discussion of the theory of osmometry, analysis parameters and examples for application will be presented. In addition, osmometry will be compared with other ways to monitor proteolysis such as pH-state and amino-group derivatization.

SESSION CC Surfactants and Detergents III: Open Forum—Current and Future Perspectives Wednesday morning

Panel discussion, no abstracts.

SESSION DD Brian L. Walker Memorial Symposium Lipids and Cancer I Wednesday morning

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FAT, CALORIES AND CANCER. David Kritchevsky, Maxine M. Weber and David M. Klurfeld, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104.

Early studies by Tannenbaum, Lavik and Baumann, Boutwell and others have suggested that caloric intake affects progression of both spontaneous and chemically-induced tumors in mice and rats. We have re-investigated this phenomenon in female Sprague-Dawley rats given 7,12-dimethylbenz(a)anthracene to induce mammary tumors. One group of rats was fed ad lib. a diet containing 3.9% fat (2.9% coconut oil, 1% corn oil; 9.7% of calories) and 10.1% fiber. Another group was pair-fed to provide 60% of the calories ingested by the first group. The diet fed to the second group was designed to provide (in the restricted calories), 8.4% fat (7.8% coconut oil, 0.6% corn oil; 34% of calories) and 10.1% fiber. The pair-fed group gained significantly less weight than did that fed their diet ad lib. Tumor incidence in the rats fed ad lib. was 58% (14/24) with a yield of 2.8 ± 0.5 tumors per tumor-bearing rat (T/TBR). Those fed the restricted diet exhibited no tumors. Carroll and others have shown that rats treated with a carcinogen and fed unsaturated fat exhibited more tumors than rats fed saturated fat. We repeated the calorie restriction experiment using corn oil as the sole source of fat. Tumor incidence was 80% (16/20) in the rats fed ad lib. and 20% (4/20) in those whose caloric intake was restricted. T/TBR was 4.0 ± 0.5 in the control group and 1.0 ± 0 in those fed the restricted diet. In another study, colon tumors were induced in male Fischer rats by administration of 1,2-dimethylhydrazine. The diet contained corn oil as the sole source of fat. Tumor incidence in the control and calorie-restricted group was 100% (19/19) and 53% (10/19), respectively. T/TBR was 3.5 ± 0.4 and 2.1 ± 0.6 in the two groups and the incidence of extracolonic tumors 32% (6/10) and 11% (2/19). Caloric intake may be a greater determinant of tumor enhancement than level of dietary fat.

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RETINOID INHIBITION OF BREAST CANCER. Richard C. Moon, R.G. Mehta and D.L. McCormick, IIT Research Institute, 10 West 35th Street, Chicago, IL 60616.

Several retinoids have been evaluated for prevention of mammary carcinogenesis in rats and mice. Of those which were active, retinyl acetate (RA) and 4-hydroxyphenyl retinamide (HPR) proved most effective. In rats, dietary administration of the retinoids reduced the incidence and number, and increased the latency of N-methyl-N-nitrosourea (MNU)-induced mammary cancers. HPR reduced the number of hyperplastic alveolar nodules (HAN) in MTV-mice and the number of tumors HAN in MTV^+ mice. Other studies indicate that the synergistic effect of retinoid administration and hormonal deprivation is more efficacious in prevention of MNU-induced mammary cancer than is either modality alone. Suppression of either the ovarian steroid (ovariectomy) or prolactin (CB-154) levels in combination with retinoids almost completely blocks induction of mammary cancer with MNU. Furthermore, retinoids alone and the combination of retinoid and ovariectomy inhibit the appearance of mammary cancers following the surgical removal of the first cancer. Again, the combined modality was the most effective. Retinoids also exert an antiproliferative effect on the mammary epithelium in vivo which is represented morphologically by a bare duct system with little branching, end buds, and few, if any, alveoli. In organ culture, retinoids inhibit mammary ductal and end bud proliferation induced by insulin and prolactin. These changes in mammary gland morphology are accompanied by a decrease in mammary DNA synthesis both in vivo and in vitro.

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ENHANCEMENT OF MAMMARY TUMORIGENESIS IN RATS FED A DIET HIGH IN LARD. Adrianne E. Rogers, Department of Pathology, Boston University School of Medicine, 80 E. Concord Street, Boston, MA 02118.

Chemical carcinogenesis in the mammary gland is enhanced in rats fed diets that contain 20-25% (by wt) polyunsaturated or monounsaturated vegetable oils or lard compared to rats fed a lower but nutritionally adequate amount of fat, 5% by wt. The effect of the polyunsaturated oils, of which corn oil has been the most intensively studied, is exerted primarily after carcinogen exposure, and is related quantitatively to duration of feeding. In constrast, lard, which is a mixture of saturated, monounsatuated and polyunsaturated fats, enhances tumorigenesis when fed only before as well as when fed before and after carcinogen exposure. The effect is increased if feeding is continued after exposure and is, as with corn oil, quantitatively related to duration of feeding. The mechanisms by which dietary fats influence mammary carcinogenesis are not known. The activity of lard at the time of carcinogen exposure suggests that it may have components that are carcinogens or co-carcinogens or that it may alter carcinogen pharmacokinetics or cellular metabolism or that it may alter susceptibility of the gland by altering DNA synthesis. Experiments were performed to examine certain of these mechanisms. In the first, female Sprague-Dawley (S-D) rats were fed purified nutritionally complete, calorically balanced diets that contained 1% corn oil and 4% or 23% lard. Groups of rats were fed the high lard diet throughout the experiment or from weaning to 53 days of age or from 57 days of age to termination. The control, 4% lard diet was fed at all other times and was fed to one group throughout the experiment. At 55 days, rats were given 7,12-dimethylbenzanthracene (DMBA), 0.25 mg, by injection into the R3 mammary gland and surrounding fat pad. Tumor latency was reduced significantly ($P=0.03$) compared to controls in rats fed the high lard diet throughout. It was reduced also, but less markedly, in the 2 groups fed high lard either before or after administration of DMBA and was inversely proportional to duration of feeding the high lard diet. Therefore, the effect of the diet is exerted at the gland and is not due to pharmocokinetic alterations. Evaluation of two different lard preparations was made in the DMBA model. Lard was obtained from 2 producers (A&B) as prepared for human consumption except that the antioxidants butylated hydroxyanisole (BHA) and butylated

hydroxytoluene (BHT) were not added to Lard B by the producer. Both lards were assayed for fatty acids, BHA and BHT, halogenated hydrocarbon pesticides and other contaminants. Groups of 20 rats were fed the purified, nutritionally complete diets, containing 1% corn oil and either 4% or 23% Lard A, Lard B, or Lard B with addition of 10 ppm or 100 ppm each of BHA and BHT. The diets were fed by the protocol given above. DMBA, 2.5 mg, was administered by gastric gavage at age 55 days. Analysis of the 2 lards showed almost identical fatty acid composition and no significant contaminants. Lard A contained ca. 10 ppm each of BHA and BHT; the amounts of the 2 antioxidants added to Lard B were confirmed by analyses at the beginning and end of the experiment. Tumor latency was reduced by all high lard diets when they were fed throughout the experiment, and there was no detectable effect of the 2 antioxidants. In contrast to the earlier experiments, the effect appeared to be exerted primarily after DMBA exposure.

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ROLE OF DIETARY FATS IN PANCREATIC CARCINOGENESIS. B.D. Roebuck, Dartmouth Medical School, Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH 03756.

In the United States, human pancreatic cancer is the fifth most common cause of death due to cancer. The incidence of pancreatic cancer has risen in the last few decades. The etiology of this important disease is poorly understood. One of the epidemiologically identified risk factors associated with pancreatic cancer is a high intake of dietary fat. Studies with experimental animal models generally corroborate this finding. Additionally, these animal studies indicate that the ingestion of diets with high levels (20% by weight) of fat enhances pancreatic carcinogenesis not during the initiation phase, when the carcinogen is given, but during the postinitiation phase after carcinogen exposure has ceased. Furthermore, from these models, there is evidence that large intakes of unsaturated fats are generally more effective than similar intakes of saturated fats. In this regard, there appears to be a parallelism between the breast and pancreas for the role of fat in carcinogenesis. A short-term rat model has been developed and extensively characterized for the study of pancreatic cancer. In this model, azaserine (a known pancreatic carcinogen) induced populations of putative, preneoplastic foci of atypical acinar cells. Within 2-4 months following initiation by azaserine (30 mg/kg b.w.) of pancreatic carcinogenesis in 14-day-old, male Lewis rats, the number and size of these foci can be quantitated. We evaluate H&E stained pancreatic tissue for foci by light microscopy. Measurements are accomplished with the aid of a computer x-,y-digitization system. The ingestion of a high level of unsaturated fat, but not saturated fat, following azaserine pretreatment results in larger and more numerous foci. The feeding of these unsaturated fat diets to azaserine-initiated rats increased the ^{3}H-thymidine labeling index of focal cells but not of non-focal acinar cells. Similar levels of saturated fat in the diet do not enhance the labeling index of focal cells. These results indicate that high dietary levels of unsaturated fat affect selectively either the growth rate of azaserine-induced foci or possibly the outgrowth of initiated cells to become foci.

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DIETARY POLYUNSATURATED FAT IN RELATION TO MAMMARY CARCINOGENESIS. Kenneth K. Carroll and Laura M. Braden, University of Western Ontario, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 5C1.

High-fat diets have been shown to promote mammary carcinogenesis in rats, and polyunsaturated fats are more effective than saturated fats. This difference appears to be due to a requirement for essential fatty acids in the development of mammary tumors. Feeding a mixture of 3% ethyl linoleate and 17% saturated fat to rats treated with 7,12-dimethylbenz(a)anthracene (DMBA) to induce tumors led to a much higher tumor yield than feeding 20% of the saturated fat alone in the same type of semipurified diet (Hopkins et al. JNCI 66:517, 1981). In that study, feeding 3% menhaden (fish) oil with 17% saturated fat gave a tumor yield comparable to that obtained with the ethyl linoleate-saturated fat mixture. This suggested that the requirement for polyunsaturated fat was not specific for n-6 fatty acids like linoleate, since most of the polyunsaturated fatty acids in menhaden oil belong to the linolenate (n-3) family. In a subsequent experiment, diets containing 3%, 10% or 20% of either corn oil or menhaden oil were fed to DMBA-treated rats for further comparison of the effects of n-6 and n-3 fatty acids. At the 3% level, rats fed menhaden oil developed more tumors than those fed corn oil. At the higher levels, the mammary tumor yield was enhanced by corn oil, as observed previously, but was markedly inhibited by menhaden oil. This result, together with evidence from other laboratories that indomethacin, a cyclooxygenase inhibitor, counteracts tumor promotion by n-6 polyunsaturated fatty acids, suggests that the promoting effect may be mediated by prostaglandins derived from n-6 fatty acids.

SESSION EE Special Processes and Oil Sources
Wednesday morning

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FAT SPLITTING IN AN IMMOBILIZED LIPASE ENZYME REACTOR. Frank Taylor and Dennis J. O'Brien, USDA Eastern Regional Research Center, Philadelphia, PA 19118.

Lipase enzyme catalysis of the hydrolysis of fats and oils is being studied as an alternative to the high-pressure steam splitting method for production of fatty acids and glycerol. Enzyme immobilization would permit continuous process operation, simplify product separation and enable reuse of enzyme. Data will be presented for the conversion at 50 C of beef tallow by a thermostable lipase from *Thermomyces lanuginosus* immobilized in a microporous membrane filter cartridge. Immobilized enzyme activities of up to 15 units per square decimeter of membrane area and half-lives of over 2 months have been demonstrated. Factors affecting half-life, activity and efficiency of immobilization have been investigated.

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PROCESSING AND PROPERTIES OF SEED OILS OBTAINED BY SUPERCRITICAL CO2 EXTRACTION AT HIGH TEMPERATURES AND PRESSURES. G.R. List and J.P. Friedrich, Northern Regional Research Center, ARS-USDA, 1815 N. University Street, Peoria, IL 61604.

Previous work has shown that extraction of flaked cottonseed, soybean and corn germ with supercritical CO2 (SC-CO2) at 50 C and 8,000 psi yields crude oils equivalent to expeller or solvent-extracted crudes. Since the solubility of triglycerides in SC-CO2 is rather low under these conditions, higher temperatures and pressures were studied. In batch systems, extraction times are reduced from several hours to a matter of minutes at 80 C and 12,000 psi. Corn and soybean oils obtained at high temperatures and pressures appear suitable for steam refining with a minimum of pretreatment steps. Cottonseed oil obtained at 80 C and 12,000 is less intensely colored than conventionally extracted crude oil. It does not appear suitable for steam refining but requires less caustic than expeller or solvent extracted crude. Soybean and corn oils show little or no increase in color, free fatty acids, phosphorus or refining loss when extracted at high temperatures and pressures. Cottonseed oil color increases at high temperatures and pressures but free fatty acids and other refining loss factors are unaffected. Phosphatides which protect crude oils from oxidative deterioration in storage are essentially absent in SC-CO2 oils extracted under the most stringent conditions. Although the tocopherol contents of CO2-extracted oils are comparable to expeller- or solvent-extracted oils, tocopherol alone is not sufficient to protect CO2-extracted oil from oxidation. Preliminary work on the mechanism of deterioration of CO2-extracted oil will be presented.

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LABORATORY THIN-FILM CONTINUOUS DEODORIZER FOR VEGETABLE OILS. E.D. Bitner and J.P. Friedrich, Northern Regional Research Center, ARS-USDA, 1815 N. University Street, Peoria, IL 61604.

A laboratory-scale continuous thin-film deodorizer based on

a modified Snyder distillation column was constructed to simulate commercial deodorization of alkali-refined and bleached vegetable oils. Results of taste panel evaluations show that the quality of soybean oils deodorized over a temperature range of 194-260 C is equivalent to commercial salad oils. Oil flow rates are 1-2 ml/min and contact time is ca. 5 min; a vacuum of 0.5-1.0 mm Hg is maintained with countercurrent steam flow of 1-5% of the oil weight. The bubble cap design of the column and countercurrent steam flow produce a thin oil film and promote good contact. Small samples of oil 250-1000 ml are readily accommodated in this equipment.

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IDENTIFICATION OF THE CHEMICAL CHANGES OCCURRING DURING THE TRANSIENT INJECTION OF SELECTED VEGETABLE OILS. Thomas W. Ryan III, Southwest Research Institute, Post Office Drawer 28510, 6220 Culebra Road, San Antonio, TX 78284, and M.O. Bagby, Northern Agricultural Energy Center, U.S. Department of Agriculture, 1815 N. University, Peoria, IL 61604.

Four different types of vegetable oils (degummed soybean oil, once-refined cottonseed oil, once-refined peanut oil, and once-refined sunflower oil) were injected into a high-pressure, high-temperature environment of nitrogen. The injection process was transient in nature, lasting for ca. 3 milliseconds. With the oil temperatures maintained at 40 C, the peak injection pressure was ca. 100 MPa. The oils were injected into a nitrogen atmosphere maintained at 4.1 MPa and 480 C. A sonic velocity, water-cooled sample probe, fast acting sample valve, and cold traps were used to collect samples of the injected oils as they were being injected. Typical residence time of the oils in the high temperature environment was 400 microseconds before collection. Chemical analyses of the samples indicated that major chemical changes occurred during the injection process. The predominant changes involved the formation of lower molecular weight species, C_4 - C_{16}, from the $C_{18:3}$ and $C_{18:3}$ fatty acids.

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SIMULTANEOUS INTERPENETRATING NETWORKS PREPARED FROM SPECIAL FUNCTIONAL GROUP TRIGLYCERIDE OILS: CASTOR OIL, *LESQUERELLA PALMERI* AND OTHER WILD PLANT OILS. M.A. Linne, L.H. Sperling and J.A. Manson, Lehigh University, Materials Research Center, Coxe Lab #32, Bethlehem, PA 18015.

Among the energy conservation research programs started on account of the oil crisis, renewable resource materials as replacements or substitutes for products derived from coal and petroleum are becoming increasingly realistic as oil costs are rising. The Lehigh oil program started with the investigation on polymerized castor oil as an elastomer. The research was broadened with lunaria, linseed and vernonia oils, and the most recent focus was an oil native to Arizona, Lesquerella palmeri or so-called "popweeds." Lesquerella oils contain 55% of lesquerolic acid triglyceride, the rest of the oil is a variety of saturated and unsaturated triglycerides. Lesquerella palmeri (LP) derived elastomers are capable of toughening plastics. These special functionality oils are attractive because of their special chemical activity like hydroxyl groups (castor oil, LP) and epoxy groups (Vernonia). Special groups can also be added to oils bearing unsaturation by epoxidation techniques. These functionalities allow polycondensation polymerization to be carried out readily. The preferred crosslinking synthesis of the oil-based elastomer is polycondensation either with sebacic acid (SA)—being itself a castor oil residue—or 1,4,toluene diisocyanate (TDI) to form, respectively, a fully renewable polyester or a polyurethane, or with a mixed SA-TDI to obtain the polyesterurethane. The reactivity of LP was shown to be improved by epoxidation of the oil prior to elastomer formation. The glass transition temperatures of the elastomers are typically -60 C for crude oil polymer and -28 C for the epoxidized oil polymer. Both elastomers were used as the elastomeric phase to toughen divinylbenzene (DVB) crosslinked polystyrene (PS) in the form of both sequential interpenetrating polymer networks (IPNs) and simultaneous interpenetrating networks (SINs), where the networks are formed either sequentially or simultaneously in the immediate vicinity of each other by non-interfering polymerization reactions. Series of IPNs and SINs with PS as the plastic phase were made as prototype materials. The morphology of these samples was found to depend on the synthesis method, the sequential IPN having smaller domains than the SINs. Modulus-temperature, stress-strain and transmission electron microscopy (TEM) studies of a number of compositions showed that the SINs based on LP oil are tough plastics or reinforced elastomers depending on the composition. Mid-range compositions yielded leathery products. Also the toughness of the crude oil-based materials was significantly higher than those made from epoxidized oil. TEM analysis disclosed the latter to be less phase separated. There must be an optimum between reactivity enhancement due to increased number of condensation sites in the aftermath of epoxidation and the capability of the SIN reacting system to undergo proper phase separation between the PS and oil phase.

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NUTRITIONAL SIGNIFICANCE OF UNCONVENTIONAL OILS. Dr. C. Rukmini, National Institute of Nutrition, Hyderabad-500007, A.P., India.

Acute shortage of edible in India has focused attention on vast untapped potentials of unconventional oils, which can provide 1.5 million tons of oil if properly exploited. Most people have a very low intake (14 g - 20 g/day/capita) providing 8-10% of the total energy as against the ICMR recommended allowance of 34 g/day/capita which includes 15 g of EFA. Hence high priority to oil seeds is given to meet the consumer requirement. A comprehensive and systematic study involving chemical evaluation, nutritional quality and toxicological safety by multigeneration breeding studies in rats, including biochemical, histopathological, mutagenic and teratogenic investigations was designed. This protocol has been specially designed taking into consideration the food safety regulations of DGHS/EEC/FDA/WHO. Such an exhaustive study was carried out on four different oils namely Mango kernel *Cleoma Viscosa*, *Hibiscus Sabdariffa* and *Terminelia bellerica* (Myrobalans) and communicated.

SESSION FF H.W. Kircher Memorial Symposium Chemistry, Biosynthesis and Function of Sterols I Wednesday morning

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OXYSTEROLS: CHEMICAL SYNTHESIS, BIOSYNTHESIS, AND BIOLOGICAL ACTIVITIES. Edward J. Parish, Department of Chemistry, Auburn University, Auburn University, AL 36489.

As a class of compounds, oxysterols have demonstrated a wide variety of biological properties. Some of these include cytotoxicity, atherogenicity, carcinogenicity, mutagenicity, hypocholesterolemia and effects on specific enzymes. The specific inhibition of cholesterol biosynthesis in mammalian cells by oxysterols has been shown to result primarily from a decrease in cellular levels of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA) activity. Recent evidence suggests that these cellular responses may be mediated by an oxysterol binding protein found in the cytosol of many lines of cultured cells. In certain instances, oxysterols have been shown to be produced in biological systems. These results support the supposition that oxysterols may regulate sterol biosynthesis at the cellular level. Due to the general interest in these compounds, new methods of chemical synthesis have been developed to provide these compounds for biological investigation. Other approaches to chemical synthesis have included the streamlining of existing or "classical" methods of synthesis.

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THE SQUALENE-2,3-EPOXIDE CYCLASE AS A MODEL FOR THE DEVELOPMENT OF NEW DRUGS. Luigi Cattel, Laura Delprino, Gianni Balliano, Franca Viola and Maurizio Ceruti, Istituto di Chim. Farm. Appl., Università di Torino, c.so Raffaello 31, 10125-Torino, Italy, and Pierre Benveniste, Institut Botanique, Univ. Strasbourg, France.

The 2,3-oxido squalene (SO) cyclases represent a group of enzymes that convert SO into polycyclic triterpenoids such as lano-

sterol, cycloartenol, cucurbitadienol or amyrin. Taking into account the postulated model of the enzymic cyclization of SO, we have investigated the possibility of designing compounds which would be selective and potent inhibitors of SO cyclases. Moreover, owing to the fundamental role of the sterols in the animal, higher plants and fungi tissues, these inhibitors could behave as very selective (ipocolesterolemic, antifungine or phytotoxic) drugs. Our first approach was the synthesis and the biological evaluation of 2-aza-2,3-dihydrosqualene and derivatives which, being protonated at physiological pH, would present some similarities with the C-2 carbon ion generated by the opening of the oxirane ring of SO. Microsomes from different sources (germinated pea cotiledon, maize seedlings, rat liver and yeasts) were used to determine the inhibition values of (I_{50}). From the results obtained so far, it can be concluded that the 2-aza-2-dihydro-squalene and derivatives strongly inhibited the cyclases, that the site of the enzyme responsible for the binding to the inhibitor is quite sensitive to the steric hindrance and that the degree of the inhibitory activity is greater in higher plants than in rat liver or fungi. A good correlation between the I_{50} values of the azasqualene derivatives and the antifungine activity was also found.

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STEROLS AND TRITERPENE ALCOHOLS OF CUCURBITACEAE PLANTS. Toshihiro Itoh and Taro Matsumoto, College of Science and Technology, Nihon University, 1-8, Kanda Surugadai, Chiyoda-ku, Tokyo, 1010 Japan, and Swapnadip Thakur, Department of Chemistry, University of Burdwan, and Fumiko U. Rosenstein, College of Agriculture, The University of Arizona.

