Aloha! AOCS and JOCS Members and Guests,

Welcome to the soft trade winds, warm tropical waters and white sandy beaches of Hawaii.

We trust those will not tempt you away from the excellent technical program arranged for us under the cochairmanship of Drs. Glen Fuller of AOCS and Ichiro Hara of JOCS. The program of more than 380 papers covers virtually every topic in fats and oils research, and includes several excellent symposia.

The Japan Oil Chemists' Society is cosponsoring a traditional Hawaiian luau as an optional social event on Friday evening. In addition, you are invited to our opening mixer on Wednesday evening, the awards breakfast on Thursday, the optional gala international party on Saturday evening and the inaugural brunch on Sunday morning.

While technical registrants are busy conferring with their colleagues and listening to the latest research reports by their peers, spouses' program registrants will be enjoying a house and garden tour, a fashion show and many other unique events they will remember long after our meeting ends.

Our entire local committee—including Arnold Johnson, Bob Faulkner, Elizabeth Loft, Larry Brickman, Mike Sandor, Jackie Lewis and Dennis Taylor—join me in extending a warm welcome to what promises to be one of AOCS' most exciting annual meetings.

Best regards,

Stan Loft
General chairman

American, Japanese oil chemists meet in Honolulu

Oil chemists from around the world will gather in Honolulu May 14–18 as the American Oil Chemists' Society and the Japan Oil Chemists' Society hold their third combined annual meeting.

Approximately 1,000 technical registrants are expected to attend to listen to more than 380 presentations during three intensive days of technical sessions. The abstracts for those presentations are published in this issue of *JAOCS*; the timetable for presentations will be distributed to meeting attendees in Honolulu. Approximately 90 papers will be by JOCS members. Sessions will begin as early as 8:40 a.m. and run as late as 5:30 p.m., with approximately 90 minutes in midday for lunch or a quick trip to the beach.

The mid-week start is a departure from the AOCS traditional meeting schedule, but was arranged to provide lower hotel room rates and to permit attendance at the pre-meeting short courses as well as the annual meeting in the same calendar week, reducing the time a registrant would be away from his or her office.

While the meeting officially begins at mid-day Wednesday when registration opens in the Hilton Hawaiian Village, a few participants will already have begun their activities. The AOCS Governing Board will convene on Tuesday, May 13. The tennis and golf tournament will be held Wednesday.

The annual AOCS business meeting will be held Wednesday afternoon.

The first social event is the opening mixer on Wednesday evening, May 14. The annual awards breakfast will be Thursday morning, May 15. The event will include AOCS President Joyce Beare-Rogers' state-of-the-society address as well as the acceptance address of Supelco AOCS Research Award recipient Robert Allen, being cited for his research on hydrogenation and other contributions.

The other social event included in the registration fee is the closing brunch on Sunday, May 18, when new society officers will be introduced.

Optional social events include a luau on Friday evening. This event is being sponsored, in part, by the Japan Oil Chemists' Society, so that tickets will cost only $25, which includes bus transportation to the luau site. The second optional event is a festive international party to be held Saturday evening, May 17. Tickets purchased at the meeting will be $35 per person.

Throughout the week, four dozen administrative and technical AOCS committees will be meeting. With a few exceptions, these meetings are open to any AOCS member. A tentative schedule is printed in this issue of *JAOCS*; the final schedule will be available in the meeting program in Honolulu.

The Second Annual Fat People's Fun Run or Walk will be held Friday morning. The registration fee of $8 includes prizes to the top finishers; early registrants were guaranteed tee-shirts, but all entrants will have a chance to gather Friday morning for a warm-up jog to Ala Moana Park and then a five-kilometer trek circling the park.

The AOCS and JOCS will maintain separate service desks at the meeting.

Japanese give cooking oil as gifts

Whereas U.S. residents are accustomed to giving and receiving fancy gift-wrapped packages of cheese, fruit or candy at the appropriate times of the year, the Japanese are more apt to exchange fancy gift packages of cooking oils. As much as 20% of the cooking oil used in Japanese homes is obtained through gift exchange. The following article, based on information from Nisshin Oil Mill, was prepared at the request of JAOCS *to acquaint Americans with this Japanese custom.*

In Japan, actual consumption of vegetable oils for edible use totaled approximately 1.55 million tons during 1984. About 30% of the oil was used for home cooking. Another 30% was consumed in the institutional sector, in hotels and restaurants and in school lunch programs. The remainder was allocated to various industrial applications, including production of mayonnaise, salad dressings and margarines. This ratio has remained relatively constant for the past several years.