A study was made of triterpene alcohol, 4α-methylsterol, and sterol (4-desmethylsterol) constituents of the unsaponifiable lipids separated from the seeds and/or mature plants (leaves and stems, pericarp of the fruit or roots) of the genera *Apodanthera*, *Benincasa*, *Citrullus*, *Cucumis*, *Cucurbita*, *Gynostemma*, *Lagenaria*, *Luffa*, *Momordica*, *Sechium*, and *Trichosanthes* of the Cucurbitaceae family. Isomultiflorenol was the principal triterpene alcohol in most of the seed materials studied, whereas cycloartane compounds such as 24-methylenecycloartanol were the most dominant ones for the mature plant materials. 10α-Cucurbita-5,24-dien-3β-ol, a cucurbitane triterpene alcohol, was found to occur in most of the seeds and the mature plants investigated. Two 24β-ethylated compounds, 24β-ethyl-31-nor-5α-lanosta-8,25-dien-3β-ol and 24β-ethyl-25-dehydrolophenol, in addition to 3 24-methylated compounds, cycloeucalenol, obtusifoliol and gramisterol, were the major 4α-methylsterols of the seeds. In the mature plants, the 3 24-methylenated compounds constituted the dominant 4α-methylsterols. Four Δ^7-sterols, 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol, 24-ethyl-5α-cholesta-7,25-dien-3β-ol, 24-ethyl-5α-cholesta-7,22-dien-3β-ol, and 24-ethyl-5α-cholest-7-en-3β-ol, were the principal seed sterols. The former 2 sterols possessed 24β-configuration, whereas the latter 2 occurred mostly as the C-24 epimeric mixture. The co-occurrence of the C-24 epimers was further demonstrated with 24-methyl-5α-cholesta-7,22-dien-3β-ol isolated from the seeds of *Cucumis sativus* and *Benincasa hispida*. Most of the mature plants studied contained 24-ethyl-5α-cholesta-7,22-dien-3β-ol and 24-ethyl-5α-cholest-7-en-3β-ol as the dominant sterols, and these were, as demonstrated with *Trichosanthes japonica* root sterols, the mixture of C-24 epimers in which the 24α-epimers predominated. In addition to the above, the other triterpene alcohols, 4α-methylsterols, and sterols present in minor amounts in the Cucurbitaceae were structurally and stereochemically defined.

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STEROL COMPOSITION DURING THE LIFE CYCLE OF THE SOYBEAN AND THE SQUASH. Glenn W. Patterson, Gregory P. Fenner and Penelope M. Koines, University of Maryland, Department of Botany, College Park, MD 20742.

Soybeans and squash were grown to maturity. Plants were analyzed for sterol composition at various stages of growth from seed to the mature plant. Soybeans contained campesterol, stigmasterol and sitosterol at all stages whereas the squash contained 7,22-stigmastadienol, 7,25-stigmastadienol and 7,22,25-stigmastatrienol at all stages. When expressed on a per plant basis, the total quantity of sterols increases after germination until just prior to flowering, levels off or declines slightly and then increases rapidly until maturity where the total sterol per plant again declines slightly. These changes in total sterol composition were observed in both plants studied and invite speculation on sterol conversion to other compounds just prior to flowering.

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DESIGN OF HIGH ENERGY INTERMEDIATE ANALOGS TO STUDY STEROL BIOSYNTHESIS IN HIGHER PLANTS. P. Benveniste, A. Rahier, M. Taton, P. Nave, A.S. Narula and L. Cattel, Institut de Botanique, Laboratoire de Biochimie Végétale, 28, Rue Goethe - 67083 - Strasbourg Cédex, France.

Several enzymes of plant sterol biosynthesis involve, during their catalysis, postulated or demonstrated carbocationic high energy intermediates (HEI). The aim of the presented study is to interfere with plant sterol biosynthesis by means of rationally designed species able to mimic these carbocationic HEI. It has been previously demonstrated that the design of Transition State or HEI analogs could lead to powerful and specific inhibitors of enzymes. We applied this approach to the following target enzymes: 2,3-epoxy-2,3-dihydrosqualene cyclase, AdoMet-Cycloartenol-C-24-methyltransferase, cycloeucalenol-obtusifoliol isomerase and $\Delta^8 \rightarrow \Delta^7$-sterol isomerase. Very potent inhibitors have been obtained in the 4 cases. As an example, analogs of cycloartenol substituted at C-25 by a charged heteroatom (N,As,S) have been synthesized and have been shown to be able to mimic the C-25 carbocationic HEI involved in the reaction catalyzed by the AdoMet-Cycloartenol-C-24-methyltransferase. These compounds were shown to be very potent and specific inhibitors of this enzyme both in vitro ($Ki = 2.10^{-8}$ M, $Ki/Km = 10^{-3}$ and in vivo. The inhibitors described are powerful tools to control in vivo the sterol profile of plant cells and therefore to study the structural and functional roles of sterols in cell membranes. Moreover these compounds constitute leader molecules of a new class of rationally designed inhibitors which could be of value in plant protection.

SESSION GG Trends in Edible Oil Processing and Consumption in Various Parts of the World II Wednesday afternoon

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INFLUENCES ON THE EDIBLE OIL SUPPLY AND DEMAND PATTERN IN CANADA 1960-1985. John Ward, Nabisco Brands Limited, 2509 Royal Windsor Drive, Mississauga L5J 1K9, Ontario, Canada.

Historical background and emerging domestic supply are discussed along with agricultural suitability, growing areas for oilseed crops, varieties and yields, demographics, markets, processing plant locations, flexibility from single source oils, regulatory influences on oilseed genetics, processing, formulation and usage, R&D perspectives and opportunities.

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THE PHILIPPINE COCONUT INDUSTRY. Richard H. Purdy, Richard H. Purdy, Inc., 16 Josefa Ct., Novato, CA 94947, and Norberto N. Coronel, San Pablo Manufacturing Corp.

The coconut industry is the largest earner of foreign exchange for the Philippines. It supports more than one third of its population and utilizes one third of the country's agricultural land area. Originally limited to the exporting of copra to American and European oil mills, extensive investments in domestic milling operations resulted in the Philippines becoming the major world supplier of coconut oil. Faced with overcapacity in the 70s caused by continued copra exports and droughts, the Philippine Coconut Oil Producers Association was formed to develop and implement programs to improve the industry's economy. This paper will describe in more detail the industry's organization, technical and economic capabilities and trends important to its future.

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PROCESSING SOYBEAN OIL IN THE PEOPLE'S REPUBLIC OF

CHINA. John B. Woerfel, 141 McGuire Cove, Clarksdale, MS 38614.

As a consultant for the American Soybean Association, the author made 4 trips to the People's Republic of China between December 1982 and October 1984. Visits were made to a number of soybean processing plants and oilseed research institutes in several provinces, and discussions were held with technical and management personnel. Observations and slides describe conditions and practices in the Chinese soybean industry.

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TRENDS IN EDIBLE OIL CONSUMPTION AND PROCESSING IN CANADA. Mark Pickard, Robert Wiggins and Don Loewen, CSP Foods Ltd., Box 8060, Dundas, Ontario, L9H 5E7, Canada.

Oilseed production in Canada has expanded dramatically with the development of the low glucosinolate, low erucic acid varieties of rapeseed known as canola. In the spring of 1984, tight supplies, combined with strong domestic and export demand, caused a run up in price and producers of the crop responded with an acreage increase of 24% from the 1983 level. Canadian crushing plants were able, in 1984, to process substantial quantities of canola and the other 2 domestically produced oilseeds, sunflower and soybeans. During 1983, Canadian refineries boosted the proportion of canola oil processed to an unpredecented level. Canola's share of the refined oil output edged up to 52.5% of the total vegetable oils. As a salad/cooking oil, canola oil accounted for 72.3% of the liquid oils produced by refineries. In shortening, canola oil accounted for 44% and in margarine, 38% of the oil used. Historically, the consumption trend has been increasing usage of canola oil, primarily at the expense of soybean oil. In processing, crushing companies have recognized the importance of producing a low phosphorus canola oil. This product has gained acceptance in both domestic and export markets. These companies and the canola industry at large are cognizant of the periodic problem of processing a frost damaged crop. Considerable research is being done to find a successful processing technology to alleviate the problem. Refining technology relevant to canola processing has evolved and as the industry continues to mature, further advances are anticipated.

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DYNAMICS OF SUPPLY AND DEMAND FOR OILS AND FATS. K.G. Berger, Palm Oil Research Institute of Malaysia, P.O. Box 10260, Kuala Lumpur, Malaysia.

The consumption patterns of oils and fats show regional differences which can be traced back to the products of the traditional local agriculture. With the development of large cities and of a world-wide trade in oils and fats, the oils used have diversified, but the consumer products are still similar, the most obvious being the replacement of butter by margarine. Rapid increase in demand is usually the result of increasing prosperity, South Korea being a recent example. Dynamic changes in supply are due to diverse causes. Whale oil has virtually disappeared because of overkill, whereas fish oils have become significant in human food because of improved refining technology. The European rapeseed crop has shown dynamic growth because of E.E.C. subsidies, but the Canadian rapeseed and the U.S. soybean crops show wide annual fluctuations in response to market prices. These and other examples of dynamic change will be discussed.

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PHYSICAL REFINING OF SUNFLOWER, MAIZE AND SOYA OIL–PRACTICAL RESULTS. Z. Balicer and Z. Leibovitz, H.L.S. LTD, POB 193, Petah-Tikva 49101, Israel, and C. Ruckenstein, U.S.O.P. (1974) Ltd., Israel.

The introduction of physical (steam) refining for soft oils with a low FFA content is still a much discussed subject in the edible oil industry. More installations have been built in recent years and we shall try to present the results obtained in practice from new plants processing sunflower, soya and maize oil. We will discuss the importance of the preparation of oils before deacidification, different degumming methods and their influence on the efficiency of refining. We will analyze the influence of crude oil quality; consumptions of chemicals, bleaching earth and energy utilities; yields of refined oil and by-products; limits of using steam refining and methods to overcome these limits; combining physical refining with other systems; and ecological problems. Also discussed are: practical results with a new continuous - semi-continuous deacidifier-deodorizer, advantages and disadvantages of working continuously, change of oils without time loss, example of a complete automatic computerized physical refining plant (100 MT/day), flowsheets and various practical solutions, quality of fatty acids, comparative investment of classical and physical refining.

SESSION HH New and Improved Methods for the Analysis of Lipids Wednesday afternoon

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AN EVALUATION OF CURRENT STRATEGIES FOR CONTROLLING THE HYDROLYSIS OF ENDOGENOUS LIPIDS IN CELL-FREE PREPARATIONS FROM PLANTS. Robert A. Moreau, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118.

Many plant tissues contain high levels of phospholipase B, phospholipase D, and galactolipase activities. These enzymes can cause a rapid breakdown of lipids during homogenization and fractionation of subcellular components. The rate of breakdown of endogenous phospholipid was surveyed in homogenates of 14 plant species at 4 C and 25 C. Even at 4 C, there was a significant rate of hydrolysis (3-56% of the phosphatidylcholine was hydrolyzed per hr) in homogenates of 12 out of the 14 species. Buffering the homogenates at pH 7.5-8.0 lowered the rate of hydrolysis in homogenates of most, but not all species. Several chemical inhibitors (bovine serum albumin, dibucaine, bromophenacyl bromide and others) were tested for their ability to control membrane breakdown in homogenates of various plant species. Dibucaine was found to inhibit membrane degradation in homogenates of all species, but the effective concentration varied among the species tested. The most dramatic case was with potato tuber homogenates where low concentrations of dibucaine (25-50μM) inhibited hydrolysis and high concentrations (0.5-5mM) stimulated hydrolysis.

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APPLICATION OF THE ION TRAP DETECTOR® TO FATTY ACID ANALYSES. R.G. Ackman and W.N. Ratnayake, Technical University of Nova Scotia, Canadian Institute of Fisheries Technology, P.O. Box 1000, Halifax, Nova Scotia, Canada B3J 2X4.

The Ion Trap Detector® is a potentially economical and flexible one-unit replacement for a variety of gas-liquid chromatographic detectors. Owing to the delay in introducing this novel device to our laboratory, this presentation will be restricted in application to fairly simple and well-known fatty acids and derivatives associated with marine lipids. Most of these are also known and of interest in other lipid research and biochemistry areas. The potential of this device will be evaluated in terms of claims for the absence of even-carbon chain length overlap in open-tubular GLC of methyl esters of fatty acids on bonded Carbowax-20M® columns.

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CHROMATOGRAPHIC METHODS FOR THE ANALYSIS OF OXIDIZED LIPIDS. Edwin N. Frankel, Northern Regional Research Center, ARS-USDA, 1815 N. University Street, Peoria, IL 61604.

In 1961, NRRC developed chromatographic methods permitting, for the first time, the isolation of hydroperoxides from autoxidized methyl linolenate. Since that time, a large share of the significant progress made in lipid oxidation research can be attributed to the development of new chromatographic techniques. High-performance liquid chromatography (HPLC) has proved to be a useful technique for the separation of isomeric hydroperoxides and secondary oxidation products. The combination of HPLC and proton and carbon-13 NMR has been instrumental in establishing the stereochemistry of mono- and di-hydroperoxides, hydroperoxy mono-, bis- and bicyclic endoperoxides. The structural information obtained by these techniques has led to new, modified and refined mechanisms for the stereochemistry of free radical autoxidation and singlet oxygenation

of unsaturated lipids. The combination of gas chromatography and mass spectrometry (GC-MS) is a powerful technique to determine quantitatively isomeric hydroperoxides and to serve as a diagnostic tool to distinguish between free radical autoxidation and singlet oxygenation. Capillary GC-MS is the latest sensitive technique used to analyze thermal and acid decomposition prorudcts of lipid oxidation. A better understanding is needed on the adverse effects of these decomposition products on the flavor and nutritional quality of food lipids.

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METHODS IN THE ISOLATION AND ANALYSIS OF MILK FAT GLOBULES. Stuart Patton and Gail E. Huston, University of California, San Diego, School of Medicine, Department of Neurosciences, M-008, La Jolla, CA 92093.

Milk fat globules are intracellular lipid droplets that have been secreted from the cell by envelopment in plasma membrane. Characterization of the globules structure, particularly of molecular arrangements in its surface layers, could increase our understanding of plasma membranes and lipid droplet formation and secretion. Milk fat globules are notoriously fragile and a first necessity is a nondestructive method of isolating them from fresh milk, one that avoids repeated washings and centrifugations. We have devised such a method based on a single centrifugation of globules out of the milk through an overlying buffer layer. Membrane preparations from such globules by churning, freezing-thawing or by treatment with bile salt (taurodeoxycholate) all showed satisfactory trilaminar structure in the electron microscope, although that prepared by churning contains considerable extraneous material, presumably triacylglycerol. SDS-polyacrylamide gel electrophoresis of intact globules and fractions produced from them during membrane preparation indicated that all of the major globule proteins are associated with the membrane and none are with the lipid droplet. A number of analytical procedures have been developed in our research including a method for globule protein and lipid, and a simplified procedure for globule phospholipid. These methods of isolating, fractionating and analyzing milk fat globules (human, bovine and caprine) will be described and findings from their application presented.

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STEREOSPECIFIC ANALYSIS OF SYNTHETIC AND NATURALLY OCCURRING FATTY ACID ESTERS OF CHLOROPROPANEDIOL. J.J. Myher and A. Kuksis, University of Toronto, Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Canada, M5G 1L6, and J. Cerbulis, Eastern Regional Research Center, USDA, Philadelphia, PA 19118.

The fatty acid esters of chloropropanediol isolated from goat milk fat were subjected to a stereospecific analysis by a modification of the phosphorylcholine method of Myher and Kuksis using a racemic-3-chloropropanediol dioleate as a control. The synthetic racemate was prepared by acylation of rac-3-chloropropanediol in benzene with oleoyl chloride in the presence of pyridine. The stereospecific analysis was performed following a release of the fatty acids from the primary positions of each chloropropanediol diester with pancreatic lipase. The resulting X-1-chloro-2-acyl-propanediols were then converted into the corresponding phosphorylcholine derivatives by a stepwise reaction with phosphorus oxychloride and choline chloride. The X-1-chloro-2-acyl-3-phosphorylcholine propanediols were subjected to hydrolysis with phospholipase C *(C. perfringens)*, which hydrolyzed 50% of the phosphatide within 2 min (assumed to be the sn-1-chloro-2-acyl-propanediol) and the rest of it in 2 hr (assumed to be the sn-2-acyl-3-chloro-propanediol). A hydrolysis with phospholipase A_2 *(Crotalus adamanteus)* released 50% of the total fatty acid along with the corresponding lyso compound within 10 min, after which time there was no further reaction. The hydrolysis products were assayed directly by GLC or were isolated by TLC prior to quantitation by GLC. Both naturally-occurring and synthetic 3-chloropropanediol diesters behaved similarly on stereospecific analysis and were concluded to have been racemic. It is suggested that the naturally occurring chloropropanediol diesters are metabolic acylation products of either chloropropanediol in the environment or of its 2-acyl derivative absorbed by the intestine.

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ANALYSIS OF LIPID STRUCTURE BY IN SITU REACTIONS ON THIN-LAYER CHROMATOGRAMS. Joseph C. Touchstone, S.S. Levin, J. Alvarez and S. Kleinbart, University of Pennsylvania, 3600 Spruce St., Rm. 574 Dulles, Philadelphia, PA 19104.

Conventional methodology for structural analysis of lipids, especially phospholipids, is tedious and tends to be irreproducible. The methods described herein were developed to handle minute biological samples, and use in situ reactions on thin layer chromatograms. The differentiation of molecular structure was based on results of reactions of spray reagents followed by quantitative densitometry. Copper sulfate solutions are used as the universal charring reagent. Copper acetate solutions react with the unsaturated modules. It has been shown that the vinyl-ether linkages can be selectively hydrolyzed with trichloroacetic acid-hydrochloric acid reagent. The alkyl esters can be completely hydrolyzed with dilute alcoholic sodium hydroxide solutions. All of these reactions are performed on the preadsorbent area of Whatman LK5 silica gel layers. Following reaction, the sample is extracted on the layer and deposited as a starting line by predevelopment only to the preadsorbent juncture with chloroform-methanol (1:1). Results using this methodology with sperm and amniotic fluid will be described.

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AN IMPROVED METHOD FOR OBTAINING AND QUANTITATING THE UNSAPONIFIABLE MATTER OF FATS AND OILS. Daniel P. Schwartz, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118.

In a previous report from this laboratory, a convenient method for saponifying butterfat in the absence of an organic solvent, and subsequent quantitative isolation of the unsaponifiable matter (USM), was described. This technique was later shown to be applicable to a large variety of plant, animal and marine fats and oils. This method has now been modified so that it is even faster, simpler and more economical to use. Ten g of oil or liquified fat is ground in a glass mortar with 6 g KOH pellets and 2 ml H_2O. The mortar is sealed with plastic wrap and heated at 110-120 C for 45-75 min to effect complete saponification. The soap is ground with 4 g Celite, transferred to a screw cap centrifuge bottle with 50 ml of n-hexane: CH_2Cl_2 (1:1) and let stand 30 min. Following centrifugation for 5 min, an aliquot of the supernatant is filtered through a small bed of alumina into a tared aluminum pan which is taken to constant weight. Four samples can be analyzed in about 2 hr using a total of only 65 ml of solvent/sample. The results in most instances compare very favorably with those obtained using our previous method. However, a number of oils were found to be incompletely saponified using the temperature/time conditions recommended previously.

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RAPID ESTIMATION OF GLUCOSINOLATES BY GAS LIQUID CHROMATOGRAPHY OF CARBONYL SULFIDE. Robert R. Lowry and Ian J. Tinsley, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331.

Methods currently available for the estimation of glucosinolates are time-consuming, multi-step procedures that are an impediment in the broad surveys needed in plant breeding programs. A simple procedure has been developed involving a single reaction followed by head space analysis of the gases formed using a sulfur-specific detector in a gas chromatograph. The sample of crushed seed or meal is heated with sulfuric acid at 80 C for 120 min in a screw cap culture tube fitted with a septum. After cooling for 60 min the head space is sampled with a syringe and the carbonyl sulfide and sulfur dioxide formed are separated using a Poropack Q column. Twelve samples are injected per hr and this capacity can be doubled by lapping samples. Standards of sinigrin and samples of both rapeseed and meadowfoam seed (single seeds) have been analyzed along with 2 meadowfoam meals. Peak identification was verified both by the use of commercial standards of the gases and by GLC-MS of reaction samples. Sample size has been in the 100 to 500 μg of sinigrin, however, the lower limits of detection have not been determined.

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A SIMPLE METHOD FOR QUANTITATIVE ISOLATION OF FREE FATTY ACIDS FROM FATS AND OILS. Daniel P. Schwartz, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118 and Dora G. Gadjeva, Meat Research Institute, Sofia, Bulgaria.

In a simple procedure, the free fatty acids (FFA) present in fats and oils can be isolated cleanly and without the formation of detectable artifacts due to saponification. The lipid (up to 0.5 g), dissolved in up to 5 ml of purified n-hexane, is passed over a 300 mg bed of Celite impregnated with saturated aq. Na_3PO_4 so that the solution passes over the bed in 3-4 min. After washing, the FFA are liberated from their salts by passing HCl vapor through the column bed. The acids are eluted with 1.5 ml of CS_2 or CH_2Cl_2, the solvent evaporated and the residue taken up in a small fixed volume of CS_2 for analysis by gas-liquid chromatography. The FFA are chromatographed underivatized on a 6' × 1/8" DEGS column operated isothermally at 205 C with a helium flow of 30 mls/min. FFA from $C_{10:0}$ through $C_{18:3}$ are separated in <15 min. Recovery of C_{10}-C_{18} saturated and $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ acids added at 30-150 ppm to fats and oils previously rendered free of fatty acids was near 100%. The entire procedure can be conducted in <45 min.

SESSION II Olefin Sulfonates—Versatile Surfactants for Household and Industry Wednesday afternoon

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DEVELOPMENT OF ALPHA OLEFIN SULFONATES (AOS) FOR HOUSEHOLD PRODUCTS IN JAPAN. Osamu Okumura, Lion Corporation, Development Laboratories II, No. 13-12, 7-chome, Hirai, Edogawa-ku, Tokyo, Japan.