Most vegetable oil for home cooking is either purchased at grocery stores or received as gift sets. The percentage of gift sets has steadily increased during the past 10 years. In fact, in 1984, 23% of the total home cooking oil consumed was obtained in the form of gift sets.

Gift set market

According to a market analysis concerning gifts purchased in 1984 (Fig. 1), the total market size of gift sets in Japan represented approximately $40.7 billion ($1 = 200 yen). About 42% of the gift sets were exchanged among personal friends and relatives on special occasions or to express personal gratitude. However, traditionally, cash has been the preferred gift on these occasions.

Traditional and peculiar gift-giving customs in Japan are called "Chugen" and "Seibo." Chugen is the mid-year gift-giving time, usually in July, though it also can be in August in some areas. Seibo is the year-end gift-giving time in December. As part of these customs, gift sets are exchanged not only among relatives and personal friends but also with business friends and associates.

Historically, Chugen and Seibo originated in China and were related to religious ceremony, such as Buddhism. At that time, Chugen and Seibo were gifts used at the memorial service for ancestors to express appreciation for good health and harvest.

In modern Japanese society, Chugen and Seibo are exchanged among people primarily to express appreciation for already received kindness and friendship and to ask for continued support. These customs are regarded as indispensable rituals among a majority of the Japanese to keep smooth and friendly relationships. They will, no doubt, be continued in the future.

Chugen, Seibo market

In 1984, the reported market sizes of Chugen and Seibo were $3.1 billion and $3.8 billion, respectively. In total, an estimated 370 million sets were exchanged for the two occasions.

According to one calculation, over 80% of Japanese families received approximately seven sets for each occasion during 1984. Approximately 75% of the gift sets were exchanged among personal friends and relatives, with the remaining 25% given among business friends and associates. Personal friends and relatives were parents, brothers and sisters, superiors at the office, teachers and family doctors.

About 50% of the gift sets were purchased at department stores; only about 6% were purchased in supermarkets (Tables 1 and 2). This ratio has been constant for the last several years.

However, over the past 10 years, items selected as gift sets have changed drastically. Even within the past five years, the choices have changed. Several explanations have been offered. A changing lifestyle of the Japanese people and marketing strategies of gift pack manufacturers and dealers certainly have affected the trend. Also, an increasing consciousness about product quality and wholesomeness of foods may have contributed to these changes.

In 1984, cooking oils were the second most popular item pur-

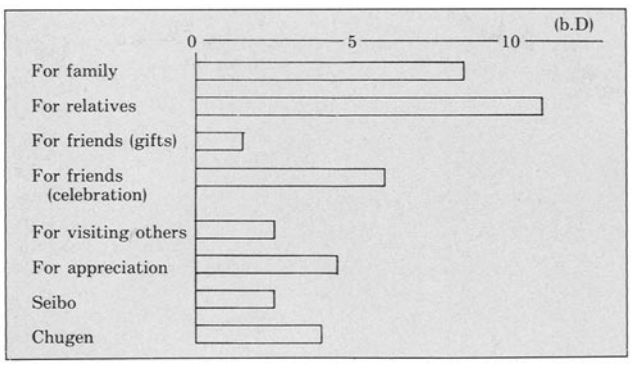


FIG. 1. Market of gift sets (1980).

TABLE 1

Market Size of Chugen and Seibo (1984)

	Total gift sets		Gift sets of cooking oil	
	Chugen	Seibo	Chugen	Seibo
Amount of sales (billion $)	3.0	3.8	0.22	0.25
Total sets (million)	173	197	13.9	15.1
Unit price ($)	17.7	19.3	15.7	15.1

chased as gift sets. The leading item that year was dried seaweed. Only a few items, including cooking oils, have been widely used as gift set items at the two gift-giving times (Table 3).

Cooking oil markets

Because of the favorable sales of cooking oil gift sets, both cooking oil manufacturers and dealers have made strong efforts to expand this market. As a result, the quantity of cooking oil used as gift sets at the two occasions has steadily increased. Currently, cooking oils hold about 8% of the market, and this share is expected to grow. Estimated sales of cooking oil gift sets at the Chugen period in 1984 totaled approximately $220 million, while sales for Seibo reached approximately $225 million.