In 1967, the Lion Corporation first introduced an AOS-based heavy-duty powder detergent in the marketplace, pioneering this product in the world. Since then, the consumption of AOS has been increasing steadily, expanding its application to other household and industrial products. This paper discusses: (a) the development of high quality AOS. Although AOS was known from 1932 by certain patents, industrial production was delayed due to the difficulty of a suitable reaction between AO and sulfonating agents. On the other hand our newly developed continuous sulfonator together with high quality AO, by the newly developed Ziegler process, make it possible commercially to manufacture AOS of high quality without bleaching. (b) Characteristic properties of AOS. AOS has many characteristic properties, such as rapid biodegradability, excellent detergency at higher water hardness, high foamability, easy drying, very good free-flowing and extremely reduced interfacial tension between oil and water in high salinity. Its safety (very low irritation to eye and skin, no sensitization, no carcinogenicity, etc.) was already proved. (c) Application of AOS to various household products. Basic properties and performances of AOS were studied in comparison with LAS, AS and AES as well as the relationship between carbon chain length of AOS and performances, and we found its versatile applicability to various products. Recently, the phosphate-free heavy-duty detergent has been socially demanded because of the problem of eutrophication in Japan. AOS is the most suitable surfactant for phosphate-free detergent owing to its excellent compatibility with enzymes in addition to above-mentioned characteristic properties. Moreover, the combination of AOS with zeolite gives excellent detergency as well as anti-redeposition properties at higher water hardness, so AOS has become one of the indispensable surfactants for heavy-duty detergents in Japan. In the light-duty detergent field, a mixture of AOS with amine oxides was found almost as mild as water to human skin in spite of high detergency and foamability for dishwashing. This combination received the top share of the dishwashing detergent market in Japan. AOS was also found to be applicable to shampoo because of its high and stable foamability and mildness to human skin. We found AOS had a versatile applicability to various products as mentioned above.

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OLEFIN SULFONATES IN CRUDE OIL PRODUCTION. D.H. Scharer, Shell Chemical Co., P.O. Box 2463, Houston, TX 77001.

Olefin sulfonates find application in several oilfield operations. Laboratory performance data relevant to the 3 following end uses will be presented and discussed: (1) existing uses in foam drilling and foam clean out of wells; (b) emerging uses as mobility control agents for steam injected in cyclic steam and steam drive enhanced oil recovery (EOR) projects; and (c) potential future EOR applications in chemical flooding processes as surfactants and co-surfactants. The emphasis will be on linear alpha-olefin sulfonates (AOS), with some data contrasting the performance of linear random olefin sulfonates or internal olefin sulfonates (IOS).

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ANALYSIS OF ALPHA OLEFIN SULFONATES VIA QUANTITATIVE CARBON-13 NMR. Peter G. Gentempo, M.K. Dickson and K.F. Guin, Shell Development Company, P.O. Box 1380, Houston, TX 77001.

Much consideration has been given to the use of alpha-olefin sulfonates (AOS) in detergent formulations. Various wet chemical methods, LC techniques, and isotactophoresis procedures have been successfully employed in the characterization of this class of compounds. The application of Nuclear Magnetic Resonance (NMR) spectroscopy to the study of AOS has also been pursued, with primary emphasis on ^1H NMR. We have exploited carbon-13 NMR to observe the carbon backbone of these compounds. Relatively pure fractions of alkene sulfonates and hydroxy alkane sulfonates were obtained by fractional crystallization of the AOS product. We have assigned the chemical shifts of the functionalized carbons of these fractions. A routine method for the determination of alkene isomer distribution and alkene sulfonate/hydroxyl alkane sulfonate ratios will be presented.

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SAFETY IN USE OF AOS MATERIALS IN JAPAN. Kenkichi Oba, Akira Mori and Toshio Nagai, Lion Corporation, 202, Tajima, Odawara-City, Kanagawa, 256 Japan.

In Japan, since an AOS-based heavy-duty powder detergent was first introduced into the market in 1967, AOS has been widely used in household products such as hair shampoos, light-duty liquid detergents and so forth. From the viewpoint of the safety of AOS materials, both AOS and AOS-based products were tested for their acute toxicity, chronic toxicity, carcinogenicity, teratogenicity, mutagenicity, skin irritation, skin sensitization, etc. A quite higher safety margin was obtained by comparison between maximum human daily intake and no effect dose, and between exposure conditions like duration, frequency and concentration and no effect levels to skin, mucosa membrane, etc. This supported the fact that various sorts of AOS-based household products have been safely used for many years. On the other hand, it was reported in the U.S. that even non-bleached AOS slurries contained skin sensitizing unsaturated sultones. The unsaturated sultones in our non-bleached AOS slurries were determined by our newly developed HPLC-GLC and GC-MS methods. Our typical AOS slurry contained unsaturated sultones at quite a low level of some 0.05 ppm. A similar level of the unsaturated sultones was found also in some AS and AES slurries. These contents of the unsaturated sultones are far lower than several ppm level found in AOS slurries in the U.S. In order to confirm the safety in use of our AOS, the animal studies and subsequent field tests in volunteers on our AOS have been carried out. The AOS did not show any sensitization potential in guinea pigs in the maximization test, and no clinical sign of suspectable allergy was found in the field test on the AOS-based light-duty liquid detergent. In the meanwhile, the long term market experience showed that AOS products were safe in human use, i.e., there has been no skin trouble complaint associated with suspected allergies.

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ALPHA OLEFIN SULFONATE—AN EXTREMELY VERSATILE SURFACTANT FOR PERSONAL CARE. Richard E. Reever, Minnetonka, Inc., Jonathan Industrial Park, Chaska, MN 55318.

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Alpha olefin sulfonates have achieved widespread application in personal care formulations in recent years. A rapid growth spurt occurred since 1979 when a new product category was formed, that of liquid soaps for hand cleaning. Since 1979, as more human exposures were safely recorded, AOS use has expanded to formulations such as all-over body shampoos or liquid shower soaps as well as specialty hair shampoos in the retail market. This paper will survey many of these retail formulations with respect to the AOS function and synergy with support ingredients. Highlighted in this analysis will be the selection of AOS because of: (a) optimum performance with low cost; (b) pH compatibility over a wide range; (c) proven cleaning effectiveness; in fact, a special human use cleaning study showed that an AOS surfactant-based liquid hand soap was more than twice as effective as a traditional bar soap; (d) ease of effective preservation to yield formulations highly resistant to microbial contamination in use; (e) good detergent properties in a high range of water hardness; (f) excellent biodegradability; (g) proven mildness in liquid skin cleansing products, as measured by a 4-year consumer use monitoring program. Special handling knowledge is required for storage, transfer and processing but all requirements are within reasonable plant capabilities. From the resultant product successes it is likely that AOS should retain a valued position in the new product formulator's surfactant selection list.

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A MECHANISTIC APPROACH TO THE THERMAL DEGRADATION OF α-OLEFIN SULFONATES. P.C. Hu, R.O. Johannessen and M.E. Tuvell, Ethyl Technical Center, P.O. Box 14799, Baton Rouge, LA 70898.

The thermal stability can be a critical parameter in selection of a surfactant for applications where the system is subjected to elevated temperatures for long periods of time. The most popular and logical approach to defining the thermal stability of a surfactant is to determine the activation energy involved in the thermal degradation process. However, determining the activation energy involved in the thermal degradation of commercial α-olefin sulfonates was found to be impractical because of the difficulties arising from the fact that α-olefin sulfonates are a mixture of hydroxyalkane sulfonates. Each of these components of AOS was found to follow a complicated thermal degradation path. We found that the thermal degradation of hydroxyalkane sulfonate is a base-catalyzed process. On exposure to elevated temperatures, hydroxyalkane sulfonates are first converted into surface-active intermediates which then degrades to non-surface active products following a second mechanism. The rate determining step of the degradation process was not found to involve a cleavage of the C-S bond as reported for other types of sulfonates. Our work indicates that the first step of the thermal degradation of alkene sulfonates involves isomerization of double bonds followed by cleavage of C-S bond. The thermal degradation of alkene sulfonates is catalyzed by acids.

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SKIN SENSITIZATION POTENTIAL OF ALPHA-OLEFIN SULFONATE (AOS) AND A PROTOTYPE DISHWASHING DETERGENT CONTAINING AOS. P.H.S. Bay and P.J. Danneman, The Procter & Gamble Company, Ivorydale Technical Center, 5299 Spring Grove Avenue, Cincinnati, OH 45217.

A program to evaluate the contact skin sensitization potential of commercial alpha-olefin sulfonate (AOS) was conducted to qualify this ingredient as safe for use in a light duty liquid dishwashing detergent (LDL) to be marketed in the United States. The program consisted of guinea pig and human sensitization studies, diagnostic patch tests and a 9-month product use test with a prototype AOS-containing LDL (AOS-LDL). Materials tested were typical commercial sodium C_{14}-C_{16} AOS pastes, a prototype AOS-LDL, some AOS-containing consumer products (AOS-CPs), mixtures of AOS pastes, AOS-LDL or AOS-CPs with hypochlorite bleach, and alkyl gamma-unsaturated sultone (US) in sodium lauryl sulfate (SLS). Materials also were assayed for US or alkyl gamma-chlorosultone (ClS) using tandem mass spectrometry to learn if sensitization correlated with level of US or ClS. In the guinea pig sensitization studies, AOS pastes, the AOS-LDL, the majority of AOS-CPs tested, and mixtures made by combining an AOS-LDL with bleach induced sensitization. The degree of response appeared to be dose-related to the amount of US present in the material being tested. In sensitization tests conducted among U.S. consumer volunteers, AOS, as paste or in an LDL (concentration of AOS up to 0.06% with US levels calculated at up to 0.002 ppm), failed to induce sensitization but did elicit strong dermal responses in one subject. These responses were interpreted as pre-existing sensitization. In addition to sensitization tests, diagnostic patch tests and a 9-month product use test were conducted in consumers who used AOS-CPs available in the U.S. In patch tests, 15 of 542 subjects (2.8%) exhibited positive sensitization responses to 1.3 ppm US in 0.046% SLS. In the product use test, 2 of 264 subjects using the AOS-LDL developed hand dermatitis and had positive reactions to patch tests with AOS-paste and/or US in SLS. None of the 248 subjects in a concurrent control group (who used a non-AOS LDL) experienced hand dermatitis. Based on results of the product use test, marketing of the prototype AOS-LDL would present a sensitization risk to U.S. consumers.

SESSION JJ Brian L. Walker Memorial Symposium Lipids and Cancer II Wednesday afternoon

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HIGH FAT DIET AND COLORECTAL CANCER IN RATS. Norman D. Nigro and Arthur Bull, Wayne State University, School of Medicine, Clinical Laboratories, 645 Mullett St., 4th Floor, Detroit, MI 48201.

Epidemiological and experimental evidence suggests that excessive dietary fat plays an important role in the etiology of cancer of the colon and rectum. The effect appears to be mediated through altered metabolism of bile acids that affects the promotional phase of carcinogenesis. An important aspect of dietary components that affect the etiology of cancer is the interaction between them. Sometimes their action is additive in either direction, that is in augmentation or inhibition. But, at times, the effect of one factor is cancelled or submerged by another. This is helpful if the reaction is in the right direction but not if a substance with an inhibitory effect is neutralized or submerged. For example, feeding rats a high fat diet will not permit the inhibitory effect of fiber to act. It has been shown in the skin of mice that ornithine decarboxylase (ODC) activity reflects the progression of carcinogenesis. It appears to be true in other organs, and we have shown in both animals and humans that colonic cancer tissue exhibits very high levels of ODC activity. Furthermore, the ODC activity of the mucosa in the colon of rats is increased significantly in those fed a high fat diet compared to controls. These findings suggest the possibility that ODC activity of colonic mucosa may serve as a marker for the identification of increased cancer risk. Studies should be done to determine whether this is true in humans.

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EFFECT OF DIETARY COMPONENTS ON THE PATHOBIOLOGY OF COLONIC EPITHELIUM: POSSIBLE RELATIONSHIP WITH COLON TUMORIGENESIS. R.P. Bird, Ludwig Institute for Cancer Research, Toronto Branch, 9 Earl Street, Toronto, Ontario M4Y 1M4, and Department of Nutritional Sciences, University of Toronto.

The concept that diet plays an important role in the initiation and/or development of various types of tumors in man and experimental animals is well documented. Although the etiology of colon cancer is complex and multifactorial in nature, there is very little information on the dietary components which may act as initiators during colon tumorigenesis. We have evaluated various dietary heterocyclic mutagenic amines present in a typical "Western diet" for their nuclear damaging effect (presumably a genotoxic response) on the colonic epithelium of C57BL/6J mice in vivo. Among the mutagenic amines studied, 2-amino-3,4-dimethylimidazo (4,5-f) quinoline (MeIQ) and 2-amino-3-methylimidazo (4,5-f) quinoline (IQ) were very potent inducers of nuclear aberrations. The colons of animals fed a high fat diet (20% by weight) vs. a low fat diet (5% by weight) were more susceptible to the nuclear damaging effects of

MeIQ and 1,2-dimethylhydrazine, a known colon carcinogen. These observations provide us with the clues that our daily diet may contain colon specific genotoxic components. Promotional effects of dietary fat and/or bile acids on colon tumorigenesis have been well studied. We have observed that orally administered fat as boluses is damaging to the colonic surface epithelium of mice and that this effect is followed by a transient increase in the mitotic activity of the colonic crypts. Similarly, cholic acid, a primary bile acid and a tumor promoter when present in the diet of the experimental animals (0.1, 0.25 or 0.5% by weight) increased cell proliferation in the colons of the animals within 2 weeks. Dietary levels of calcium (0.1, 0.5 or 1% by weight) appear to modify the toxicity of orally administered fat or cholic acid. The colons of animals consuming 0.1% or .5% calcium diet were more susceptible to the toxicity whereas the colons of those consuming a 1% calcium diet appeared more like control colons. These studies demonstrate a profound effect of dietary constituents on the pathobiology of the colonic epithelium which may have a marked influence on the colon tumorigenesis.

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COMPARISON OF LIPIDS FROM LIVER AND HEPATOMA SUBCELLULAR MEMBRANES. Randall Wood, Girish C. Upreti and Roberto J. deAntueno, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843.

Enriched subcellular fractions of nuclei, mitochondria, endoplasmic retriculum, plasma membrane, and cytosol were prepared from liver and hepatoma 7288CTC. Purity was established by marker enzymes and numerous chemical analyses. Neutral lipid and phospholipid class compositions, fatty acid compositions of lipid classes, and the levels of octadecenoate positional isomers were determined for each fraction. Hepatoma contained elevated levels of cytoplasmic RNA and endoplasmic reticulum and mitochondrial 5′nucleotidase activity, relative to liver. Cholesterol and sphingomyelin concentrations were elevated dramatically in all hepatoma subcellular fractions. Generally, the fatty acid profiles of the individual lipid classes were characteristic of the liver organelles, but not the hepatoma. Most hepatoma neutral lipid classes contained a higher percentage of C-20 and longer fatty acids than liver, whereas the phospholipids from hepatoma subcellular fractions generally contain lower percentages. Neither hepatoma nor liver neutral lipids exhibited an organelle specific distribution of octadocenoate positional isomers, but class specificity was observed in liver. Phospholipid classes from liver fractions contained higher levels of vaccenate, whereas hepatoma vaccenate and oleate levels were similar to neutral lipid classes. These data demonstrate that the quantity of the molecular species of lipids in the hepatoma membranes differs dramatically from liver.

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INHIBITORY EFFECT OF FUMARIC ACID ON 3-METHYL-4′-(DIMETHYLAMINO)-AZOBENZENE-INDUCED HEPATOCARCINOGENESIS IN RATS. Keiko Kuroda, Mitsutaro Akao and Kiyoshi Terao, Research Inst. for Chemobiodynamics, Chiba University, Inohana, Chiba 280, Japan.

In China and Japan, people have a custom of eating "seven herbs of spring" with rice gruel on January 7th to help their stomachs recover from exhaustion after the New-Year feast. Capsella bursa-pastoris (Cruciferae), shepherd's purse, is one such herb and has been used medicinally for many centuries. Our studies indicated that the herb extract had various kinds of pharmacological activities. We isolated and identified fumaric acid (FA), as the active component of the herb extract in inhibiting the growth of Ehrlich tumors in mice, by fractionating the extract using the antitumor activity as a guide. The present study was performed to examine whether FA would exhibit any inhibitory effect on azo dye carcinogenesis. Male Donryu rats were given ca. 0.5 g of 3-methyl-4′-(dimethylamino)azobenzene (3-Me-DAB) by being fed a diet containing 0.06% of the heptacarcinogen for 50 days. They were then given a diet containing 1% FA and drinking water containing 0.025% FA for 51 weeks. The administration of FA effectively suppressed the development of hepatomas and associated lesions in the livers of rats fed 3-Me-DAB. In the present study, FA was given to rats after the cessation of 3-Me-DAB intake and, therefore, it was concluded that FA inhibited the carcinogenesis by affecting the promotion step.

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ETHER-LIPIDS: ANTINEOPLASTIC ACTIVITY IN RELATION TO STRUCTURE. Wolfgang E. Berdel, Ulrich Fink, Hans D. Schick, Michael Fromm, Anneliese Reichert and Johann Rastetter, Division of Hematology and Oncology, Department of Medicine I, Technical University, Ismaninger Street 22, D-8000 Munich 80, Fed. Rep. of Germany.

In the search for new anticancer drugs, it is of importance to look for compounds with cellular toxicity-targets, other than the cell division system, which is attacked by the majority of drugs currently available. In this respect alkyl-phospholipid derivatives represent a new class of antiresponse modifiers, but additionally have been shown to exert direct toxic effects on the membrane systems of a cell. A review will be given on the results of recent in vitro and in vivo experiments concerning the antineoplastic activity of alkyl-phospholipid derivatives and other ether-lipids. In order to obtain new structures with higher direct membrane cytotoxicity, certain structure-activity relationships seem to be of importance including: (a) ether-bond in the sn-1 position of the molecule, (b) length of the aliphatic side chain in the sn-1 position, (c) modification of the 2-hydroxyl-group in the sn-2 position and (d) modification and/or substitution of the polar phosphocholine head group of the compound. Relationships of structure to in vivo side effects, e.g., due to PAF activity, and to pharmacokinetics, are discussed. Furthermore, since at least 2 enzyme systems related to lipid metabolism have been described as being defective in neoplastic tissues, (O-alkyl-cleavage-system, Snyder et al.; glycerophosphate dehydrogenase, Holzer et al.), the design of lipid structures which accumulate selectively in neoplastic cells, and thus exert a more selective tumor cytotoxicity, theoretically becomes possible. Results of early clinical trials with the first ether-lipids will be updated. In conclusion, besides their immunomodulating effects, certain lipids represent a new class of cytotoxic anticancer drugs with the cell membrane as a target of their toxicity.

SESSION KK Biochemistry of Fatty Acids Wednesday afternoon

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USE OF DEUTERIUM-LABELED FATS TO FOLLOW INCORPORATION AND TURNOVER OF *trans* AND *cis*-11-OCTADECENOIC ACID ISOMERS IN HUMAN PLASMA LIPIDS. E.A. Emken, W.K. Rohwedder, W.J. DeJarlais and R.O. Adlof, Northern Regional Research Center, ARS-USDA, 1815 N. University Street, Peoria, IL 61604, and R.M. Gulley, St. Francis Medical Center, Peoria, IL.

Triglycerides of deuterium-labeled *trans*-11-, *cis*-11- and *cis*-9-octadecenoic acid (11t-18:1, 11c-18:1, 9c-18:1) were fed as mixture to 2 healthy young adult male subjects. Plasma lipids from blood samples collected periodically for 48 hr were analyzed by gas chromatography-mass spectroscopy. The results were used to follow and compare the uptake, distribution and turnover of the stable isotope-labeled fatty acids.

Approximately equal amounts of each deuterated fatty acid were incorporated into chylomicron triglyceride (TG) samples. This finding demonstrated that the 11-18:1 isomers and 9c-18:1 were equally well absorbed. The maximum total labeled fatty acid content in the chylomicron-TG samples was 60% and indicated that a major portion of chylomicron-TG was supplied from endogenous sources.

Deuterated 9c-18:1 to 11t-18:1 and 9c-18:1 to 11c-18:1 ratios in the plasma-TG samples were both 1.33, indicating a 33% higher turnover rate for the 11-18:1 isomers. Concentration of labeled fatty acids in the plasma-TG samples reached a maximum 4 hr after feeding. At the same time, the amount of unlabeled fatty acids increased to ca. double the amount of unlabeled fatty acids in the 0 hr plasma-TG samples, which suggests that ingestion of the fed mixture results in mobilization of stored triglycerides. Percentages of the deuterated 11-18:1 isomers in. total plasma phosphatidylcholine (PC) were nearly identical to deuterated 9c-18:1, but both 11-18:1 isomers were highly concentrated in the 1-acyl PC position. The 11t-18:1 isomer was almost completely excluded from the 2-acyl PC position. Of all the plasma lipid classes, discrimination against 11c- and 11t-18:1 was strongest for esterification of cholesterol. Ratios

of 9c/11t-18:1 and 9c/11c-18:1 were 16 and 2.4, respectively. Labeled 16:1 produced by in vivo chain shortening was detected only in fatty acids from triglycerides and represented ca. 1% of the total deuterated fatty acid content. No evidence of desaturation or elongation products was detected.

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DIETARY FAT MODULATION OF LIPOPROTEIN FLUIDITY IN HUMAN SUBJECTS AND IN LABORATORY ANIMALS. Elliott Berlin, USDA, Beltsville Human Nutrition Research Center, Rm. 109, Bldg. 308, Beltsville, MD 20705.

Lipoprotein fluidity (LF) is subject to control by the lipid composition, which may in some instances be determined by dietary lipids including fatty acyl moieties, cholesterol, and α-tocopherol. Dietary fatty acid saturation alone is not an adequate predictor of lipoprotein fluidity. Rabbits fed corn oil, high in polyunsaturated fatty acid (PUFA) content, did not have more fluid lipoproteins than rabbits fed cocoa butter, which contains a high level of saturated long chain fatty acids. When 20% lard, high in saturated fatty acids, was added to a PUFA-containing stock diet and the mixture fed to minipigs, lipoprotein fluidity was not altered. In contrast, adding cholesterol (1%) to the 20% lard diet drastically reduced VLDL fluidity, but had little or no effect on LDL or HDL. The cholesterol-induced rigidity was rapidly evident with r_s, the steady-state DPH fluorescence anisotropy, changing from 0.07 to 0.15 within 4 wks of cholesterol feeding. Similarly, in a regression experiment, r_s equalled 0.07 within 4 wks of feeding the stock diet to minipigs who had been fed the atherogenic cholesterol/lard diet for 3 months. Dietary PUFA did increase LDL fluidity in humans. Healthy adult males were fed 2 controlled diets consisting of typical USA foods and containing 35% of fat energy with either 10 or 30 g linoleate daily. The increase in LDL fluidity was small but statistically significant, whereas VLDL and LDL were unaffected. When α-tocopherol (600 IU/day) was administered to women with mammary dysplasia, r_s was significantly elevated for LDL but not with HDL_2 or HDL_3. Apparently cholesterol or α-tocopherol ingestion results in more drastic changes in lipoprotein composition as the lipoproteins are responsible for their transport. Fatty acyl saturation in lipoproteins is, however, under tighter metabolic control, since LF does respond to changes in lipoprotein fatty acyl saturation when they occur. LF in HDL was indeed a statistically significant linear function of lipoprotein PUFA ($r_s = 0.240 - 2.161X$ (HDL-PUFA); $P < 0.02$, $r = 0.52$) in a group of minipigs ranging from 6 weeks to 12 years old.

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WHOLE BODY OXIDATION OF DIETARY FATTY ACIDS. M.T. Clandinin, Departments of Foods and Nutrition and Medicine, The University of Alberta, Clinical Sciences Building, Edmonton, Alberta T6G 2G3, P.J.H. Jones, Department of Nutritional Sciences, University of Toronto, and P.B. Pencharz, Division of Clinical Nutrition, The Hospital for Sick Children.

Whole body oxidation of dietary stearic, oleic and linoleic acid was measured in 6 normal resting males consuming fixed diets. Free living subjects consumed a test diet of normal foods for 16 days at a level commensurate with energy requirements. The diet contained 41.6/ energy as fat, a polyunsaturated/saturated fatty acid ratio of 0.244 and 2.2 gm fat/kg body wt/day. On days 6, 8, 11 and 14, 6-minutes breath samples were obtained from 0745 to 1745 hr and analyzed for total CO_2 content and $^{13}CO_2$ abundance. A bolus dose of 20 mg/kg body weight [1-^{13}C] stearic acid, 10 mg/kg [1-^{13}C] oleic acid or 10 mg/kg [1-^{13}C] linoleic acid was randomly assigned to and consumed with the breakfast meal on days 8, 11 and 14. Enrichment of $^{13}CO_2$ after a bolus dose was calculated over background ^{13}C abundance with diet only, and expressed as a fraction of substrate dose absorbed. Fecal excretion of labeled and diet fatty acids was determined in days 8-16 pooled stool collections. Fat extracts were saponified and methylated, and individual fatty acids were quantitated by GLC. Preparative HPLC was used to obtain fractions containing stearic, oleic and linoleic acid for combustion to CO_2 and assay of ^{13}C enrichment over background. Total fatty acid, stearic, oleic and linoleic acid excretion in subjects over the 9-day period was 41.5±7.3, 10.0±1.3, 8.8±0.1 g/day/kg body weight (x±SEM, n=6), respectively. The absorption efficiency for [1-^{13}C] stearic, [1-^{13}C] oleic and [1-^{13}C] linoleic acid was 78.0±4.5, 97.2±1.7 and 99.9±0.1%, respectively. The reduced absorption of [1-^{13}C] stearic acid observed emphasizes the importance of correcting breath test oxidation data for fecal loss of ^{13}C substrate. At hr 7-9 after the test breakfast, significant differences in percent of absorbed dose excreted with breath were observed between all 3 fatty acids. For the fatty acids contained in the test breakfast, oleate, linoleate and stearate showed significant differences in oxidation rate. These findings challenge the current understanding in energy utilization that dietary fat is oxidized at a rate independent of its long chain fatty acid composition.

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OXIDATION OF OLEIC AND ELAIDIC ACIDS BY RAT AND HUMAN HEART HOMOGENATES. A.C. Lanser, A.E. Emken and J.B. Ohlrogge, Northern Regional Research Center, ARS-USDA, 1815 N. University Street, Peoria, IL 61604.

There has been concern that dietary isomeric fatty acids in hydrogenated vegetable oils may not be as readily oxidized in tissues as the naturally occurring fatty acids. Various approaches, including measurements of $^{14}CO_2$ release and oxygen uptake, have previously been used to investigate the β-oxidation of fatty acids in rat tissues. Previous in vitro studies with human tissue have investigated oxidation of only long-chain fatty acids normally present in unhydrogenated vegetable oils. We have compared the in vitro β-oxidation of *cis* and *trans* fatty acids in tissue homogenates prepared from rat and human hearts by conducting parallel incubations with uniformly-^{14}C-labeled oleic and elaidic acids. Radioactivity in $^{14}CO_2$ and ^{14}C-chain-shortened perchloric acid-soluble products was used to assess the relative rates of oxidation. Rates of formation of $^{14}CO_2$ from oleate and elaidate were independent of geometrical configuration for both rat and human heart tissues. Oxidation rates (nmol/min/mg heart protein) based on ^{14}C-acid-soluble products suggest that oleic acid was oxidized 35-40% faster than elaidic acid by both male and female rats, whereas human heart oxidized these fatty acids at equal rates. Total oxidation rates for these fatty acids (CO_2 + acid-soluble intermediates) were higher for females than for males in both rats (0.2284 vs. 0.0898) and humans (0.1488 vs. 0.1188). Comparative rates of formation of oxidation products expressed as oleic/elaidic ratios from parallel incubations confirm that preferential oxidation of oleic acid did occur in rat heart homogenates. In contrast, both fatty acids were oxidized equally well by the human heart. These data suggest that the presence of the *trans* double bond in elaidic acid does not impair its oxidation by human heart muscle.

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EFFECTS OF n-3 FATTY ACIDS ON EICOSANOIDS: POSSIBLE TISSUE AND SPECIES DIFFERENCES. J.E. Kinsella, B. German, B. Lokesh, G. Bruckner, J. Swanson and M. Black, Institute of Food Science, Cornell University, Stocking Hall, Ithaca, NY 14853.

Rats fed menhaden oil (MO) at 5, 10 and 20 weight % corresponding to 1, 2 and 4% eicosapentaenoic acid (EPA) show no significant alterations in the hematological factors such as bleeding time or platelet aggregability and relative sensitivity to ADP or collagen through blood viscosity of animals on 20% MO decreased compared to the controls. Tissue selectivity in the uptake of n-3 fatty acids was observed, e.g. platelets selectively incorporated (EPA) at the expense of 20:4; whereas aorta epithelia incorporated both EPA and docosahexaenoic acid (CHA). Whereas serum TXB was decreased 50% by 5% MO, reduction in PGI_2 synthesis required higher dietary levels, e.g., 20% MO caused a 50% reduction in PGI_2 synthesis. Similar trends were observed when rats were fed equivalent amounts of EPA or DHA. Thus, platelets preferentially incorporated EPA compared to DHA and EPA was more effective in depressing serum TXB_2 levels. These studies revealed disparities in sensitivity of different tissues to dietary n-3 fatty acids; indicated that the relative effectiveness of n-3 fatty acids may be dose-dependent and demonstrated that platelet TXA_2 production may be more sensitive to low dietary EPA intake than endothelial PGI_2 synthesis thereby reducing overall platelet aggregability. However, species differences in sensitivity to dietary n-3 fatty acids and relative concentrations of TXA_2 and PGI_2 need to be determined to assess the validity of animal models in evaluating the effects of dietary n-3 fatty acids on eicosanoids and platelet behavior.

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SESSION LL Hydrogenation, Dehydrogenation and Interesterification Wednesday afternoon

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FATS AND OILS HYDROGENATION CATALYST PERFORMANCE EVALUATION. Douglas V. Okonek, Thomas J. Sullivan and Orest Nebesh, Harshaw/Filtrol Partnership, 23800 Mercantile Road, Beachwood, OH 44122.

Laboratory testing of hydrogenation catalysts is important to both catalyst supplier and user. Performance evaluation of catalysts is used to support catalyst development, for quality control and for establishment of process conditions. Certain factors affecting mass transfer of hydrogen need to be controlled to evaluate accurately intrinsic catalyst activity. In commercial practice, control of conditions is a compromise based on economic constraints. In the laboratory, test conditions are chosen to cause fats and oils hydrogenation rates to be controlled by intrinsic catalyst activity. Because of the variations in natural feedstock composition, catalyst performance is expressed relative to a standard catalyst. Calculated catalyst selectivity ratios also are influenced by natural feedstock composition. Induction, threshold and particle size effects are discussed as they relate to meaningful catalyst comparisons.

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HYDROGENATION OF LINOLEATE I: THE RATE OF FORMATION OF *TRANS* UNSATURATION. Robert R. Allen, Food Industry Research & Development Institute, P.O. Box 246, Hsinchu 300, Taiwan, Republic of China.

The mechanism of the hydrogenation of *cis, cis*, 9-, 12-linoleate has been established as a series of reactions. A hydrogen is removed from the active methylene group between the double bonds. This causes a shift of one of the double bonds to the conjugated position. The conjugated system is reduced to a monoene by 1,2 and 1,4 addition of hydrogen. As the one double bond shifts and hydrogenates, both positional and geometrical isomers are formed. The amount of geometrical *(trans)* isomers that are formed is a constant amount of the linoleate that is reduced to monoene. The *trans* unsaturation is formed at a rate of 0.7-0.8 times the rate of hydrogenation of the linoleate and this ratio is not dependent on the conditions of hydrogenation. Since the conjugated dienes are reduced 2-5 times faster than the pentadiene system, the rate determining step in the sequence must be the conjugation step. Thus, the amount of *trans* unsaturation formed during conjugation must be constant.

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HYDROGENATION OF LINOLEATE II: POSITIONAL AND GEOMETRICAL ISOMERS. Robert R. Allen and Dorothy Yan-Hwa Chu, Food Industry Research & Development Institute, P.O. Box 246, Hsinchu 300, Taiwan, Republic of China, and Jesse E. Covey, Jr. and Donita See, Anderson Clayton Foods.

It has been shown that the rate of formation of *trans* unsaturation is 0.7-0.8 times as fast as the reduction of linoleate during hydrogenation. Also, this rate ratio is independent of the conditions of nickel catalyzed hydrogenations. The rate-determining step is believed to be the conjugation of the diene system. Therefore, the rate of formation of *trans* must be related to the *trans* produced during conjugation and hydrogenation of the conjugated system. The isomers produced by these steps must be the same under various conditions of hydrogenation of linoleate. To try to determine the isomers produced, slightly hydrogenated linoleate was chromatographed on a 100 m quartz capillary column to separate the positional and geometrical isomers. It was found that *trans* unsaturation is formed by the conjugation reaction and by the 1,2 and 1,4 hydrogenation of the dienes to a monene. However, unless the hydrogenation is extremely selective, the monoene is isomerized to an equilibrium ratio.

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POSITIONAL ISOMERS OF OCTADECENOIC ACIDS IN THE HYDROGENATED PRODUCTS OF CANOLA OIL. S.S. Köseoğlu, The Cambrian Engineering Group Limited, 1465 Cawthra Road, Suite 112, Mississauga, Ontario, Canada L5A 3P2, and L.L. Diosady, L.J. Rubin and W.F. Graydon, Dept. of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, M5S 1A4, Canada.

The positional distribution of the double bond in octadecenoic acids was determined in hydrogenated products of canola oil catalyzed by nickel, arene-$Cr(CO)_3$, and various mixtures of nickel and methyl benzoate-$Cr(CO)_3$. The method involved a chain-length fractionation system based on thin-layer chromatography (TLC) of the methoxybromomercuri adducts of the total methyl esters to isolate groups of acids of a common degree of unsaturation, and then high performance liquid chromatography (HPLC) on a reverse-phase column. The octadecenoic acid fraction was subsequently ozonized, and the fragments were analyzed by using a GC-mass spectrometer. The isomers ranged from Δ6 to Δ15 with the major one being Δ9 for nickel catalyzed hydrogenation products. Benzene-$Cr(CO)_3$, methyl benzoate-$Cr(CO)_3$ and toluene-$Cr(CO)_3$ catalyzed reaction products contain only Δ9, Δ10, Δ11 and Δ12 isomers of octadecenoic acid. For the mixed catalyst, at higher chromium to nickel ratios, the positional isomer distribution was similar to that given by methyl benzoate-$Cr(CO)_3$ alone. However, at low chromium-to-nickel ratios, the characteristic isomer distribution of nickel was observed. The mixed catalyst systems have some practical importance because of their high selectivity and relatively high activity when methyl benzoate chromium tricarbonyl is used in low concentrations at which it would have no activity when used alone.

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CONTINUOUS ULTRASONIC HYDROGENATION–OPERATING CONDITIONS AND OIL QUALITY. K.J. Moulton, Sr., S. Koritala, K. Warner and E.N. Frankel, Northern Regional Research Center, ARS-USDA, 1815 N. University Street, Peoria, IL 61604.

Soybean oil was hydrogenated continuously at different temperatures, pressures and catalyst concentrations and ultrasonic energy. Flavor and oxidative stability of deodorized products were compared with a batch commercial product. The extent of hydrogenation (ΔIV) was not affected by temperature within the range tested (270-290C) but was greater at 106 psig than at 65 psig hydrogen pressure. The ΔIV increased linearly with nickel concentration from 0.004% to 0.015%. The ΔIV increased up to 50% when ultrasonic energy was applied compared to identical processing without use of ultrasonic energy. Energy expended per ΔIV was 30% less when the power supply to the processing cell was reduced from 100% to 40% of maximum. Under conditions used in this study, the linolenate selectivities and specific isomerizations (% *trans*/ΔIV) were not affected by varying the operating parameters. Flavor evaluation of the deodorized oils showed no difference between ultrasonic and control (non-ultrasonic) hydrogenated oils when tested initially or after storage at 60 C for 8 days. Flavor scores of commercial batch hydrogenated oil were significantly lower than those of oil hydrogenated continuously with ultrasonic energy when tested at the 2% linolenate level. Use of ultrasonic energy to enhance continuous hydrogenation may lessen the cost of produce hydrogenated vegetable oils.

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LOW PRESSURE HYDROGENATION OF CASTOR OIL. R.K. Trivedi and A.K. Vasishtha, Harcourt Butler Technological Institute, Kanpur- 208 002, India.

Castor oil was hydrogenated at low pressures (2.0-2.5 kg/cm^2) and low temperatures (125-135 C), using nickel catalyst to obtain saturated waxy product rich in hydroxy-stearic acid. High catalyst concentration was required to obtain good results. However, the catalyst could be recycled for use. The final product had an iodine value below 3.0, hydroxyl value of 155, and slip point 84 C.

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CATALYTIC ISOMERIZATION OF METHYL LINOLEATE–KINETICS, MATHEMATICAL MODELING AND REGENERATION STUDIES. C.S. Narasimhan, D. Mukesh, V.M. Deshpanda

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and R.G. Gadkari, Alchemie Research Centre, Private Limited, CAFI Site, P.O. Box 155, Belapur Road, Thane-400601, Maharashtra, India.

Kinetics of isomerization of methyl linoleate were studied using 5% ruthenium on carbon support as catalyst in the temperature range 200-270 C. The reaction was carried out in an autoclave with protic as well as aprotic solvents. The protic solvents used were methanol, isopropyl alcohol and tertiary butylalcohol and the aprotic solvents were hexane and cyclohexane. Very interesting variations of activity and selectivity with change of solvent type and its composition (in IPA + cyclohexane mixture) were observed. IPA gave very high activity and selectivity for hydrogenation (99% conversion with 90% selectivity) whereas cyclohexane led to high activity and selectivity for conjugation of double bonds (42% conversion with a selectivity of 74%). These differences are attributed to variation of density of Ru-H active sites on the catalyst surface. It was found that at a methyl linoleate to solvent ratio of 1:30, conjugation and hydrogenation were the main reactions. When the above ratio was maintained below 1:10, the reaction was complicated by polymer formation. Further, analysis of geometric isomers of conjugated methyl linoleate showed the operation of kinetic effect on the distribution of *cis-cis* and *trans-trans* isomers. Mathematical modeling of the network of reactions revealed that hydrogenation proceeds via conjugation whereas polymer formation resulted directly from non-conjugated methyl linoleate. The kinetic parameters of the model are estimated by fitting the simulation with the transient experimental data. Regeneration of catalyst was investigated by ESCA studies. The changes in surface ruthenium concentration were correlated with deactivation and regeneration processes.

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CASTOR OIL DEYDRATION WITH COPPER SULFATE CATALYST. T.A. Khan, R.K. Trivedi and A.K. Vasishtha, Harcourt Butler Technological Institute, Kanpur-208 002, India.

Castor oil was refined and dehydrated in inert atmosphere in the presence of anhydrous copper sulfate catalyst. Optimum conditions for obtaining high conjugated unsaturation were established. The final product was monomeric having an iodine number of 138.4 (Wij's) and donjugated dienoic fatty acids at 47.6%.

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DIRECTED INTERESTERIFICATION OF *SHOREA ROBUSTA* FAT. Madhu Bajpai and A.K. Vasishtha, Harcourt Butler Technological Institute, Kanpur-208 002, India.

The fat of *Shorea robusta*, popularly known as sal fat in India, was interesterified using sodium methoxide catalyst. The fat was first randomized at 90 C and subsequently directed interesterified at 30 C. The interesterification was also carried out in solution in n-hexane at 50 C and 9 C as randomization and directed interesterification temperatures, respectively. The slip points of the oil increased during randomization and directed interesterification and GS_3 content of the oil samples also showed a simultaneous rise. A linear relationship between rise in slip points and GS_3 content was observed. Dilatation durves of directed interesterified samples indicated a sharp decrease in dilatation at ca. 20 C in the case of samples interesterified without solvent. Dilatation curves of directed interesterified samples in solvent after 24 hr showed a steep dilatation at 38 C. The melting curves of the different samples do not correlate with their respective slip points.

SESSION MM K.W. Kircher Memorial Symposium Chemistry, Biosynthesis and Function of Sterols II Wednesday afternoon

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OBSERVATIONS ON THE REGULATION OF PLANT STEROL BIOSYNTHESIS. L.J. Goad, University of Liverpool, Department of Biochemistry, P.O. Box 147, Liverpool L69 3BX, U.K.

Much is now understood about the mechanismsm of sterol biosynthesis in plants. However, by contrast to the situation in mammalian tissues, relatively little is known about the regulatory factors which operate to control the rate of sterol production in plant tissues, and attention is now being focused on this facet of plant sterol biochemistry. In order to investigate some aspects of the regulatory mechanisms operating in plants, reliable methods are required for the measurement of the rate of sterol synthesis. The applicability of various radioactively labeled sterol precursors for this purpose will be considered. Any investigations on the determination of the rate of plant sterol synthesis may be complicated by the fact that plant tissues usually contain a mixture of sterols. Those most often encountered are 24-methylcholest-5-en-3β-ol ("campesterol"), 24-ethylcholest-5-en-3β-ol (sitosterol) and 24-ethylcholesta-5,22-dien-3β-ol (stigmasterol) and evidence will be presented which now appears to indicate that these components may be synthesized at differing rates. The incorporation of radioactive precursors such as mevalonic acid and acetate by plants often results in a relatively high labeling of the steryl ester fraction. The possible significance of this observation in relation to the regulation of plant sterol synthesis will be discussed.

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A COMPARISON OF STEROL BIOSYNTHESIS IN PATHOGENIC FUNGI AND THEIR HOST PLANTS. Rick C. Heupel and W. David Nes, U.S. Department of Agriculture, Plant Physiology and Chemistry Research Unit, 800 Buchanan Street, Albany, CA 94710.

Sterol biosynthesis is variable among fungi, whereas crop plants, as well as all tracheophytes, possess a complete sterol pathway. Shunting of early sterol precursors, e.g., MVA, can occur in plants, although an apparent developmental switch operates to control substrate flow. Evidence will be presented which shows that, in the latter stages, fungi possess a lanosterol-based pathway whereas tracheophytes utilize the cycloartenol route to 4-desmethylsterols. In fungi, e.g., Oomycetes, where the sterol pathway is assumed to have been present in their evolutionary history but now has apparently become repressed or absent, the first missing step is notably squalene epoxidation. The functional loss of this important enzyme is correlated with the absence of some but not other types of sterol metabolism, e.g., lack of lanosterol demethylation but capability of reducing Δ^7-steroids to produce Δ^5-steroids in *Phytophthora cactorum* (Oomycetes). Although the functional end-product synthesized by pathogen and host may be the same, e.g., 24-β-methylcholesterol produced by *Gibberella fujikuroi*, a stem rot fungi of sorghum, the sequence and intermediates involved in the pathway are different. The loss of sterol synthetic capabilities has special physiological ramifications. How such information is important to the fungal disease cycle is discussed.

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THE FATE OF RADIOLABELED 22,25-DIDEOXYECDYSONE AND ECDYSONE IN ADULT TOBACCO HORNWORM OVARIES. Malcolm J. Thompson and James A. Svoboda, Insect Physiology Laboratory and Bioenvironmental Bee Laboratory, ARS, USDA, Beltsville, MD 20705.

The major free ecdysteroid of *Manduca sexta* during pupal-adult development at peak titer of molting hormone activity is 20-hydroxyecdysone, along with lesser quantities of ecdysone, 20,26-dihydroxyecdysone and 3-epi-20,26-dihydroxyecdysone. Five days later in pupal-adult development, 20,26-dihydroxyecdysone is the major ecdysteroid. Interestingly, in ovaries of 93-hr-old-adult female and newly-laid eggs (0- to 1-hr-old) the ecdysteroids are present mainly as conjugates (>95%) and 26-hydroxyecdysone, which has not been detected in any other stages of development of the tobacco hornworm, accounts for more than 95% of the total ecdysteroids. Although in eggs of different age groups (4-hr-old or older) 26-hydroxyecdysone accounts for 80-90% of the total free ecdysteroids, the identification of ecdysone, 20-hydroxyecdysone and 20,26-dihydroxyecdysone in these eggs has led to the suggestion that there are at least 2 biosynthesis pathways for ecdysteroids during embryonic development of the tobacco hornworm: the pathway to 26-hydroxyecdysone as the principal route and the formation of 20-hydroxyecdysone as a minor pathway. Ecdysone could serve as an intermediate in both pathways. The fate of labeled 22,25-

dideoxyecdysone and ecdysone in ovaries of 96-hr-old females was determined by injecting pupae with the labeled ecdysteroids 5-6 days before adult emergence in order to determine whether either steroid is an intermediate in the pathway of 26-hydroxyecdysone. The discussion will include the identification and role of ecdysteroid conjugate(s) in ovaries of *Manduca*.

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HYDROXYMETHYLGLUTARYL-CoA REDUCTASE, A KEY ENZYME IN PHYTOSTEROL BIOSYNTHESIS? Thomas J. Bach, Botanical Institute, University of Karlsruhe, Kaiserstr. 12, D-7500 Karlsruhe, Federal Republic of Germany.