Market figures show that while corporate bodies give 25% of the total gift sets versus 75% given by individuals, 34% of the cooking oil gift sets are given by corporate bodies. Sixty-three percent of the cooking oil sets were purchased at department stores (Tables 1 and 2).

The popularity of cooking oils as gift sets is credited primarily to their practicality and storage stability (Table 4). Also, at least three distinctive differences are perceived between the marketing of cooking oils for daily use and that of cooking oils as gift sets. First, about 70% of cooking oil for daily use is purchased at supermarkets. On the other hand, only 10% of cooking oil gift sets are handled in supermarkets. Secondly, perhaps the most important marketing factor for cooking oils for daily use is their price competitiveness. The market for cooking oil gift sets, meanwhile, tends to be strongly affected by quality and the health consciousness of their customers. Finally, for gift sets, a smaller package size, of about 600 to 800 g, is preferred. Currently, cylindrical can packages are very popular, with about 60% of oils for gift sets packaged in this way.

Because of the nature of the gift set market, those manufacturing cooking oil gift sets see the need for different market strategies than those for cooking oil products sold for daily use. The gift set market, they say, offers a challenging opportunity for those in the Japanese edible oil industry.

TABLE 2

Sales Outlets and Senders of Chugen and Seibo (1984)

	Total gift sets (%)	Gift sets of cooking oils (%)
Sales outlet		
Department stores	49	63
Supermarkets	6	9
Others	45	28
Sender		
Corporate bodies	25	34
Personal	75	66

JOCS leaders

Akira Mori, president of the Japan Oil Chemists' Society, and M. Nagayama, a JOCS vice-president, will be leading the delegation of JOCS members participating in the joint AOCS-JOCS annual meeting.

Other JOCS vice-presidents include A. Adachi, H. Kaneko and K. Yoshitomi.

Ichiro Hara served as chairman of the JOCS steering committee for the joint meeting. Dr. Hara is a past president of JOCS. Other steering committee members are H. Seino, Y. Tanizaki, T. Hashimoto, T. Itoh, M. Kayama, T. Niki, K. Fujimoto, O. Suzuki, E. Araki and Y. Miura. All the members of the steering committee will serve as chairmen or cochairmen for technical sessions during the meeting.

TABLE 3

Popular Gift Items (in Order of Popularity)

Chugen		Seibo	
1980	1984	1980	1984
Beer	Beer	Japanese sake	Dried seaweed
Cooking oils	Cooking oils	Cooking oils	Cooking oils
Dried seaweed	Dried noodle	Dried seaweed	Japanese sake
Soap	Dried seaweed	Soap	Fresh foods
Dried noodle	Soap	Dried noodle	Ham
*	Whiskey and wine	*	Appetizers
Whiskey and wine	*	Whiskey and wine	Fruit

*Soft drinks fermented with Lactobacillus.

TABLE 4

Reasons Given for Selecting Cooking Oils as Gift Sets on Chugen (1983)

Reason	Percentage citing
Practicality	86
Happy when received	60
Storage stability	52
Select every year	32
Useful for gift recipients	29
Good for health	18
Reduce housewives' shopping loads	16

Spouses' events

Tours of the Honolulu area and other parts of the island of Oahu are planned for persons registered for the spouses' program at the annual meeting.

On Thursday, May 15, registrants will tour the Honolulu area. Sites scheduled to be visited include the Punchbowl Crater, the Summer Palace, the state capitol and the Hawaiian War Memorial, plus others.

On Friday, May 16, registrants will visit a Hawaiian family estate, with lunch at the Over Family Estate at the edge of a wildlife bird sanctuary called Paika Lagoon. After lunch, the tour will visit a lookout above Hanauma Bay, salt water geysers, Makapuu Beach, Waimanalo Town, the Koolau mountain range and Pali Lookout.

On Saturday, May 17, there will be a fashion program to illustrate Hawaiian history.

Spouses' program registrants also will receive tickets to the all-meeting opening mixer on Wednesday evening, May 14, and the Inaugural Brunch on Sunday, May 18. Spouses' program registrants may purchase tickets for optional events, including a luau on Friday evening, May 15, and an international party on Saturday evening, May 17.

Witting honored

Lloyd A. Witting, an AOCS member for more than 30 years, has been selected as recipient of the 1986 AOCS Award of Merit, to be presented during the annual meeting.

Witting is the technical director in biochemical research and manufacturing for Supelco Inc. in Bellefonte, Pennsylvania.