Hydroxymethylglutaryl-CoA reductase ("HMGR," EC 1.1.1.34) regulates the synthesis of mevalonic acid ("MVA"), the precursor of the myriad of isopentenoid compounds functional in plant cells, with phytosterols representing one class of major importance. Recently, for the first time, it has been possible to solubilize and to purify the membrane-bound plant enzyme from a heavy membrane fraction (P 16000 × g) isolated from a cell-free homogenate of etiolated radish seedlings. Some details of the experimental procedures developed will be reported as well as what is presently known about the molecular and kinetic properties of radish HMGR. Mevinolin, a highly specific competitive inhibitor of HMGR, has been used as a valuable research tool towards studying the regulatory role of HMGR activity for the growth and development of intact seedlings and of plant cell cultures. The results obtained indicate that a primary effect of inhibition of MVA biosynthesis is a significant decrease in the phytosterol content whereas the accumulation of other end-products of the multi-branched isopentoid pathway, such as ubiquinone in the mitochondria, or chlorophylls and carotenoids in the plastids, is less or not at all affected. This and other data can be interpreted to mean that the organelles are autonomous in the capacity to synthesize MVA. The mevinolin-induced decrease of the ER-bound HMGR activity in vivo is specifically parallelled by a drop in free sterol accumulation as well as of plant growth retardation, thereby suggesting a rate-limiting role of HMGR activity for phytosterol synthesis and a normal development of plants. New aspects of the regulation of HMGR in plant cells will be discussed by comparison with recent information obtained from studies using mammalian cells as an enzyme source.

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PHYSIOLOGICAL ROLES FOR STEROLS IN YEAST. Leo W. Parks and Russell J. Rodriguez, Microbiology Department, Oregon State Univ., Corvallis, OR 97331-3804.

Analyses with a yeast sterol auxotroph indicate that there may be at least 4 different levels of function for sterols. We have designated these functions sparking, critical domain, domain, and bulk. Growth of yeast sterol auxotrophs on cholestanol is precluded unless minute amounts of ergosterol (10ng/ml) are available. We have designated this phenomenon the sparking of growth in which cholestanol satisfies an overall membrane sterol requirement and ergosterol fulfills a high specificity sparking function(s). The level of ergosterol necessary for sparking growth on cholestanol is insufficient to support growth alone or to regulate overall plasma membrane fluidity. The critical domain role for sterol is observed under conditions of lanosterol availability where slightly higher levels of ergosterol (10 times that necessary for sparking on cholestanol) are required for growth. The sterol functions designated domain and bulk are illustrated by assessing cellular free sterol levels and plasma membrane properties of a sterol auxotroph after growth on different concentrations of exogenously supplied sterol, 1μg/ml and 5μg/ml respectively. Although growth rates were unaffected by the concentration of exogenous sterol, growth yields varied significantly. Plasma membranes isolated from auxotrophs with either domain or bulk levels of sterol underwent no lipid phase transitions, as determined by fluorescence anisotropy measurements, whereas plasma membranes from cells grown with critical domain levels of sterol did undergo a lipid phase transition. The ability of sterol to modulate phospholipid metabolism has also been investigated. Fluorescence anisotrophy measurements indicated that lipid phase transitions occurred in the plasma membranes of non-ergosterol synthesizing yeast sterol mutants but not in the plasma membranes of an ergosterol wild-type. Parallel experiments with model membrane liposomes verified that the membrane lipid changes observed in the sterol mutants are dependent on the sterol present, and not on the phospholipid composition. In addition, the membrane lipid changes observed in liposomes derived from wild-type phospholipids were eliminated by addition of ergosterol but persisted in the presence of cholesterol, cholestanol, ergostanol or sterols from the sterol mutants. No membrane lipid changes were observed, however, in plasma membranes from a sterol auxotroph even when the auxotroph was grown on cholesterol or cholestanol. The lack of membrane lipid phase transitions in the sterol auxotroph corresponds to changes in phosphilipid patterns and may reflect the ability of the auxotroph to modify its phospholipid composition to most efficiently utilize the available sterol.

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THE ROLE OF PHYTOSTEROLS IN HOST PLANT UTILIZATION BY CACTOPHILIC *DROSOPHILA*. James C. Fogleman, Department of Biological Sciences, University of Denver, Denver, CO 80208, and Susann M. Duperret and Henry W. Kircher, Dept. of Nutrition and Food Science, University of Arizona.

The Cactus-*Drosophila* Model System of the Sonoran Desert consists of 4 endemic species of *Drosophila (D. mojavensis, D. nigrospiracula, D. mettleri* and *D. pachea)* and 5 species of columnar cacti (agria, organpipe, saguaro, cardon and senita). Extensive collection records clearly indicate a 1:1 relationship of *Drosophila* species to cactus species with 2 of the insect species exhibiting host plant shifts between different geographic regions of the desert. The elimination of 6 of the 20 possible combinations of *Drosophila* species and cactus species can be directly attributed to phytosterols. *Drosophila pachea* have a strict requirement for Δ^7-sterols such as 7-cholestenol and 7-campestenol. Since Δ^7-sterols are found only in senita cactus, *D. pachea* cannot use agria, organpipe, saguaro or cardon as host plants. The lipid fractions of agria and organpipe are chemically similar and contain high concentrations of several 3β, 6α-dihydroxysterols. Larval viability tests using chemical constituents of organpipe cactus demonstrate that the sterol diols are toxic to *D. nigrospiracula* but not to the resident species, *D. mojavensis*. Agria and organpipe are, therefore, unsuitable as host plants for *D. nigrospiracula*. These results suggest that phytosterols play a major role in determining host plant utilization by cactophilic *Drosophila* in the Sonoran Desert.

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STEROL-POLAR LIPID INTERACTIONS AND THE DOMAIN STRUCTURE OF FUNGAL PLASMA MEMBRANES. Charles E. Martin, Department of Biological Sciences, Rutgers University, New Brunswick, NJ.

Plasma membranes of *Neurospora crassa* exhibit large changes in sterol/phospholipid ratios when grown over a wide range of growth temperatures. Modification of these ratios is due to increases in the levels of sphingolipids at low growth temperatures. Although the sterol/phospholipid ratio differs widely with respect to temperature, sterol/polar lipid ratio remained relatively constant, suggesting that the membrane sterol content may be under stringent regulatory control. Plasma membrane bulk fluid properties appear to be closely regulated in spite of the introduction of large amounts of sphingolipids into the membrane at low growth temperatures. Fractionation of plasma membrane lipids showed that the sphingolipids by themselves produce highly ordered bilayers. Reconstruction of total plasma membrane lipids into vesicles produced highly fluid bilayers, suggesting that interactions between the different lipid classes are an important factor in maintaining bulk membrane fluidity. We have also used the technique of fluorescence photobleaching recovery (FPR) to monitor lateral diffusion rates of fluorescent phospholipid probes in the plasma membrane of a cell wall-less mutant of *Neurospora crassa*. In plasma membranes of 37 C-grown cells, diacylindocarbocyanine probes with acyl chain lengths of 16 carbons and 18 carbons exhibit different thermotropic behavior when lateral diffusion coefficients are measured over a temperature range from 5-40 C. These probes have been demonstrated to exhibit similar diffusion coefficients in uniformly fluid lipid bilayers, suggesting that the *Neurospora* plasma membrane contains domains of differing fluid properties into which these probes segregate. To test this hypothesis

further, we performed resonance energy transfer experiments in purified plasma membrane vesicles using the same C_{16} and C_{18} probes as energy donors, and a C_{18} probe with altered spectral characteristics as the energy acceptor. Under conditions where FPR data suggested that domain formation occurred, fluorescence energy transfer indicated that probes with different chain lengths segregated from each other whereas probes with similar chain lengths remained either at a constant distance or became more closely associated. These data suggest that discrete fluid lipid domains may exist in *Neurospora* plasma membrane, whose size and composition may vary as a function of temperature.

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SELECTIVE STEROL TRANSFER IN THE HONEY BEE: ITS SIGNIFICANCE AND RELATIONSHIP TO OTHER HYMENOPTERA. J.A. Svoboda, E.W. Herbert, Jr., M.J. Thompson and M.F. Feldlaufer, Insect Physiology Laboratory and Bioenvironmental Bee Laboratory, ARS, USDA, Beltsville, MD 20705.

The honey bee, *Apis mellifera*, is one of only a few species of phytophagous insects that is known to be unable to convert C-24 alkyl phytosterols to cholesterol. Regardless of the dietary sterols available to the worker bees, they are able to rear brood whose major tissue sterol is always 24-methylenecholesterol, with sitosterol and isofucosterol the next most abundant sterols. Normally, very little or no cholesterol is present in honey bee sterols. This maintenance of high levels of certain sterols is accomplished through a selective transfer of sterols from the endogenous sterol pools of the workers to the developing larvae through the brood food material secreted from the hypopharyngeal and mandibular glands and/or the honey stomach of the workers. Selective uptake and transfer of various radiolabeled C_{27}, C_{28} and C_{29} sterols have been studied to correlate these aspects of sterol utilization with the discovery of an unusual molting hormone (ecdysteroid) in honey bee pupae as the major ecdysteroid of this stage of development. The discussion will include phylogenetic implications of this phenomenon in the honey bee and comparison with sterol metabolism in certain other hymenopteran species to emphasize the diversity of this area of insect biochemistry.

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THE INFLUENCE OF OXYGEN ON THE UPTAKE OF STEROLS BY *SACCHAROMYCES CEREVISIAE*. William R. Nes, William J. Pinto and Inder C. Dhanuka, Department of Biological Sciences, Drexel University, Philadelphia, PA 19104.

Work in this and other laboratories has shown that the ability of the yeast, *Saccharomyces cerevisiae*, to adsorb sterols depends on the presence of an active apparatus to form these compounds endogenously. Under anaerobic conditions which block synthesis, certain (but not all) sterols can enter the cells readily. Under fully aerobic conditions substantial amounts of various sterols can also enter mutant cells with a block in sterol synthesis, but aerobically grown cells of wild type yeast will absorb only traces of either cholesterol or ergosterol. With radioactive substrates, we find incorporation of these sterols amounts to less than ½% of the total sterol in either case. Masking the hydroxyl group as the methyl ether does not improve uptake. This apparent inability to study the fat and utilization of exogenous sterols in wild type yeast in the presence of oxygen has been overcome through the use of intermediate conditions. A semiaerobic system has been devised with an atmosphere of 10% air. Growth occurs but to an extent which is limited by restricted sterol synthesis, and the cells will now absorb certain (but not all) sterols presented to them. That certain of the absorbed sterols are functional can be shown by increased growth and the metabolic fate of the sterols can be followed by an examination of extracts.

SESSION NN Poster presentations Wednesday afternoon

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METABOLISM OF STEROLS BY *SACCHAROMYCES CEREVISIAE*. William J. Pinto and William R. Nes, Department of Biological Sciences, Drexel University, Philadelphia, PA 19104.

Saccharomyces cerevisiae might be useful as a tool in biotechnology for the production of commercially important steroids. Although under fully aerobic conditions the organism will not absorb significant amounts of exogenous sterols, uptake readily occurs when the amount of oxygen is reduced. Some of the absorbed steroids are metabolized. For instance, as reported earlier, desmosterol, the 24-dehydro-derivative of cholesterol is converted to 24β-methylcholesterol (also known as 22,23-dihydrobrassicasterol). We now wish to report the conversion of 24,25-dihydrolanosterol to 7-dehydrocholesterol, an intermediate in the biosynthesis of vitamin-D_3. Dihydrolanosterol was adsorbed by the cells and entered the sterol biosynthetic pathway by mimicking lanosterol. It was demethylated at C-4 and C-14 ultimately yielding 7-dehydrocholesterol. The results indicated that the $\Delta^{24(25)}$-bond is not essential for demethylation to take place. Moreover, the absence of cholesta-5,7,22-trienol in the cells showed that the introduction of the Δ^{22}-bond does not readily occur in the absence of a C_1-group at C-24.

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STEROLS OF THE FAMILY *CUCURBITACEAE*—AN EVOLUTIONARY RECAPITULATION. Vipin K. Garg and William R. Nes, Department of Biological Sciences, Drexel University, Philadelphia, PA 19104.

The members of the family *Cucurbitaceae* seem to be unusual in terms of their sterol structures. We have recently characterized a large array of 14 different sterols in the seeds of *Cucurbita maxima*. These include the dominant Δ^7-sterols, some "mainline" Δ^5-sterols, and sterols characteristic of organisms (algae and fungi) much lower on the evolutionary scale. We now wish to report on developmental changes which occur in the sterol patterns. The percentage as well as the absolute amount of Δ^5-sterols progressively decreased during germination, and no Δ^5-sterols could be detected in mature plants. This would suggest that the Δ^5-sterols may have a specific function during germination. However, the percentage and amount (on a fresh weight basis) of 24β-alkylsterols, which accounted for 50% of the total sterols in the seeds, was unaffected by germination, but as the seedlings developed into mature plants the percentage of the total sterols which had the 24β-configuration fell to 5%. This seems to represent an evolutionary recapitulation, since sterols of the majority of the investigated nonvascular plants contain only 24β-c alkylsterols, while sterols in most of the tracheophytes have mainly the 24α-configuration.

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COMPARISON OF HEART LIPIDS ISOLATED FROM MICE FED DIETS CONTAINING MARGARINE OR CONTROL FAT. Janet Kerr, Mark Keeney and Joseph Sampugna, Department of Chemistry, University of Maryland, College Park, MD 20742.

Hearts were isolated from male mice (C57B1/6J) reared on experimental (E) or control (C) diets containing margarine or control fat, respectively. Control fat was blended to give a fatty acid composition similar to that of the experimental fat, except that oleic acid replaced *trans* isomers. At 2 and 6 months of age, lipids were extracted and analyzed. With regard to phospholipid classes, phosphatidylinositol was significantly higher and sphingomyelin was significantly lower in the E group compared to the C group at both ages. At both ages, total saturated fatty acids were lower in the E group compared to the C group. In the E animals, the percentage of saturated fatty acids was inversely related to the percentage of *trans*-18:1, which decreased from 5.1% at 2 months of age to 3.3% at 6 months of age. These results are consistent with a partial replacement of saturated fatty acids by *trans* isomers. Other differences in the fatty acid patterns of E mice compared to C mice were lower percentages of 22:6(n-3) at both ages and a higher ratio of 18:1(n-6)/20:3(n-6) and a lower ratio of 20:3(n-6) to 20:4(n-6) in the 2-month old mice. Differences in the ratios of (n-6) fatty acids have generally been ascribed to inhibition of desaturase(s) by *trans* isomers and the lack of similar differences at 6 months of age may be due to the lower levels of *trans* isomers found in heart tissue at that age.

Meetings

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MODIFICATION OF MOUSE LIVER MICROSOMAL MEMBRANES BY DIETARY *TRANS* FATTY ACIDS: CHANGES IN MONOENOIC FATTY ACID COMPOSITION. Mary G. Enig, Mark Keeney and Joseph Sampugna, Department of Chemistry, University of Maryland, College Park, MD 20742.

Changes in monoenoic fatty acid composition of total lipids (TL), polar lipids (PL) and neutral lipids (NL) in liver total microsomes (TM), rough + smooth ($R+S_IM$) and smooth II ($S_{II}M$) microsomes isolated from C57B1/6J mice fed an experimental (E) diet (10% fat w/w) containing 25% of the fatty acids (FA) as *trans*-octadecenoates from margarine oil compared to a control (C) diet (10% fat w/w) matched in overall FA composition but devoid of *trans*-FA have been measured. Whereas the naturally occurring *trans*-FA from biohydrogenation seen in the control mice represented from 0 to 5.6% of the microsomal membrane monoenes, the *trans*-FA from the partially hydrogenated vegetable fat seen in the experimental mice represented from 15.2 to 38.8% of the membrane monoenes. *Trans*-FA represented 0 to 1.8% of the total C-FA and 6.9 to 10.2% of the total E-FA in the microsomal membranes.

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CHOLESTEROL OXIDES I. ISOLATION AND DETERMINATION OF SOME CHOLESTEROL OXIDATION PRODUCTS. Gerhard Maerker and Joseph Unruh Jr., USDA-Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118.

The lability of cholesterol toward oxidation, especially in the presence of air and at elevated temperatures, has been known and studied for some years. Mixtures of the numerous cholesterol oxidation products, as well as several individual products, have been reported in recent years to produce various adverse health effects in test animals. Reported biological activity includes cytotoxicity, angiotoxicity, atherogenicity, mutagenicity and carcinogenicity. Some of the deleterious cholesterol oxidation products have been observed in foods, especially in spray-dried eggs, certain cheeses and intermittently heated tallow. The detection and measurement of cholesterol oxidation products in foods are difficult because analytical procedures are inadequate and cumbersome, and cholesterol oxidation products, if present at all, exist at very low concentrations. Furthermore, isolation and measurement procedures may themselves give rise to artifacts that interfere in the intended determinations. In the current study isolation and analytical procedures were examined to determine the extent to which HPLC-purified cholesterol is oxidized during lipid extraction, saponification, preliminary HPLC cleanup and on-column capillary GC determination. Cholesterol in oxide-free triolein was subjected to saponification by three methods, followed by HPLC separation of unreacted cholesterol and on-column, capillary GC determination of underivatized oxides. Total oxides found per mg cholesterol exposed to the methods follow: AOAC saponification (method 28.081) - 2.69 μg, "dry column" saponification - 1.1 μg, dry column saponification in presence of BHT - 0.71 μg. HPLC separation and GC analysis together were responsible for the formation of 0.26 μg oxides per mg cholesterol.

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CHOLESTEROL OXIDES II: ANALYSIS OF THE 5,6-EPOXIDES DURING CHOLESTEROL OXIDATION IN AN AQUEOUS DISPERSION. Gerhard Maerker and Frank J. Bunick, USDA-Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118.

Cholesterol is the principal sterol of animal tissue and is present in small but significant amounts in most animal derived foods and hence in our diets. The ease with which cholesterol oxidizes in air especially at elevated temperatures is well known; numerous oxidation products have been characterized. Some of these products, notably the α-epoxide and its hydration product the 3,5,6-triol, have been cited as producing pathological conditions in test animals. Their formation has been observed both in model systems and in various food products with the relative amount of the α-epoxide and its non-toxic β-epoxide isomer varying greatly depending on the oxidation system. To gain more information about the formation of the isomeric epoxides and their relative ratio, a model system of sodium stearate in water at pH 8.0 containing cholesterol was analyzed periodically during oxidation by air at 50 and 80 C. Analysis of total oxidation products by TLC showed that at both temperatures, the cholesterol hydroperoxides were the major initial products formed. Subsequently, the epoxides, the 7-keto cholesterol, the 7α- and 7β-hydroxy isomers, and the 3,5,7-triol were observed to increase with time. Late in the 80 C oxidation, almost no hydroperoxides remained while the 7-keto and 7-hydroxy isomers were the major products present along with lesser amounts of the epoxides, the 3,5,6-triol and a large number of minor oxidation products. Resolution of the α- and β-epoxides was accomplished by recovery of the epoxide band from TLC plates followed by capillary gas chromatography of the underivatized isolates. Although the total amount of the epoxides increased throughout both oxidations, no significant variation was seen in the α/β-epoxide ratio with time; 0.35 ± 0.02 at 50 C and 0.43 ± 0.04 at 80 C.

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IMPROVING QUALITY OF USED DEEP-FRYING FATS. H.F. Al-Shaikh, J. Mancini-Filho and L.M. Smith, Food Science and Technology, 1480 Chemistry Annex, University of California, Davis, Davis, CA 95616.

Per capita consumption of edible fats and oils in the U.S. is now over 130 pounds per year. Frying fats account for an important share of this consumption. The quality and stability of frying fats are of concern to food technologists and nutritionists. During deep-fat frying, changes eventually occur in the oil that adversely affect food flavor and may impair nutritional value. The current study was conducted to explore the use of selected chemicals in extending the quality and stability of frying fats. Series A: Samples of an animal-vegetable shortening were obtained from a commercial restaurant after 10, 20, 30 and 40 hours of cooking at 360 F. These were treated with different mixtures and concentrations of activated bleaching clay, activated carbon, magnesium oxide and Celite. Treatment conditions were as follows: fat temperature, 85 F; chemicals added and mixture stirred for 15 min; mixture filtered at 85 F to obtain clear oil. Samples were analyzed for free fatty acids, changes in dielectric constant, absorbance at 420 nm, total polar materials and fatty acid profiles. A mixture of 45% clay powder, 5% carbon powder, 25% magnesium oxide and 25% Celite was effective in lowering deterioration parameters of the commercially used shortenings. However, when these treated fats were used to fry more french fried potatoes in the laboratory, foaming soon occurred and the frying operation was terminated. Series B: Animal-vegetable shortening used commercially to deep-fry battered seafood and french fries for 40 hours at 360 F was treated with 5% of 10% of the above adsorbent mixture plus different amounts of dimethylsiloxane, butylated hydroxyanisole and ascorbyl palmitate. The treated shortening was re-used to fry french fries and frying fat samples were again analyzed at the end of 10-hr cooking periods. The combination of adsorbent treatment plus antifoam agents and antioxidants retarded deterioration and increased the useful life of the shortening.

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COMPARISONS OF PLATELET LIPIDS ISOLATED FROM MICE FED DIETS CONTAINING MARGARINE OR CONTROL FATS. Lynn D. Kurfess, Mark Keeney and Joseph Sampugna, Department of Chemistry, University of Maryland, College Park, MD 20742.

Lipids of murine platelets were isolated from animals reared for two generations on experimental or control diets containing 10 or 20% fat (by weight). Experimental diets differed from control diets only in the inclusion of geometrical and positional isomers of 18:1, derived from margarine. *Trans* fatty acids were present in platelets as 6% and 8% of the total fatty acids from the 10% and 20% experimental diets, respectively. Compared to platelets isolated from control mice, those from experimental mice gave higher phospholipid to protein ratios, lower percentages of 16:0, 18:0 and 22:6(n-3) and increased percentages of 18:2(n-6) and 18:3(n-3). These results are consistent with a partial replacement of saturated fatty acids by *trans* isomers and an inhibitory effect of *trans* isomers on delta-6 desaturase. That significant decreases in 20:3(n-6) and 20:4(n-6) were not found, despite the apparent inhibition of desaturase activity, may be related to the reported presence of an acyl-CoA synthetase in plate-

lets which facilitates preferential incorporation of these fatty acids into platelet phospholipids.

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POTENCY OF DIETARY SOY LECITHIN AND SUBFRACTIONS IN GROWTH, LIVER STATUS AND COAT QUALITY OF THE GERBIL. Tom R. Watkins and E. Caetano, CUNY, School of Health Science, 425 E. 25th St. W709, New York, NY 10010.

Lecithin has been defined as a generic class of compounds in the Food Chemicals Codex. Purified fractions of this mixture may exert distinct effects in biological systems based upon structural differences. The purpose of the present study was to compare the potency of mixed soy phospholipids (Lecithin, LEC) with phosphatidylcholine (PC) and phosphatidylinositol/phosphatidylethanolamine (PI/PE) fractions isolated from LEC in the gerbil model. PC material was greater than 98% pure; PI/PE contained 2/3 PI and nearly 1/3 PE by TLC. LEC used was the purified, degummed, deoiled soy isolate for food use. Body weight gain, liver weight as a percentage of body weight, and liver lipid accumulation were measured; coat condition was recorded on film. Weanling, female gerbils *(M. unguiculatus)*, 33-36 g, housed in groups of six to eight animals, were fed purified diet ad libitum with or without phospholipid supplement. The diet contained (weight %); casein, 15.0; corn starch, 52.4; cellulose, 6.0; salts, 6.0; vitamins, 0.6; coconut oil, 13.0; corn oil, 3.0; and phospholipid, 4.0. Diet without phospholipid contained added coconut oil to make all diets isocaloric. After 23 days of supplementation or deprivation, mean body weights differed markedly: +LEC, 44.9 g ± 1.8, compared with 25.4 g ± 1.4 in the deprived group (P 0.001). During the repletion period, the formerly deprived group, now continuously supplemented with LEC, attained the greatest weight, 45.5 g ± 3.7, followed by the +PC group, 36.9 g ± 5.4 (P 0.005), and the +PI/PE group, 33.2 g ± 6.0 (P 0.005). During the dietary depletion period, the livers enlarged in the groups deprived of LEC or its constituents. Mean weights, stated as a percentage of body weight, were: +LEC, 3.45 ± 0.1; +PC, 3.78 ± 0.2 (N.S.); +PI/PE, 4.28 ± 0.1 (P 0.005); and, -LEC, 4.92 ± 0.3 (P 0.01). Increased liver weight could be accounted for largely as lipid, which was extracted by the method of Bligh and Dyer (Can. J. Biochem., 1959). Mean liver lipid, expressed as mg/g dry weight, was lowest in the +LEC group, 66.4 ± 10; in the +PC group, 74.6 ± 9.4 (N.S.); in the -LEC group, 108 ± 28 (P 0.01); and greatest in the +PI/PE group, 124 ± 23 (P 0.01). PC fractions exerted the most liver lipid mobilizing potency. A concomitant alopecia occurred in all groups derived of dietary LEC. Upon depletion, each -LEC animal developed complete baldness of snout, back and belly. +PI/PE supplement restored half of the normal coat; the +PC addendum supported full coat restoration, as did +LEC. These observations will be discussed in light of known roles of phospholipid fractions in other organs.

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THE RELATIVE LEVEL OF CHOLESTEROL TO PHOSPHATIDYLCHOLINE IS INCREASED IN RED BLOOD CELL LIPIDS OF RATS DUE TO THE DIETARY EXCLUSION OF LINOLEATE. G. Ananda Rao, Gail Lew and E.C. Larkin, Veterans Administration Medical Center, 150 Muir Road, Martinez, CA 94553.

We reported earlier that if rats are fed a diet depleted of linoleate (18:2), the normal discoid shape of red blood cells (RBC) is altered, echinocytes are produced and the level of 18:2 in red cell lipids is reduced markedly. However, when a diet supplemented with 18:2 is fed to the 18:2-deficient rats, the 18:2 level in RBC lipids and the percentage of discocytes in red cells are increased to normal levels. Subsequently, we observed that the discocyte to echinocyte transformation can occur in vivo even when the level of 18:2 did not decline in RBC. This finding suggested that the 18:2 level is not related to the structural changes of red cells. In the present study, we determined whether the contents of various lipid classes were altered during the morphologic changes in RBC. In rats fed an 18:2-depleted diet, the contents of cholesterol, phosphatidylethanolamine, phosphatidylserine and spingomyelin per ml of packed RBC were similar to the corresponding levels in the controls which were fed the 18:2-supplemented diet. On the other hand, the levels of lysolecithin and free fatty acid increased approximately 60% and phosphatidylcholine (PC) decreased about 40% in RBC of rats fed the 18:2-deficient diet as compared to the controls. Hence, during the morphologic transformation of discocytes to echinocytes in vivo, the relative levels of cholesterol to PC and of PC to other phospholipid classes are altered.

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AN IMPROVED METHOD FOR QUANTITATION OF FREE FATTY ACIDS IN CREAM LIPOLYSATES. Lisa G. Lambert, C.L. Wolf, J.D. Bernstein, L.H. Posorske, Novo Laboratories, Inc., 59 Danbury Rd., Wilton, CT 06897-0820, and R.G. Jensen, University of Connecticut, Storrs, CT.

A modification of the method of Needs et al. has been applied to the recovery of free fatty acids (FFA) from lipolyzed cream. This method employs a different extraction procedure, one that avoids centrifugation with a highly flammable solvent. Furthermore, the analysis can be performed in less time. After the lipolysates are extracted from the cream, the FFA's are bound to an ion exchange resin. Quantitative binding to resin of up to 100 mg of FFA from standard mixture has been achieved, and is not influenced by the molecular weight of each acid. The FFA are removed from the resin by a methylation procedure adapted from Edwards-Webb. The methyl esters are quantitated by gas chromatography. The gas chromatograms have reproduced the same FFA profile as the original standard mixture.

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THE CATALYSIS OF LINOLEATE OXIDATION BY SOLUBLE CHICKEN MUSCLE PROTEINS. Eric A. Decker and Edward G. Schanus, Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164.

Lipid autoxidation is a major problem in mechanically deboned muscle used in the food industry. The accelerated rate of lipid autoxidation in deboned muscle has been attributed to myoglobin and hemoglobin. The soluble fraction of a chicken *musculus gastrocnemius* muscle homogenate was used to catalyze the oxidation of linolate after removal of compounds 700 daltons by Sephadex G10 gel filtration. The oxidation rate with desalted muscle extract exhibited a pH optimum of 6.0, over a range of 4.75 to 7.75 and negative correlation existed between oxidation rate and temperature (50 to 90 C). The addition of EDTA (10^{-2}mM) had no effect on the oxidation rate. The crude extract was separated into three major protein fractions by Sephadex G200 gel filtration. The intermediate molecular weight fraction catalyzed linoleate oxidation and contained protein bound heme as evidenced by a Soret band. Cyanide/ferricyanide did not significantly decrease oxidation catalyzed by the intermediate Mw fraction. The hemoprotein enriched fraction was resolved into five protein fractions with anion exchange chromatography and only the hemoprotein fraction catalyzed linoleate oxidation. The active fraction was resolved into six protein bands with native protein electrophoresis. Four of the protein bands catalyzed the oxidation of linoleate. The total catalytic activity of chicken muscle cannot be attributed solely to myoglobin or hemoglobin.

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CONCLUSIVE TEST FOR AFLATOXIN RESISTANCE IN PEANUTS. Timothy H. Sanders, P.D. Blankenship, R.J. Cole, K.H. Ashley and R.A. Hill, USDA, ARS, National Peanut Research Lab., 1011 Forrester Drive, S.E., Dawson, GA 31742.

Data from four years were compiled to determine that a 5-cm soil temperature mean of ca. 29.4 C and severe drought conditions 40-50 days before harvest resulted in *Aspergillus flavus* invasion and aflatoxin production in preharvest peanuts. These conditions in 6.1 m × 12.2 m plots were used to evaluate four peanut genotypes (A7404, A72118, UF791041 and UF77316) ascribed resistance to *A. flavus* in a laboratory screening assay using cured, rehydrated peanuts. A deep fruiting genotype (P1331334) and Florunner, a commercially grown genotype, were also included in the evaluations. The stress environments were imposed on growing plants 98 days after planting. Peanuts were harvested 143 days after planting, dried, shelled and separated into commercial categories. Peanuts in all categories of all genotypes contained aflatoxin and assessment of

the microflora revealed extensive colonization by *A. flavus* group fungi. These data indicate the inability of the laboratory screening method to predict *A. flavus* invasion/aflatoxin in preharvest peanuts of these four genotypes and suggest the need for careful evaluation of other "resistant" genotypes.

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MODEL FOR PLASMALOGEN OXIDATION: OXIDATION OF LONG-CHAIN ALK-1-ENYL ETHERS IN THE PRESENCE OF ETHYL LINOLEATE. William N. Marmer, E. Nungesser and T.A. Foglia, Eastern Regional Research Center, USDA, 600 E. Mermaid Lane, Philadelphia, PA 19118.

Simple alkyl-enyl ethers such as ethyl hexadec-1-enyl ether were prepared from appropriate aldehydes and isolated in their *cis* form by medium pressure liquid chromatography. Oxidation experiments were carried out in sealed vials at 86 C on neat materials under air. Ethyl stearate, which was inert to oxidation under the experimental conditions, served as internal standard. Disappearance of alk-1-enyl ether, linoleate and oxygen was followed by GC, and formation of conjugated diene, indicative of the presence of linoleate hydroperoxide, was followed by UV absorption at 235 nm. In the absence of linoleate, alkenyl ether underwent slow but measurable oxidation. In the presence of linoleate, however, disappearance of the ether was greatly accelerated, and proceeded at a rate comparable to linoleate. The results suggest that oxidation of the alk-1-enyl ether moiety of plasmalogens should not be ignored as a factor that contributes to the oxidative instability of animal tissue or the development of rancidity in meat products.

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PHOSPHOLIPID AND FATTY ACID COMPOSITIONS OF PLATELETS FROM HORSE, PIG, RAT AND HUMAN SUBJECTS. B.J. Holub and T. Wilkinson, Department of Nutrition, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

There has been much interest recently in the accelerated metabolism of various phospholipids, including phosphatidylinositol (PI) and phosphatidylcholine (PC), which accompany platelet responses to various physiological agonists and provide for the release of free arachidonic acid. Since blood platelets from several animal species have been used in this area of research, it was of interest to compare the phospholipid and fatty acid compositions in horse, pig and rat platelets with those found in human subjects. The phospholipid compositions (mol % of total) were generally similar across different species although the abundance of sphingomyelin in the rat (13 mol %) was below that for the horse (23%), pig (20%) and human (19%). Fatty acid analyses revealed that the mol % of arachidonate in the individual phospholipids of the horse was 32-89% lower than that found in the human platelet and well below the pig and rat. Dramatic differences were found also across species in the percentages of oleate and linoleate in phosphatidylcholine(ethanolamine0(serine) (inositol) and sphingomyelin. These results suggest that, before extrapolating to the human platelet, caution may need to be exerted in evaluating results on the contribution of arachidonoyl molecular species to phospholipid turnover and arachidonic acid release when derived from experiments with non-human stimulated platelets.

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QUALITY CHARACTERISTICS ASSOCIATED WITH THE SEED COAT COLOR OF CANOLA. J.K. Daun and D.R. DeClercq, Agriculture Canada, Grain Research Laboratory, 1404-303 Main Street, Winnipeg, Manitoba, Canada R3T 0Z6.

Partially yellow seeded canola varieties (*Brassica campestris* L. cv. Candle and Tobin) from both pedigreed seed and commercial production were separated into yellow and dark seeded portions. The portions were examined for oil content, protein content, fibre content, glucosinolate content, fatty acid composition, and chlorophyll content. The pedigreed seed samples, (breeders and certified seed) contained 65-85% yellow colored seeds compared with 50-65% yellow colored seed in the commercially grown which suggested the presence of some volunteer grown black seeded varieties. The yellow seeds had higher oil and protein than the dark seeds for the pedigreed seed samples. These differences were smaller in the commercially grown samples with the dark seeds being slightly higher in oil content and equal in protein content to the yellow seeds. Erucic acid levels were higher in the dark colored fraction of all samples examined. The fiber content and chlorophyll content was consistently higher in the dark seeded fraction.

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BROADENED MESOPHASES IN 19-NORCHOLESTERYL ESTERS. Wolfgang Sucrow and Susanne Howard, University of Paderborn, Warburger St. 100, D-4790 Paderborn, Germany.

From our investigations of perhydro-2-phenanthrenol esters we understood that axial angular methyl groups markedly reduce the mesomorphic (nematic) range of flat aliphatic systems. We concluded that, in the opposite direction, the removal of angular methyl groups from the well known mesogenic cholesteryl esters should broaden their cholesteric ranges. This has now been verified for a number of 19-norcholesteryl esters. 19-Norcholesterol was prepared following known procedures and esterified with some aliphatic and aromatic acid chlorides. Phase transitions were recorded on heating the pure 19-norcholesteryl esters under a polarizing microscope. The cholesteric ranges of the aliphatic esters were three to four times larger than for their cholesteryl counterparts, those of the aromatic esters showed a broadening only in the case of the anisate. A tentative explanation for this behavior is given on the grounds of the role which axial methyl groups play in the crystal lattices of the cholesteryl esters.

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SEQUENCING OF STEROL BIOSYNTHESIS IN *SAPROLEGNIA FERAX*. Phu H. Le and W.D. Nes, Plant Physiology and Chemistry Research Unit, WRRC-ARS/U.S. Department of Agriculture, 800 Buchanan St., Albany, CA 94710, and Edward J. Parish, Department of Chemistry, Auburn University, Auburn, AL.

Culture of *Saprolegnia ferax* were grown in the presence of various inhibitors of sterol biosynthesis to determine the route from squaleneoxide to 4,4,14-trisdesmethyl end-products. Incubations with [^{14}C] squaleneoxide, [2-^{3}H] lanosterol, [2-^{3}H] cycloartenol, [2,4-^{3}H] desmosterol, [2,4-^{3}H] 24-methylenecholesterol, and [2,4-^{3}H] dehydropollinastanol have allowed us to assess the lack of importance of a cycloartane-based pathway in this fungus and the involvement of Δ^{8}-sterols versus Δ^{5}-sterols as precursors to the four natural end-products—cholesterol, 24-methylenecholesterol, desmosterol and fucosterol. 24-Iminolanosterol (the synthesis of which will be described) failed to inhibit alkylation in this fungus, however in other mycelial fungi e.g., *Gibberella fujikuroi*, alkylation can be repressed. The inability for 24-iminolanosterol to affect the alkylation mechanism in *S. ferax* is assumed to be related to sequence and substrate specificity. The importance of these findings to sterol biosynthesis and the fungal disease cycle is discussed.

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STUDY ON THE HALF-LIFE OF THE TERMINAL CATABOLITE OF E PROSTAGLANDINS IN STORED HUMAN URINE. Aldo Ferretti, USDA, Beltsville Human Nutrition Research Center, Bldg. 308, Beltsville, MD 20705.

The terminal catabolite of E prostaglandins (PGE-M) is an important urinary parameter to monitor the aggregate systemic production of prostaglandins E_1 and E_2 in the human. A GC-MS-SIM method for PGE-M quantification developed in our laboratory is based on the use of the diethyl ester of the metabolite as the internal standard (IS). Because the decay rate of this IS cannot be assumed to be identical to that of PGE-M, the half-life of both species must be determined so that the necessary data adjustments can be made when urine specimens are stored for a prolonged time, e.g., during large clinical studies. Our study shows that at -22 C PGE-M decays according to the following concentration-time relationship: $\ln c = \ln c_0 - 0.00305t$ (half-life 227 days). The decay equation for the diethyl ester is $\ln c = \ln c_0 - 0.00230t$ (half-life 301 days). On the basis of these findings, it can be readily shown that if the storage time is less than 60 days, data adjustments for the difference in decay rates can be omitted, the consequent error being less than 4.4%.

Meetings

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WIND-DRIVEN *ASPERGILLUS FLAVUS* INOCULATION OF COTTON BOLLS IN ARIZONA. L.S. Lee and L.V. Lee Jr., USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179, and T.E. Russell, University of Arizona, Phoenix, AZ.

Aflatoxin in Arizona cotton can follow *Aspergillus flavus* infection of seed in bolls that apparently are not insect damaged. Since *A. flavus* propagules in soil increase dramatically in August, and this is also the monsoon season when high winds blow soil particles prior to heavy rains, we investigated inoculation by fungal propagules in wind-driven soil. Spores of *A. flavus* were mixed with autoclaved Arizona soil and blown by a modified commercial leaf blower at 125 mph onto cotton plants that had bolls in all stages of maturity. Half the plants were sprayed with water following inoculation. After a month all bolls were harvested and examined for bright-green-yellow fluorescence (BGYF) of lint, a property associated with *A. flavus* infection. No BGYF bolls were found on noninoculated control plants. In contrast, 5% to 17% of the bolls from inoculated plants sprayed with water exhibited some BGYF whereas only 2% to 3% of those not wetted had BGYF lint. Bolls fully fluffed at the time of inoculation were not infected but bolls that had opened in the 30-day period following inoculation were among those exhibiting BGYF. Fluorescence often followed the central placentral membrane indicating possible fungal infection through external nectaries. *A. flavus* in wind-driven soil preceding heavy August rains may be this source of inoculum.

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FORMATION OF AMMONIA DEGRADATION PRODUCTS OF AFLATOXIN B_1 IN THE ABSENCE AND PRESENCE OF COTTONSEED MEAL. L.S. Lee, M.G. Legendre and S.P. Koltun, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Degradation of aflatoxin B_1 by ammoniation is influenced by constituents of cottonseed meal. When aflatoxin B_1 alone was heated at 100 C for one to three hours in a laboratory Paar bomb with ammonium hydroxide, 5-10% B_1 remained unreacted, 10-30% was degraded to a 286 MW compound and 3-10% was converted to a 206 MW compound. In a second reaction in which aflatoxin B_1 was ammoniated in a pilot plant chamber for 30 min at 40 psi at 100 C in the absence of meal, no 286 MW compound was formed and 20% degraded aflatoxin B_1 was accounted for as the 206 MW compound. Following laboratory-scale ammoniations of cultured and naturally contaminated cottonseed meal, an average of 0.15% B_1 remained unreacted and 0.1% was converted to the 286 MW compound; no 206 MW compound was detected. Similarly, after ammoniation of naturally contaminated cottonseed meal at 100 C for 30 min at 40 psig, 0.1% aflatoxin B_1 remained unreacted and neither the 286 MW nor 206 MW compounds were detected. Mass spectral analyses confirmed the absence of these compounds in ammoniated cottonseed meal. Meal constituents must interfere with the formation of either the 286 MW or 206 MW compounds during ammoniation of aflatoxin contaminated cottonseed meal under pilot plant conditions reported.

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EFFECT OF EXPERIMENTAL DIABETES ON THE OUTFLOWS OF PROSTAGLANDINS FROM PERFUSED RAT MESENTERIC VASCULAR BED. K. Fujii, Y-S. Huang, M.S. Manku and D.F. Horrobin, Efamol Research Institute, P.O. Box 818, Kentville, Nova Scotia, Canada B4N 4H8.

It has been postulated that prostaglandins (PGs) may be involved in the pathogenesis of atherosclerosis in diabetes. This study was to examine the effect of streptozotocin-induced diabetes on PG production from the rat mesenteric vascular beds. Male SD rats weighing 200g were rendered diabetic by a single i.p. injection of 75 mg/kg body weight of streptozocin. The controls received only saline. Both control and diabetic rats were maintained on a semipurified diet supplemented with 10% safflower oil (containing 80% linoleic acid) for four weeks. The animals were anesthetized with ether. The vessels of the mesenteric bed separated from intestine were perfused with Krebs-Henseleit buffer gassed with 5% CO_2 in oxygen at a flow rate of 3 ml/min. Aliquots of the effluent were collected for 1 min at 15, 30, 60, 90, 120, 150 and 180 min after starting the perfusion. The levels of 6-keto-$PGF_{1\alpha}$ PGE_2 and TxB_2 were measured by direct radioimmunoassay. In comparison with that from the normal rats, the mesenteric outflows of 6-keto-$PGF_{1\alpha}$ and PGE_2 from diabetic rats were significantly higher, and that of TxB_2 were also increased but not statistically significant. The release patterns of three PGs were also different. The phospholipid fatty acid compositions extracted from the mesenteric vascular bed showed the levels of arachidonic acid (AA)—the immediate precursor of PGs—were not significantly affected by diabetes. Thus, increasing outflow of PGs is unlikely due to increasing synthesis or incorporation of AA. However, it is possible that the conversion of esterified AA in phospholipid of mesenteric vascular bed to PG production is increased without significantly affecting the levels of AA in mesenteric artery phospholipids.

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LIPID COMPOSITION OF CHICKPEA CULTIVARS. Frank W. Sosulski, University of Saskatchewan, Dept. of Crop Science and Plant Ecology, Saskatoon, Sask. Canada S7N 0W0, and Hamad M. Gadan, University of Baghdad.

The lipid compositions of 60 cultivars of chickpeas, *Cicer arietinum* L., grown in India, Iraq and Canada were evaluted by silicic acid fractionation and column chromatography. The average hull content of Desi cultivars was 11.6% as compared to 4.4% for cultivars of the large seeded Kabuli type. These differences in hull percentages resulted in crude fiber levels of 7.7 and 3.4%, resp. Protein contents were highly variable (18.4-25.3%) but the lipid contents were associated with location: Canada—7.3%, India—6.1% and Iraq—5.3%. Neutral lipids constituted nearly 80% of the chloroform/methanol extractable lipids with phospholipids occurring in much greater proportion, 20%, than glycolipids, 1%. The fatty acid compositions of the neutral lipids were 10% C16:0, 2% C18:0, 21% C18:1, 65% C18:2 and 3% C18:3. The phospholipids and glycolipids averaged 16% C16:0, 2% C18:0, 33% C18:1, 46% C18:2 and 3% C18:3. Relative to soybean, the lipoxygenase activities were nearly 40% for Desi cultivars and 64% for Kabuli genotypes. It appeared that, relative to other starchy legumes, storage stability and shelf-life would be a problem for processed chickpea products.

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STUDY OF THE POLYMORPHISM AND INTERSOLUBILITY OF SOME PALMITIC, STEARIC AND OLEIC TRIGLYCERIDES: PPP, PSP AND POP. V. Gibon and F. Durant, Laboratoire de Chimie Moléculaire Structurale, Département de Chimie, Facultés Universitaires Notre-Dame de la Paix, Rue de Bruxelles B-5000 Namur, Belgium, and C. Deroanne, Chaire de Technologie Agro-Alimentaire, Facultés des Sciences Agronomiques, Avenue de la Faculté B-5800 Gembloux, Belgium.