An internationally known specialist in chromatography, Witting has participated in numerous AOCS activities. He has been an associate editor for *Lipids* and book review editor for *JAOCS*. He has served on the education committee, as organizer and chairman of a short course and as editor of a monograph on glycolipids. He has served on committees investigating new analytical methods.

Committees

The following is a tentative schedule for meetings of AOCS technical and administrative committees in Honolulu. With a few exceptions, these committee meetings are open to any AOCS members. The final schedule with meeting rooms will be published in the program distributed in Honolulu.

Day/Date/Committee	Time
Tuesday, May 13	
Governing Board	1:30-6:00 p.m.
Wednesday, May 14	
Membership Admissions	1:00-2:00 p.m.
Public Relations	1:00-2:00 p.m.
1987 World Conference	2:00-4:00 p.m.
Flavor Nomenclature	2:00-3:00 p.m.
Aflatoxin	2:00-3:00 p.m.
Soap & Synthetic Detergents Analysis	3:00-4:00 p.m.
Mycotoxins	3:00-5:00 p.m.
Lecithin and Co-Products	3:00-4:00 p.m.
Dibasic Acids	3:00-4:00 p.m.
Atomic Absorption	4:00-5:00 p.m.
Hydrogenated Oil	4:00-5:00 p.m.
Committee on Program Evaluation	4:00-5:00 p.m.
Thursday, May 15	
World Conference Planning	9:00-10:00 a.m.
Bleaching Methods	9:00-10:00 a.m.
Soap & Detergent Steering	Noon-2:00 p.m.
Advertising	2:00-3:00 p.m.
Uniform Methods	2:00-4:00 p.m.
International Relations and Development	3:00-4:00 p.m.
Honored Student	3:00-4:00 p.m.
Cellulose Yields	3:00-4:00 p.m.
Environmental	3:00-4:00 p.m.
Awards Administration	4:00-5:00 p.m.
Technical Safety & Engineering	4:00-6:00 p.m.
Monograph	4:00-6:00 p.m.
Meeting Logistics	4:00-5:00 p.m.
Seed & Meal Analysis	4:00-5:00 p.m.
Membership Development	5:00-6:00 p.m.
Friday, May 16	
Lipids Advisory	8:00-10:00 a.m.
Chromatography	8:00-9:00 a.m.
Commercial Fatty Acids	8:00-9:00 a.m.
Commercial Fats & Oils Analysis	9:00-10:00 a.m.
Industrial Oil & Derivatives Analysis	9:00-10:00 a.m.
Investments	10:00-11:00 a.m.
Protein & Co-Products Luncheon	Noon-2:00 p.m.
Examination Board	2:00-5:00 p.m.
1987 Annual Meeting	4:00-5:00 p.m.
Nutrition	4:00-5:00 p.m.
Protein & Co-Products Session Chairpersons	4:00-5:00 p.m.

(continued on page 386)

4 students cited

Three U.S. graduate students and one Canadian graduate student will be recognized during the annual meeting as AOCS Honored Students.

The awardees are Kenneth E. Hundrieser of the University of Connecticut; David B. Josephson, University of Wisconsin; Christopher C. Parrish, Dalhousie University; and Laura A. Woolett, Iowa State University. A fifth student selected for the award had to withdraw when she decided to take her degree early to qualify for a specific employment opportunity.

Students receive a complimentary registration for the meeting, as well as travel and housing funds.

In addition to presenting technical papers and attending technical sessions, the honored students are invited to participate in a variety of social events to enable them to talk with experienced fats and oils researchers.

Among the companies contributing to the AOCS Honored Student Fund this year were

Akzo Chemie America, Chicago, Illinois
Anderson Clayton Foods, Dallas, Texas
Canada Packers Inc., Toronto, Canada
Cargill Inc., Minneapolis, Minnesota
CasChem Inc., Bayonne, New Jersey
Colgate-Palmolive, New York, New York
CPC International/Best Foods U.S., Union, New Jersey
Eagr Development Group, State College, Pennsylvania
Fabrica de Jabon la Corona S.A., Mexico City, Mexico
The French Oil Mill Machinery Co., Piqua, Ohio
Gerber Products Co., Fremont, Michigan
Kraft Inc., Glenview, Illinois
Lehn & Fink Products Group, Sterling Drug Inc., Montvale, New Jersey
LSI Bulk Terminals, Division of LSI Inc., Oakland, California
Nu Chek Prep Inc., Elysian, Minnesota
Shell Chemical Co., Houston, Texas
Somes-Nick & Co., Chicago, Illinois
Travenol Laboratories, Deerfield, Illinois
Union Camp Corp., Savannah, Georgia
U.S. Borax Research, Anaheim, California