Triglycerides can crystallize into four polymorphic forms (sub-α, α,β', and β) with a particular hydrocarbonated chains packing. Structural informations are available only for the β-form (monocrystal X-ray diffraction); for the other forms, IR spectroscopy, powder X-ray diffraction, differential scanning calorimetry, and NMR spectroscopy allow us to formulate some hypothesis. Transitions can occur between those forms and are principally irreversible ($\alpha \rightarrow \beta' \rightarrow \beta$); the sub-$\alpha \Leftrightarrow \alpha$ transition seems however to be reversible. Because of close structural properties (long chains compounds), triglycerides can form mixed crystals by intersolubility. We present here some results concerning the polymorphism, the kinetic of this polymorphism, and the intersolubility of tripalmitin (PPP), 2 stearo-dipalmitin (PSP) and 2 oleo-dipalmitin (POP). These molecules differ essentially by the carbon's number (P → S) and the saturation (S → O). The organization of the hydrocarbonated chains in the more stable polymorphic forms (β' or β) has been investigated by temperature variable NMR spectroscopy. The study of the proton relaxation times T_1, $T_1\rho$, and T_2 may lead to structural informations. The kinetic of the important $\beta' \rightarrow \beta$ transition has been studied by temperature variable powder X-ray diffraction for PPP, and PPP-PSP and PPP-POP mixtures. The molecular interactions created into the mixed crystals have been explained by using the Arami's model and an empirical isolation method. The construction, by differential scanning calorimetry and X-ray powder diffraction, of

binary phase diagrams (PPP-PSP and PPP-POP) indicates the formation of solids solutions or monotectic interactions, depending from the nature of the triglycerides and the polymorphic forms.

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LIPID ANALYSIS OF PLANKTON FROM THE BENGUELA UPWELLING SYSTEM (SOUTH AFRICA). I.E. Horgan and J.A. Barrett, University of Cape Town, Department of Zoology, University of Cape Town, Private Bag Rondebosch 7700 South Africa.

During the course of an extended survey of lipid composition at different tropic levels in the Benguela region (South Africa), wild phyto- and zooplankton samples, collected in the same area, were analyzed monthly. Phytoplankton samples were either dominated by diatoms or dinoflagelates. The predominant neutral lipid (NL) was generally triacylglycerols (TAG) but unexpectedly, almost similar amounts of wax esters (WE) were detected in several samples of diatoms. The identity of WE was ascertained. WE were present in phytoplankton as 4-14% of the total lipids (TL). Differences in lipid composition between diatoms and dinoflagelates may be due to species-specificity, though seasonal variation cannot be discarded. Alkyldiacylglycerols (ADG) were detected only in a November sample of diatoms, coinciding with the highest amount of WE and the lowest of TAG of all specimens analyzed. In fact, WE were in higher proportions than TAG. This is probably associated with environmental conditions during this bloom. Zooplankton samples were dominated in general by copepods. Unlike phytoplankton, zooplankton showed more fluctuations in lipid composition during the period studied. Bioaccumulation of TL and NL by zooplankton seems indicated, following earlier peaks in phytoplankton. TAG levels fluctuated and was the most abundant NL class, except in April and May. Conversely, WE content was constant, following closely the phytoplankton trend and in April, WE levels started to increase, predominating over TAG, with concomitant presence of ADG. The data suggest that some of the zooplankton WE may be dietary.

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WATER AND LIPID AUTOXIDATION IN FOOD. Jennifer L. Kahl and E.G. Schanus, Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164-6330, and William E. Artz, Department of Food Technology, University of Illinois.

A unique relationship exists between water and lipid autoxidation in food systems. In an effort to determine the primary factors affecting lipid autoxidation in food systems, a new model system was developed, consisting of a thin film of methyl linoleate adjusted to a known water activity (A_W). Oxygen uptake was used to monitor the rate of oxidation. The oxidation of pure methyl linoleate exhibited a maximum at A_W=0.3 and a minimum at A_W>0.6, counter to that observed in food systems. Previous studies have shown that the initial addition of water to an oxidizing lipid system accelerated oxidation through solvation and stabilization of the propagation transition state. Further addition of water beyond the maximum slowed the rate through solvation of the peroxy radical, which in then sterically hindered from entering a propagation reaction. The addition of cobalt (1ppm) to the model system resulted in maximum rates at A_W<0.3 and A_W>0.7 with a minimum between A_W 0.3-0.7, as observed in food systems. Cobalt accelerated oxidation at low and high A_W and had no effect on oxidation at intermediate A_W. Therefore, the rapid oxidation observed at low and high A_W in food systems cannot be attributed to the effect of water alone.

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EFFECTS OF DIETARY GEOMETRICAL FATTY ACID ISOMERS ON MITOCHONDRIAL COMPOSITION AND FUNCTION. Remi De Schrijver, University of Ghent, 19, Heidestraat, 9220 Merelbeke, Belgium, Orville S. Privett, Fred C. Phillips and Warren L. Erdahl, The Hormel Institute, University of Minnesota, 801, 16th Avenue, N.E., Austin, MN 55912, and Robert W. Anderson, Hennepin County Medical Center, 701 Park Ave. S., Minneapolis, MN 55415.

In previous experiments, it was shown that EFA-sufficient diets containing 1.0% or 2.5% linolelaidate reduced hepatic oxidative function, whereas dietary elaidate did not. We have extended the study to find out whether mitochondrial lipid composition (especially cardiolipin) of liver and heart could be related to the effects observed. Two groups of rats were fed a lab chow diet to which 2.5% elaidate or 2.5% linolelaidate was added. Reference groups were fed the lab chow diet with 2.5% supplemented oleate or linoleate, respectively. Quantitative analysis of the mitochondrial lipid classes was performed by HPLC-FID. As compared to the linoleate-group, the animals fed the supplemented oleate, elaidate or linolelaidate diets showed elevated levels of hepatic cardiolipin, whereas this effect was not observed in the heart. Contrary to phosphatidylcholine and phosphatidylethanolamine, the incorporation of elaidate and linolelaidate into cardiolipin was remarkably low (<2%). A parallelism was observed especially between linolelaidate feeding and reduced linoleate content of cardiolipin. Examination of mitochondria by means of electron microscopy did not reveal any as related to membrane lipid composition will be discussed.

SESSION OO Gas Chromatography in the Measurement of Food Safety Thursday morning

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ANALYSIS OF TRACE AMOUNTS OF GEOSMIN IN WATER. H.P. Dupuy and G.L. Glic, Virginia Polytechnic Institute and State University, Food Science Department, and A.J. St. Angelo and R.L. Ory, USDA Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Trace amounts of geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol) were concentrated from relatively large volumes of water by vegetable oil extraction. After stirring the two phases for one hour, the dispersed oil was allowed to separate. The oily layer was removed and centrifuged to break the emulsion and separate the two layers. The direct gas chromatographic technique was used to resolve the geosmin from other volatile components on a carbowax 20 M column. Volatiles were eluted from the oil by securing an aliquot of the oil layer on volatile-free glass wool in the glass liner of the special GC inlet system. Geosmin was determined at the parts per billion level with this simple and rapid technique.

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EVALUATION OF EDIBLE OIL ADDITIVES BY VOLATILE PROFILE ANALYSIS. Joseph L. Gensic, Endre F. Sipos and Bernard F. Szuhaj, Central Soya Co., Inc., Box 1400, Fort Wayne, IN 46801.

A direct gas chromatographic analysis for volatile compounds was evaluated for analysis of edible oil additives and potential contaminants. The gas chromatographic system employed an external inlet block and automated valve for unattended operation. Compounds analyzed included antioxidants, BHA, BHT and TBHQ; heat exchanger fluids, Dowtherm A, Therminol-55 and Therminol-66, and residual hexane. Effects of modified inlet block conditions were studied to access operating limits. Utilization of the direct gas chromatographic analysis system in a routine QC environment is discussed.

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VOLATILE FLAVOR COMPONENTS IN PASTEURIZED SEAFOODS. Stephen S. Williams, Gloria M. Rodriguez, Cameron R. Hackney, Carmen A.O. Pantoja, Stanley L. Biede and Darrell L. Gerdes, Louisiana State University, Department of Food Science, Baton Rouge, LA 70803.

Volatile flavor components of shrimp, crabmeat and crayfish pasteurized at equivalent lethalities in flexible retort pouches and aluminum cans were compared after 0, 3, 6 and 9 weeks of storage. Volatile contents were analyzed by direct gas chromatography injection and mass spectrometry. Changes in the volatile components were used to predict an optimum pasteurization process. A high correlation between volatile profiles and flavor scores demonstrated the usefulness of volatile "fingerprinting" by direct gas chromatographic injection. Seafood lipids, characterized by their high percentage of polyunsaturated fatty acids, undergo deteriorative

reactions during processing and storage which adversely affect seafood flavor. Lipid analysis was performed to determine the contribution of fatty acids to flavor in these pasteurized products as lipids significantly affect the sensory quality attributes in seafoods.

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A RAPID METHOD FOR DETERMINING RESIDUES OF FUMIGANTS IN FOODSTUFFS WITHOUT SAMPLE PREPARATION. Esmeralda Baptiste and Joseph H. Ford, Natl. Monitoring & Residue Analysis Laboratory, U.S. Department of Agriculture, 3505 25th Ave., P.O. Box 3209, Gulfport, MI 39505-3209.

The External Closed Inlet Device (ECID) is being used at the National Monitoring and Residue Analysis Laboratory (NMRAL) for the rapid screening of fumigants and other pesticides in a variety of sample matrices. This technique has proven invaluable for the determination of residues of the more volatile fumigants such as methyl bromide. The ECID has eliminated sample handling and other time consuming techniques for the detection of residues of fumigants.

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SOME FACTORS IN THE DIRECT DETERMINATION OF VOLATILE PROFILE BY GAS LIQUID CHROMATOGRAPHY TO MEASURE PEANUT QUALITY. Norman V. Lovegren, USDA Southern Regional Research Center, 1100 Robert E. Lee Blvd., P.O. Box 19687, New Orleans, LA 70179.

The direct determination of volatile profile is compared with concentration and head space methods. Some factors discussed are stripping temperature, carrier gas flow, moisture content of the sample and sample preparation. The range and types of volatiles present in normal and unacceptable peanut samples and typical volatile profiles are given. The more useful peaks for prediction of quality are identified. Another valuable use of this direct volatile profile method is to measure changes in the peanut sample before and after various treatments such as storage under good and poor conditions, frost damage, blancing and roasting. Rates of increase of lipid oxidation products may be measured when the concentration of oxidation products is still well below the threshold of taste.

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APPLICATIONS OF HEADSPACE GAS CHROMATOGRAPHY TO FOODS AND INGREDIENTS. Edwin L. Anderson and Thomas W. Gaylord, Kellogg Company, Chemistry Department, 235 Porter St., Battle Creek, MI 49016.

High resolution fused silica gas chromatography/mass spectrometry is a powerful tool for characterization of flavors and aromas in food systems. The method of sample introduction into the chromatographic system is a critical parameter of the analysis. Three sample introduction methods will be compared and contrasted: static headspace, dynamic headspace and the Dupuy direct sampling method. Each method has unique strengths and limitations and each serves as a viable tool in the chromatographer's arsenal. Practical application of each method will be shown for analysis of food flavors.

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DIRECT GAS CHROMATOGRAPHIC ANALYSIS OF FLAVORS IN BROWN SUGARS AND MOLASSES. M.A. Godshall, Sugar Processing Research, Inc., USDA Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

The direct gas chromatographic method (Dupuy inlet) has been used to evaluate volatile components in sguar products as part of an integrated research program to determine flavor quality in brown sugar. The versatility of the inlet has provided information on the source of targeted components and allowed their quantitation. Important volatile materials that originate in the sugar cane plant, such as dimethyl sulfide, have been followed through to the final molasses product. Other compounds, such as acetic acid and diacetyl, were found to have their origins in the refinery. Certain volatiles in brown sugar profiles could be related to sugar quality and to the manufacturing process of the sugar.

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SAMPLING TECHNIQUES FOR ENHANCEMENT OF FOOD FLAVOR COMPONENT YIELD IN DIRECT GAS CHROMATOGRAPHY. John R. Vercellotti, V-LABS, Inc., Covington, LA 70433, and Gulf South Research Institute, P.O. Box 26518, New Orleans, LA 70186.

Drying or sterilization of food materials frequently introduces oxidative and browning products. The intermediates in these reactions are most often colorless and tasteless. In order to sample flavor volatiles and quality in the aromas of products such as cocoa or chocolate, whey solids, milk, cheese or cooked eggs, a new techniques has been devised based on the Legendre-Dupuy external inlet. Dynamic headspace chromatography or direct gas chromatography with heated external inlet on such products risks introducing extraneous flavor peaks not actually in the aroma. In the modified device the sample (1-15 g) is placed in a 500 cc glass pressure vessel with an equal quantity of water, the bottle wrapped with a heating blanket, and the top closed with a rubber stopper through which two 1/16-inch stainless steel tubes and fittings are pressed. A Parr hydrogenation apparatus clamp serves well to effect secure closure over the rubber stopper. One 1/16-inch tube is attached to the carrier gas inlet with the second tube as exit to the trap column. The sample is then heated at the desired temperature (e.g., 80 C) with occasional manual shaking and the headspace continually purged for 0.5 to 1 hr onto a cold column of Porapak P or Q, or Tenax GC with 8% polymetaphenyl ether. Volatile peaks are then separated by temperature programming the column from 50 C. With this technique dramatic differences in aroma volatile profiles result, compared to simply packing the sample in the Legendre-Dupuy glass cartridge and purging the heated sample in inert gas. Much less total organic volatile results from the headspace aroma in the pressure vessel but certain distinct peaks are detected. From chocolate so analyzed, gas chromatographic peaks corresponding to retention times of isobutyraldehyde, isovaleraldehyde, methylfuran and diacetyl were prominent. Eggs freshly scrambled in a Teflon frying pan without vegetable oil likewise yielded ethanol, butyraldehyde, isobutyraldehyde, valeraldehyde and phenylacetaldehyde. A good quality dried whey in aqueous suspension possessed as the principal aroma volatiles acetone, isobutyraldehyde, diacetyl, isovaleraldehyde, 2,3-pentanedione, octane, hexanal, furfural (largest peak) and 2-acetyl furan. The furfural is diagnostic of glycosylamine or furosine linkages in the whey. Using conventional Legendre-Dupuy technique, it also has been found that for routine residual volatiles such as hexane, or other solvents, codistillation with two to three times the quantity of water to sample injected throughout the solid (e.g., 500 mg H_2O with 250 mg sample at 140 C for 25 min) improves residual volatile recovery in meals, flours, flakes and industrial hydrocolloids such as guar, xanthan and many substituted polysaccharides. Similarly, by the same technique plastic packaging materials on foodstuffs which they contact also can be tested for residual volatiles as potentially offensive off-flavors.

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THE USE OF DIRECT GAS CHROMATOGRAPHY TO MONITOR FRUIT QUALITY. Stanley L. Biede, Ricardo Fuenmayor and Nahida Saker, Louisiana Agricultural Experiment Station, Department of Food Science, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, and Roysell Constantin, Hammond Research Station.

Cultivars of fresh strawberries, blueberries and dewberries were harvested twice weekly during the 1983 and 1984 harvesting period. Titratable acidity and dissolved solids were determined at the time of harvest and the remaining samples were quick frozen and stored at −20 C. Direct gas chromatography was used to determine the volatile composition of the berries. Tubes were prepared using 0.1-0.5 g of frozen berries and approximately 0.5 g of sodium sulfate to prevent liquid from entering into the system. Inlet temperature was set at 75 C to prevent caramelization of the sugars. Volatiles were purged onto the inlet end of a cold 2.7 meter 10% poly MPE on Tenax column for 30 min. Column temperature was then raised to 75 C in 2 min and then programmed to 200 C at 2 C/min. Effluent from the column was split 1:1 with one part going to a sniffing and sampling port while the other part went to a

flame ionization detector. Volatile patterns were then used to determine differences between cultivars and harvesting period. Results indicated the harvesting period has a greater influence on the presence of volatiles than does cultivar. Sugar and organic acid content were measured on the stored samples. Results indicated that both sugar content and organic acid content varied significantly during the harvesting period as well as between cultivars of specific berry type. Berries picked early in the harvesting period had significantly less sugar and higher organic acid content than those picked during the later stages of harvest.

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APPLICATION OF THE DIRECT SAMPLE INJECT GAS CHROMATOGRAPHIC METHODS FOR SEAFOODS AND FRYING OILS. G.J. Glick Jr., Virginia Polytechnic Institute and University, Blacksburg, VA.

Abstract not available at press time.

SESSION PP Surfactants and Detergents IV: Dispersions Thursday morning

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THE EFFECT OF CARBOXYLIC ACID ON 2-ETHYL HEXYL ACRYLATE AND STYRENE LATEX PARTICLES. F.V. Loncar, M.S. El Aasser and J.W. Vanderhoff, Lehigh University, Bethlehem, PA.

Abstract not available at press time.

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ELECTROKINETIC PROPERTIES OF POLYMER COLLOIDS IN AQUEOUS AND NON-AQUEOUS MEDIA. F.S. Micale, C.M. Ma, R.V. Mann, R.S. Ells and B. Baumart, Lehigh University, Bethlehem, PA.

Abstract not available at press time.

SESSION QQ High Performance Liquid Chromatography of Proteins Thursday morning

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REVIEW OF PROTEIN SEPARATION BY REVERSED-PHASE LIQUID CHROMATOGRAPHY. Alan P. Goldberg, E. I. du Pont de Nemours & Co., Biomedical Products Department, Quillen Building—Concord Plaza, Wilmington, DE 19898.

The usefulness of reversed-phase chromatography (RPC) for the separation of small molecules is well known; so much so that approximately 75% of all liquid chromatographic separations utilize reversed phase. Among the advantages of RPC are rapid equilibration, ease of methods development and column stability. Recently these same features have been applied to macromolecules, specifically peptides and proteins. A variety of mechanisms and approaches to the reversed-phase separation of these materials have been proposed. There seems to be, however, significant differences between these suggested methodologies, so much so that the average practitioner is often left to "reinvent the wheel" when it comes to developing separations of his or her own materials. In this review, we will attempt to put into perspective the often conflicting variables which can be adjusted to effect the desired separations. These variables include column pore size and bonded phase structure, mobile phase constituents, gradient steepness and flow rate. The effectiveness of these to enhance peak capacity (resolution) and sensitivity will be discussed. Also, a step-by-step procedure for gradient optimization will be demonstrated using specific protein digests as an example.

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HYDROPHOBIC INTERACTION HPLC: A REVIEW. Steven C. Goheen, Bio-Rad Laboratories, 2200 Wright Ave., Richmond, CA 94804.

Hydrophobic interaction chromatography (HIC) is a method that separates macromolecules according to solvophobic interactions, or essentially according to their hydrophobic nature. To accomplish this, samples are eluted in a descending salt gradient often starting with 1-3 M ammonium sulfate. At the higher concentration of salt, compounds adsorb to the hydrophobic ligands in the column. As the salt concentration is lowered, macromolecules desorb from the matrix and separate according to their hydrophobicity. Water soluble proteins can generally be separated in this manner, but those which are strongly hydrophobic require special procedures for elution. These include the use of either organic solvents or detergents as modifiers. When salts alone are used for elution, recoveries and activities of enzymes are generally 90-100%, but the use of modifiers can potentially influence both recovery and enzyme activity. Column selectivity can be influenced by elution time, flow rate, column temperature, and the eluting solvent composition. Some columns designed for hydrophobic interaction chromatography can also be used as if they were reversed phase columns. Under these operating conditions, up to 100% acetonitrile and 0.05% trifluoroacetic acid can be used. An advantage to using hydrophobic interaction columns in reversed phase chromatography is that proteins are often eluted at lower solvent concentrations and in many cases, recoveries are improved. Hydrophobic interaction chromatography columns are commercially available with silica based or hydroxylated polyether based materials. Separations are similar with either of these columns, but there are some advantages and disadvantages of each. Some chromatograms will be presented to accentuate the features of hydrophobic interaction HPLC of proteins.

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MAJOR FACTORS INFLUENCING RESOLUTION IN THE HIGH PERFORMANCE ION EXCHANGE CHROMATOGRAPHY OF PROTEINS. Lawrence A. Haff, Waters Chromatography Division of Millipore Corporation, Maple St., Milford, MA 01757.

Recently, ion exchange resins designed for the high-performance ion exchange liquid chromatography of proteins have been developed. Many of these packings are silica-based. While these packings produce high resolution, they are often limited in lifetime due to alkaline hydrolysis of the silica base. Polymer-based ion exchangers such as Protein-Pak DEAE and SP can be used for anion exchange and cation exchange chromatography of proteins, respectively without any of the disadvantages of the silica-based products. Such packings must have extremely high exclusion limits, over about 10^6 daltons for linear polysaccharides and greater than 10^7 daltons for globular proteins, to minimize gel permeation effects allowing for "pure" ion exchange to take place. Because polymeric materials are pH stable, separations in the pH range from 2-12 are possible. Some biological extracts, such as venoms, contain proteins with high isoelectric points. Such proteins can only be separated on polymeric supports at high pH. It is generally better to use packings containing high, uniform distributions of anion or cation exchange groups with extreme pKa's. Generally, the chromatographic behavior of complex mixtures of proteins can be more easily determined using these "strong" exchangers than "weak" exchangers because over a wide range of pH only the charge of the proteins varies, and not the charge on the column packings. The high resolution of these packings can be demonstrated by separations such as those of hemoglobin varients, in which complete separations of proteins differing in only one amino acid substitution can be effected in a few minutes. Conditions for separation hemoglobins A,C,S, Alc and F have been determined using cation exchange. A major benefit of high speed separations is the ability to recover greater yields of biological activity of labile proteins than obtained with slower, traditional soft gel techniques. The primary influence upon resolution was found to be separation time, buffer pH and ionic strength. Column length is a small factor in resolution of biological polymers.

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ANALYSIS OF APOPROTEIN OF SERUM LIPOPROTEIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. I. Hara

and M. Okazaki, Scientific Instrument Division, Toyo Soda Mfg. Co., Hayakawa, Ayaseshi, Kanagawa, Japan 252; and M. Okazaki, Toyko Medical and Dental University, Kohnodai, Ichikawashi, Chiba, Japan 272.

Serum lipoprotein in general, contains several kinds of apoproteins. The apoproteins in urea solution prepared from delipidated HDL or VLDL are examined by HPLC procedure. This procedure includes delipidation process. A simple and rapid method for apoprotein analysis is performed by HPLC using aqueous gel permeation column (TSK GEL G3000SW). Native HDL fraction is treated with 0.1 M phosphate buffer solution (pH 7.0) containing 0.1% SDS at 60 C for 5 minutes and applied onto G3000SW. By monitoring the absorbance at 280 nm. The clearly separated patterns of apoproteins, apo E,apo A-I,apo A-II and apo C mixture are observed in this order. The patterns using G4000SW or G2000SW are not so clear as G3000SW. The elution patterns of native HDL treated with phosphate buffer containing SDS do not coincide with those by protein moiety. This means that lipid components, cholesterol and phospholipid form micelle solution giving different elution volume from protein portion.