(continued from page 384)

Saturday, May 17

JAOCS & Publications	8:00-10:00 a.m.
NMR	9:00-10:00 a.m.
1988 Jojoba Conference	10:00-noon
AOCS Foundation	11:00-noon
Finance	Noon-2:00
National Program Planning	3:00-5:00
Sections	4:00-5:00
Smalley	4:00-6:00
Education	4:00-6:00

Sunday, May 18

Governing Board	11:00 a.m.-5:00 p.m.

Potts awardee

Jonathan Blitz, a doctoral graduate student at Colorado State University in Fort Collins, Colorado, has been selected as the 1986 recipient of the Ralph G. Potts Memorial Fellowship.

His graduate work has been on how to determine detailed structural relationships to biological activity for immobilized antimicrobial agents on silica surfaces. The paper he will present at the annual meeting is entitled "Fourier Transform Infrared Investigations of Immobilized Antimicrobial Agents."

The Potts award includes a cash stipend and a plaque as well as travel and housing funds for the annual meeting. The award is named for the late Ralph Potts, a pioneer researcher on fatty acids and nitrogen derivatives. The award fund was established by Akzo Chemie America, the successor firm to Potts' original employer, the Armour organization.

Meeting exhibit

The annual exposition by suppliers of equipment and services to the fats and oils industry will be held in conjunction with the annual meeting in Honolulu.

Firms that had reserved exhibit space (with booth number in parentheses) as of mid-February include

Anco/Votator Division of Cherry Burrell, PO Box 35600, Louisville, KY 40232 USA (Booths 20 and 21). The Votator equipment lines for the fats and oils industry will be shown through photo displays. Included will be oil deodorization plants, shortening and margarine processing equipment, and a thin-film evaporator for drying lecithin and fatty acid stripping.

Bio-Rad Laboratories, 2200 Wright Ave., Richmond, CA 94804 USA (14). HPLC systems for the analysis of a wide variety of compounds will be exhibited. New HPLC columns for hydrophobic chromatography, normal and reverse phase chromatography and ion exchange chromatography will be shown. An applications chemist will be available for consultation.

The Cambrian Engineering Group Ltd., 2200 Argentia Rd., Mississauga, Ontario L5N 2K7 Canada (58 and 59). Models, photographs and diagrams of the "campro" line of deodorizers and steam refiners will be on display, including those available from our Far Eastern licensees. Material also will be available on the "campro" continuous hydrogenation system for edible oils and on some new oil treatment systems.

The Chemithon Corp., 5430 W. Marginal Way SW, Seattle, WA 98106 USA (6). Featured will be information on and photographs of Chemithon's sulfonation/sulfation plants and equipment, and detergent spray drying and agglomeration equipment. Production samples of superior quality products from Chemithon plants also will be displayed.

Crown Iron Works Co., PO Box 1364, 1229 Tyler St. NE, Minneapolis, MN 55440-1364 USA (19). Crown Iron Works Co. designs and manufactures solvent extraction equipment. Photos, brochures and technical literature will be on display.

Desert Whale Jojoba Co. Inc., PO Box 41594, Tucson, AZ 85717 USA (38). The exhibit will feature information on jojoba oil, seed, wax and derivative products. General information about jojoba oil will be available along with samples of jojoba oil and its various products.

Eastman Chemical Products Inc., 1133 Avenue of the Americas, New York, NY 10036 USA (18). Eastman will provide information on Tenox antioxidant and monoglycerides.

EMI Corp., 3166 Des Plaines Ave., Des Plaines, IL 60018 (23). EMI representatives Arnold Gavin, David Tandy and Bill McPherson will be at the booth. The display will include slides and literature describing the firm's physical refining systems and award-winning edible protein processing systems, as well as plant pictures and process flowsheets for solvent extraction of oilseeds, fats and oils refining, fatty acid production processes and complete plants as offered by EMI.

Equipment Engineering, 757 E. Murry St., Indianapolis, IN 46227 (16 and 17). Equipment Engineering specializes in the repair and remanufacture of high speed and decanter centrifuges. Other services include replacement parts, custom-engineered control panels, outside service and purchase and trade-in of unwanted centrifuge equipment.

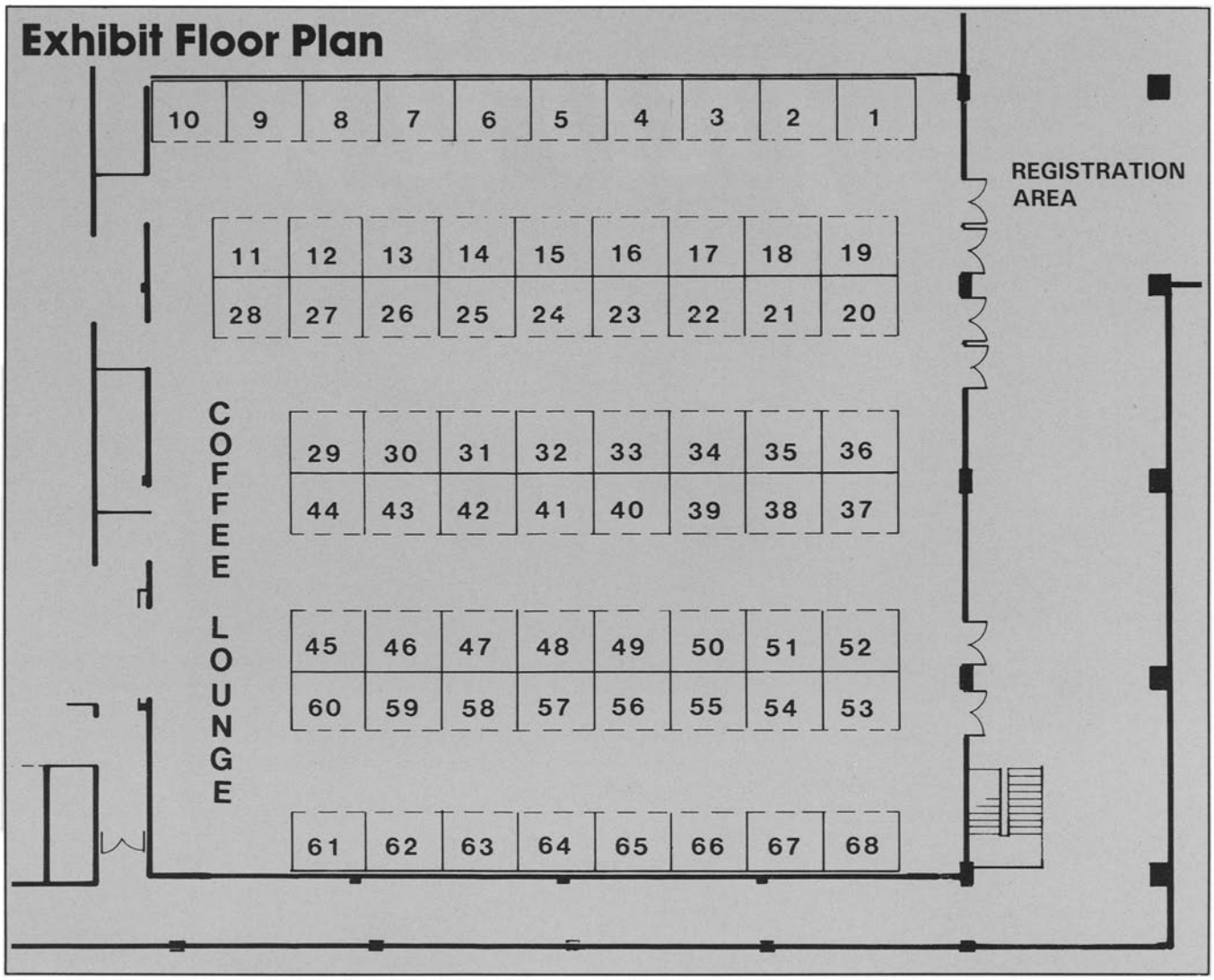
Extraction De Smet S.A., 265 Prins Boudewijnlaan, B-2520 Edegem, Belgium (43 and 44). De Smet specializes in agro-industrial turnkey operations for vegetable oil, sugar, animal feed, starch and glucose as well as loading, unloading, storage and handling of cereals and other products. The firm also designs systems for processing animal by-products and gelatin as well as wool scouring and lanolin refining.