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SIZE-EXCLUSION CHROMATOGRAPHY OF PROTEINS AND PEPTIDES. Paul E. Antle, E. I. Du Pont de Nemours & Co., Biomedical Products Department, Quillen Building–Concord Plaza, Wilmington, DE 19898.

This presentation will introduce the technique of size-exclusion chromatography (also known as gel filtration or gel permeation) and present applications of this technique for the separation of protein and peptide samples. Size-exclusion chromatography (SEC) performs separations on the basis of molecular size. The penetration of molecules into the porous structure of the stationary phase provides the sample retention. Smaller molecules spend a greater amount of time in the pores and elute later than larger molecules. Solutes differing significantly in molecular size can be separated, or isolated, and, in many cases, the approximate molecular weights of components in the sample can be determined. With this technique, adsorptive and partitioning interactions between the sample and the stationary phase must be eliminated. The primary factor controlling SEC separations is the porosity of the stationary phase. The advantages and disadvantages of this technique follow directly from its mechanism. The technique is quite simple to use, but the selectivity is limited. The entire chromatogram is finished in a single column volume. This makes the run fast and predictable, but limits the number of peaks which can be resolved. Traditionally, this technique utilized organic polymer beads as the stationary phase. More recently the introduction of more rigid, mechanically stable stationary phases has cut the time required for an average SEC run from 12 hours to 12 minutes. Early applications of rigid-bead technology to separations of proteins and peptides revealed problems with denaturation, low recovery and/or poor column stability. Improved surface modification procedures have been developed to solve these problems.

SESSION RR Protein Quality
Thursday morning

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PROTEIN QUALITY AND REQUIREMENTS: TWO SIDES TO ONE PROBLEM. R.D. Phillips, Department of Food Science, The University of Georgia College of Agriculture, Experiment Station, GA.

Abstract not available at press time.

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PROTEIN BIOUTILIZATION AS A FACTOR IN DETERMINING FOOD PROTEIN QUALITY. Constance Kies, Dept. of Human Nutrition and Food Service Management, University of Nebraska - 316 Ruth Leverton Hall, Lincoln, NE 68583-0807.

If quantitative and qualitative amino acid requirements of humans were well established, protein quality of any food source could easily be established in vitro by simple comparison of analytical values of amino acid proportionality patterns of the food protein with standards of excellence based on human amino acid requirement patterns. For several reasons, these theoretically computed evaluations of protein quality and in vivo evaluations using human subjects do not always yield similar results. Protein bioutilization is one factor contributing to this lack of agreement. Maximal protein utilization involves protein digestibility, efficient use of mechanisms for absorption of di- and tripeptides as well as for simple amino acids, avoidance of competition for amino acid absorption sites, and timing of absorption of individual amino acids for use within the body. Non-protein components of the test food or diet may be influential in determining protein bioutilization as well as physiological characteristics of the person consuming the protein. Development of parenteral and enteral formula diets for use by patients has created a need for better understanding of protein utilization as a factor in protein quality.

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ABSORPTION OF PRODUCTS OF PROTEIN DIGESTION IN HUMAN INTESTINE. Siamak A. Adibi, Montefiore Hospital, 3459 Fifth Ave., Pittsburgh, PA 15213.

Studies within the past decade have shown that the products of protein digestion in the gut lumen are amino acids, and to a greater extent, oligopeptides. An important function of the brush-border membrane of the intestinal mucosa is to absorb these products. This function is achieved by uptake of amino acids by neutral and basic amino acid transport systems, and uptake of dipeptides and tripeptides by a peptide transport system. The peptide transport system appears to be more efficient than any of the amino acid transport systems as far as the absorptive function is concerned. The absorbed peptides, once inside the mucosal cell, are largely hydrolyzed by cytoplasmic peptide hydrolases to free amino acids. Peptides with more than three amino acid residues are not substrates for the peptide transport system, but are converted to absorbable products by the action of brush-border peptide hydrolases. Unlike cytoplasmic peptide hydrolases which are active only against di- and tripeptides, the brush-border peptide hydrolases can hydrolyze oligopeptides with as many as eight amino acid residues. This coordinated function between transport and hydrolytic systems allows a highly efficient assimilation of dietary proteins in the intestine. In patients with pancreatic insufficiency the luminal digestion of protein is reduced resulting in increased loss of nitrogen in the stool. In patients with mucosal disease the intestinal assimilation of amino acids and oligopeptides is reduced also resulting in increased loss of nitrogen in the stool. In all these patients dipeptides and tripeptides are superior to either intact proteins or free amino acids as substrates for enteral nutrition.

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AMINO ACID OXIDATION TO STUDY PROTEIN AND AMINO ACID REQUIREMENTS. Henry S. Bayley, Department of Nutrition, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Amino acids absorbed from the digestive tract can be used either as fuels, or for protein synthesis. Their partition between these two functions depends on the maturity and energy status of the animal, and on the nutrient balance of the diet. In a well balanced diet the use of amino acids as fuels is minimized; it is the objective of nutritionists to define the intake of each nutrient to provide such a well balanced diet for growing animals. Utilization of dietary amino acids for protein deposition has been studied measuring nitrogen balance, and more recently monitoring the fate of the carbon moiety by measuring $^{14}CO_2$ production from labeled amino acids. Incrementing the dietary intake of an amino acid from deficient to sufficient results in no change in $^{14}CO_2$ release as long as the amino acid being studied is first limiting in the diet. Increasing the dietary level from sufficient to excess results in increasing production of $^{14}CO_2$, signifying an increasing use of the amino acid as a fuel. Experiments such as this have allowed definition of the dietary level of lysine needed to maximize its use for protein deposition in rats, sheep and pigs. Feeding graded levels of the amino acid being studied results in a change in pool size necessitating estimates of pool size and assumptions about the appropriate model to use in

analyzing the experimental data. The need for these assumptions can be minimized by using a second amino acid, to indicate the effects of changing the dietary intake of the amino acid being studied on the metabolic fate of all the other amino acids. L-[1-^{14}C] phenylalanine is a suitable indicator amino acid: it is predominantly oxidized in the liver, the carboxyl carbon is lost as carbon dioxide at the first step in its catabolism, and it is readily available from commercial suppliers. Phenylalanine oxidation is reduced when the level of lysine (or any other essential amino acid) is increased from deficient to sufficient in a series of experimental diets. Increasing the lysine level from sufficient to excess results in no further change in the oxidation of the phenylalanine. This technique measures the metabolic response to dietary manipulation directly, and it has been shown that the animal responds to the dietary change rapidly. It has been possible to estimate the dietary requirement for a number of essential amino acids and total protein to maximize their retention in young pigs in the first three weeks of life.

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APPROACHES TO PROTEIN QUALITY EVALUATION USING AMINO ACID DATA. C.E. Bodwell, Chief, Energy and Protein Nutrition Lab, Room 214, Building 308, BHNRC, BARC-E, Beltsville, MD 20705.

The official U.S. method for protein nutritional quality evaluation for regulatory purposes is the Protein Efficiency Ratio rat growth assay. The requirements of the growing rat for essential amino acids, and in particular for total sulfur amino acids, are different than those of humans of all ages (except for the human infant). Thus, an evaluation based on the level of essential amino acids in a protein source or upon one or more amino acids which may be indicative of protein nutritional value might be useful for assessing protein nutritive value more accurately than by any rat growth assay. Examples of the use of a non-essential amino acid, hydroxyproline, as an indicator of protein quality in meat, poultry and their products will be presented. Likewise, examples of the use of amino acid composition data, with or without corrections for amino acid bioavailability or nitrogen digestibility, will be discussed. Lastly, research yet needed for the validation of the use of amino acid data for regulatory purposes will be considered.

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PROTEIN QUALITY IN CLINICAL CONDITIONS: RELATIONSHIP BETWEEN PROTEIN AMINO ACID COMPOSITION AND AMINO ACID REQUIREMENTS. Lon O. Crosby, Central Laboratory, CSP #221, Room A-405-R, Surgical Research, VA Medical Center, University and Woodland Avenues, Philadelphia, PA 19104.

Protein quality, in the absence of nutrient deficiencies or amino acid imbalances, is defined by the relationship between the protein's amino acid profile and the individual's amino acid requirement. Human protein (amino acid) requirement varies substantially across and within the groups and are altered by environmental and hormonal factors. The protein requirements of normals have been estimated at 0.4-0.8 g/kg/d. The imposition of a moderately severe stress, e.g., surgery, increases the requirement 0.8-1.5 g/kg/d. Severe stress, i.e., burns or head trauma, may increase requirements to 3.4-4.3 g/kg/d. The limited number of studies available on amino acid requirements in stress suggest that the increase in protein requirement reflects non-uniform, unique changes in essential amino acid requirements rather than a uniform increase in all amino acids. This results in a real but artificially elevated estimate of protein requirement. In addition, there are genetic of environmentally induced alterations in the requirements of selected amino acids which alter the evaluation of protein quality as it relates to individuals. Clinically applicable techniques are available to rapidly determine amino acid requirements of a single individual and to define protein quality biochemically.

SESSION SS General
Thursday morning

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ANALYSIS OF THE FATS AND FATTY COMPONENTS OF NON-DAIRY IMITATION PRODUCTS. M.A. Amer, McGill University, Ste. Anne de Bellevue, Quebec, Canada, and W.J. Mullin, Agriculture Canada, Ottawa, Ontario, Canada

Abstract not available at press time.

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ADDITION OF FAT TO DAIRY CATTLE RATIONS. D. Sklan, Medical College of Pennsylvania, Philadelphia, PA, and W. Chalupa and D.S. Kronfeld, University of Pennsylvania, Philadelphia, PA.

Abstract not available at press time.

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COOXIDATION OF PLANT PIGMENTS BY NATIVE AND MODIFIED SOYBEAN TYPE-2 LIPOXYGENASE. B.P. Klein, University of Illinois, Urbana, IL, and B. Cohen, S. Grossman and A. Pinsky, Bar-Ilan University, Ramat-Gam, Israel.

Abstract not available at press time.

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THE DETERMINATION OF UNREACTED FREE AMINES IN LONG CHAIN AMINE OXIDES BY POTENTIOMETRIC TITRATION—REVISITED. James A. McDonell, Economics Laboratory Inc., 840 Sibley Memorial Highway, St. Paul, MN 55118.

Long chain amine oxides have found increasing use in many industrial and consumer products in the last decade as surfactants and emulsifiers. The amine oxides are produced commercially by oxidation of tertiary amine oxides with hydrogen peroxide. The quality control of amine oxides requires the determination of residual free amine in the presence of product amine oxide. Known methods of analysis for amine oxides include reduction, TLC, GC, polarography and nonaqueous titration. The latter is most widely used because it affords both free amine and amine oxide. A well established titration method uses a derivatization with methyl iodide with two titrations which is time consuming for process quality control. Bezinger has reported a one step nonaqueous titration method with no derivatization. Two equivalence points are produced due to "anomalous" salts. One of these is due to free amine plus half the amine oxide and the other break is due to the other half amine oxide. Metcalf and Wang expanded Bezinger's work to determine limitations and set up a modified solvent and titrant system to rapidly and accurately determine long chain amines and their oxides in MEK or acetonitrile. Metcalf and Wang report equivalence point assignments opposite to that of Bezinger. We have found the assignments to be solvent dependent and present this and related data on the titrations.

SESSION TT Physical and Organic Aspects of Fatty Acids and Derivatives
Thursday morning

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EFFECTS OF IONIZING RADIATION ON LIPIDS IN MONOLAYERS. Lung-Bin Hau and W.W. Nawar, Department of Food Science & Nutrition, University of Massachusetts, Amherst, MA 01003.

In order to study the effect of molecular orientation on the behavior of lipids when exposed to high-energy radiation, model systems of palmitic acid or ethyl palmitate adsorbed on silica were irradiated with ^{60}Co at 25 Mrad under vacuum, and the volatile products compared with those of control samples irradiated in bulk. Qualitative and quantitative analyses were accomplished by combination GC/MS. Striking quantitative differences were observed. The monolayer samples produced more of the shorter-chain alkanes than the bulk samples. In contrast, more of the Cn-1 alkane relative to the shorter-chain homologs was formed in the bulk samples. The Cn-2 alkene and Cn aldehyde also were formed in greater quantities in bulk. These observations are explained on the basis of a reduced perferential cleavage near the Carbonyl group in the case of monolayers. Hydrogen bonding and the relative mobility of certain free radical intermediates appear to be important factors.

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PHASE TRANSFORMATIONS AND CRYSTALLIZATION BEHAVIORS OF NEW POLYMORPHS OF ULTRA-PURE OLEIC ACID. Masao Suzuki, T. Ogaki, Research Laboratory, Nippon Oil & Fats Co., No. 56, 1-Chome, Ohama-Cho, Amagasaki-City, 660 Japan, and Kiyotaka Sato, Faculty of Applied Biological Science, Hiroshima University, Japan.

New polymorphic modifications of ultra-pure (>99.9%) oleic acid were investigated by X-ray powder diffractometry and DSC at temperatures ranging from −20 to 20 C. X-ray diffraction spectra revealed three different polymorphs named α, β and γ, which differs from each other most significantly in the short-spacing spectra. DSC analyses have clarified the transformation as well as the crystallization processes of three polymorphs. A first-order solid state transformation was found to occur between α and γ at −2.6 C on heating. No direct transformation between β-α and β-γ was detectable. The melting temperatures of α and β were 13.1 and 16.2 C. The crystallization behaviors were strongly dependent on the thermal treatment. A rapid cooling always yielded α (higher temperature) and γ (lower temperature). The β crystal only crystallized at extremely low crystallization rates when the temperature was kept between two melting points of α and β. The enthalpies and entropies of the melting (α and β) and of the transformation (α-γ) will be shown. The thermodynamic stability among α, β and γ was also evaluated by measuring each solubility at the above range of temperatures. β is the most stable form, while α and γ are metastable. It was inferred that the kinetic effects on the crystallization processes may favor the occurrence of α and γ.

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HIGH RESOLUTION PULSED NMR IN THE STUDY OF FATTY AMINE REACTIONS. Edward H. Fairchild and Floyd E. Friedli, Sherex Chemical Company Inc., 5777 Frantz Rd., Dublin, OH 43017.

Our ongoing study of fatty amine chemistry has relied extensively on NMR spectroscopy. Various pulse techniques now allow the spectroscopist to obtain a great deal of structural information on components of an ongoing chemical reaction, without purturbing the reaction mixture in any way. A survey of many of these methods, using proton, ^{13}C and ^{15}N NMR techniques, will be accompanied by examples obtained in fatty amine studies. Novel branched amines were produced during nitrile to secondary amine conversions. The structures of these products were characterized by NMR and will be discussed.

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REACTION OF FATTY ACIDS WITH 3,3′-IMINOBIS-PROPYLAMINE, Raymond G. Bistline Jr., Warner M. Linfield, William B. Wise and Philip E. Pfeffer, Eastern Regional Research Center, Philadelphia, PA 19118.

Reaction between two moles of fatty acid and one of 3,3′-iminobis-propylamine is somewhat analogous to the previously reported reaction between fatty acid and diethylenetriamine, but there are significant differences. Amidation to the diamide (I) $HN(CH_2CH_2CH_2NHCOR)_2$ proceeds much more rapidly than the analogous reaction of fatty acid with diethylenetriamine. The diamide (I) is obtained in only about a 60% yield and by-products are obtained whereas the reaction with diethylenetriamine gives an almost quantitative yield of diamide. Cyclization to the pyrimidine (II) $RCONHCH_2CH_2CH_2NCH_2CH_2CH_2N{=}CR$ is extremely slow and incomplete, in contrast to the analogous imidazoline which is obtained almost quantitatively. The AOCS wet method for amine titration gives erroneous structural information, as in the case of the diethylenetriamine reaction product. Whereas ^{13}C NMR indicates that diamide (I) is a symmetrical compound and thus a secondary amine, the AOCS titration always indicates the presence predominantly of primary amine. The diamide forms a Schiff base with salicylaldehyde, which would indicate that acyl migration had taken place. The water repellency of soils treated with a homologous series of diamides (I) and pyrimidines (II) was studied, and it was found that there is essentially no difference between the behavior of this series and the previously reported analogous one from diethylenetriamine.

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TRANSESTERIFICATION KINETICS OF SOYBEAN OIL. B. Freedman, R.O. Butterfield and E.H. Pryde, USDA Northern Regional Research Center, 1815 N. University St., Peoria, IL 61604.

Transesterification of soybean oil and other triglycerides (TG) with alcohols (ROH), in the presence of a catalyst, yields fatty esters (R^1CO_2R) and glycerol ($C_3H_8O_3$). The reaction proceeds by reversible steps. Specific rate constants, k, have been determined for each reaction with a computerized kinetic program. The rates of the forward reactions are considerably greater than those of the reverse reactions. Alkali-catalyzed reactions with methanol and butanol were studied at 20, 30, 40, 50 and 60 C. Acid-catalyzed reactions with methanol and butanol were studied at 20, 30, 40, 50 and 60 C. Acid-catalyzed reactions with butanol were examined at 77, 87, 97, 107 and 117 C. Energy of activation was determined for each reaction from plots of log k vs. 1/T. For example, these activation energies were 13-16 kilocalories for the pseudo first order reactions at a molar ratio of butanol/soybean oil of 30:1, with 1% sulfuric acid catalyst at 77-117 C.

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PREPARATION OF METHYL 8*c*,11*c*-OCTADECADIENOATE-17,17,18,18-d_4 AND METHYL 8*c*,11*c*,14*c*-OCTADECATRIENOATE-17,17,18,18-d_4. R.O. Adlof and E.A. Emken, USDA Northern Regional Research Center, 1815 N. University Street, Peoria, IL 61604.

Multigram quantities of the all-*cis* dienoic and trienoic esters were synthesized for use in human metabolism studies. The 8*c*,11*c*-octadecadienoic-d_4 ester (>97% d_4) was synthesized in 8 steps (14% overall yield) and began with the catalytic deutration of the tetrahydrophranyl (THP) ether of 5-hexyne-1-ol by tris (triphenylphosphine) chlororhodium (I) catalyst. The THP ether was converted to the iodide, coupled to 3-butyne-1-ol to yield 3-decyne-1-ol-d_4 and reduced to the olefin using Lindlar's catalyst. The alcohol was next converted to the bromide via PH_3PBr_2 and the triphenylphosphonium bromide salt was prepared. The double bond in the $\Delta 8$ position was obtained by coupling of the phosphonium salt and 7-formylheptanoate, which was prepared by ozonolysis of cyclooctene. The 8*c*,11*c*,14*c*-octadecatrienoic-d_4 ester (>96% d_4) required 11 steps to synthesize (10% overall yield) and began with the catalytic deuteration of the THP ether of propargyl alcohol, hydrolysis to the alcohol and conversion to the iodide by H_3PO_4/KI. The iodopropane-d_4 was coupled to propargyl alcohol to obtain 2-hexyne-1-ol-d_4. the bromide of the alcohol was coupled to 3-butyne-1-ol to yield 3,6-decadiyne-1-ol and reduced to the diene via Lindlar catalysis. The alcohol was next converted to the bromide and the triphenylphosphonium bromide salt was prepared. Again, the double bond in the $\Delta 8$ position was formed by the Wittig coupling of this compound with 7-formylheptanoate. The final products contained 5 to 15% *trans* isomers which were removed by silver resin chromatography.

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SYNTHESES OF DEUTERATED METHYL 9,15-OCTADECADIENOATE AND METHYL 9,12,15-OCTADECATRIENOATE GEOMETRIC ISOMERS. Henry Rakoff, USDA Northern Regional Research Center, 1815 N. University St., Peoria, IL 61604.

Methyl 9,15-Octadecadienoate-6,7-d_2 isomers were obtained by Wittig coupling between methyl 9-oxononanoate-6,7-d_2(1) and 6-nonenyltriphenylphosphonium bromide(2). Compound 2 was prepared from methyl 6-nonenoate(3) by reduction with sodium bis(2-methoxyethoxy) aluminum hydride, bromination with triphenylphosphine dibromide and further reaction with triphenylphosphine. Synthesis of compound 3 was achieved by Wittig coupling between propyltriphenylphosphonium bromide and methyl 6-oxohexanoate(4) which was obtained by ozonolysis of cyclohexene. Compound 1 was prepared through the dimethoxy derivative from methyl 8-dioxanyloctanoate-6,7-d_2 which was obtained by deuteration of methyl 8-dioxanyl-6-octenoate(5) with tris-

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(triphenylphosphine)-chlororhodium and deuterium gas. Compound 5 was obtained from compound 4 by chain extension with [2-(1,3-dioxan-2-yl)ethyl] triphenylphosphoium bromide(6). Chain extension of 1 with 6 yielded methyl 11-dioxanyl-9-undecenoate-6,7-d_2. This was converted to methyl 12-oxo-9-dodecenoate-6,7-d_2 which was treated with 3-hexenyltriphenylphosphonium bromide and butyl lithium to give methyl 9,12,15-octadecatrienoate isomers. Geometric isomers formed during each of the Wittig reactions were separated by silver resin chromatography.

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QUANTITATION IN THE ANALYSIS OF TRANSESTERIFIED VEGETABLE OILS BY CAPILLARY GAS CHROMATOGRAPHY. B. Freedman, W.F. Kwolek and E.H. Pryde, Northern Regional Research Center, USDA/ARS, 1815 N. University St., Peoria, IL 61604.

A quantitative capillary gas chromatographic method has been developed for studying transesterification of soybean oil to fatty esters. Standard solutions containing methyl linoleate, tri-, di- and monolinolein were analyzed using a 1.78 meter X 0.32 mm SE-30 column. Prior to analysis, the di- and monoglycerides were silylated with N,O-bis(trimethylsilyl)trifluoroacetamide. Tridecanoin was used as an internal standard. From plots of area and weight relationships, response factors for all four compounds were determined. Agreement between the measured and calculated compositions of the standard solutions was good; the overall standard deviation was 0.5. Response factors were also determined for soybean oil and its methyl esters as well as funflower oil and its methyl, ethyl and butyl esters. Complete separation of the ester, mono-, di- and triglyceride was obtained in 12 min by temperature programming from 160 to 350 C. This method of analysis gave excellent results when used in a kinetic study of soybean oil transesterification.