Extraktionstechnik GmbH, PO Box 76 03 69, Humboldtstrasse 56, D-2000 Hamburg 76, West Germany (40 and 41). The booth will provide a survey of the activities comprising the supply of complete plants as well as unit equipment for the processing of all vegetable oil-bearing materials and all vegetable oils and fats. The exhibit will feature the firm's latest improvements in extraction, refinery technology and pollution control.

The Foxboro Co., Bristol Park (52-1) (Dept. 120), Foxboro, MA 02035 USA (60). "Exact Control," a package application of artificial intelligence for the process industries, will be demonstrated using the new single station microcontroller with a PC-based operator interface package. The control system is designed to eliminate the need for loop tuning, provide faster startups and offer improved control. Other products include a new FlowExpert Totalizer/Batcher, along with a variety of measurement devices for temperature, pressure, level, flow and composition.

French Oil Mill Machinery Co., 1035 W. Greene St., PO Box 920, Piqua, OH 45356 USA (35 and 36). French will display model machinery demonstrating its extractor with a new patented rotating extractor bottom, as well as models of current prepresses and rolls. The firm 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 prepressing and solvent extraction plants. Brochures will be available.

Harshaw/Filtrol Partnership, 30100 Chagrin Blvd., Cleveland, OH 44124 USA (29 and 30). The Harshaw/Filtrol booth will have material describing a broad line of Filtrol clay products and Harshaw hydrogenation catalysts used in refining and processing edible and inedible oils. Technical representatives will be available to discuss specific products and applications.



Heinz Schumacher V.D.I., Hoperfeld 26, 2050 Hamburg 80, West Germany (10). Featured will be processes such as DTDC that already are licensed for manufacture, as well as newly developed processes still available for licensing.

Herzog-Hart Corp., 185 Dartmouth St., Boston, MA 02116 USA (32 and 33). Herzog-Hart, a full service engineering company, will exhibit the Buss Loop Reactor System, with heat recovery, for the hydrogenation of fats, oils and fatty acids. The Buss system is designed to provide excellent product reproducibility and attractive operating economics relative to conventional hydrogenation systems.

Industrial Filter & Pump Mfg. Co., 5900 Ogden Ave., Cicero, IL 60650 USA (28). Industrial Filter & Pump Mfg. Co. will display an array of filtration equipment featuring type 122 and 114D fully automated filtration systems. Process applications include crude oil bleaching, hydrogenation, winterizing, and polish plus tank loading, etc.

Marcel Dekker Inc., 270 Madison Ave., New York, NY 10016 USA (52). Marcel Dekker will be displaying the latest volumes of its *Handbook of Fiber Science and Technology* series. These include *Volume III: High Technology Fibers, Part A* (Lewin/Preston) and *Volume IV: Fiber Chemistry* (Lewin/Pearce). Also on display will be *Electrical Phenomena at Interfaces: Fundamentals, Measurements and Applications* (Kitahara/Watanabe) and the journal *Food Review International* as well as other new titles and topic-related journals.

Maschinenfabrik Gustav Eirich, PO Box 1160, D-6969 Hardheim, West Germany (49). Eirich will be introducing its new-age process (Armour-Dial Eirich) for the preparation of soap and soap/synthetic detergent products from raw materials normally used in the manufacture of such products as fatty acids, triglycerides and caustics. The raw materials are subjected to intensive countercurrent mixing whereby saponification takes place in a relatively short time and requires no further drying for most uses. Large amounts of additives can be added at this stage and, through

further intensive mixing, yield free flowing nontacky granules. The resulting product can, if desired, then be subjected to plodding, extrusion and stamping to form soap in bar form.

Milton Roy Co., 201 Ivyland Rd., Ivyland, PA 18974 USA (53). Milton Roy will exhibit a methods development system for supercritical extraction process studies. Also on display will be the firm's Critical Extraction Monitor, an on-line instrument designed to enable researchers to make rapid solubility measurements at supercritical conditions. Technical specialists speaking Japanese and English will be available to discuss SCE applications. Literature will be available in both languages.

MirOil Division, PO Box 298, Allentown, PA 18105 USA (22). MirOil's exhibit will feature (a) fry quality quick screening test for 24–27% polar material according to IUPAC-AOAC standards; (b) quick screening test for alkaline contaminant materials (ACM) according to AOCS Method Cc 17-79; (c) adsorptive powder for removing ACM from used frying oils; and (d) high performance reusable filters for restaurant use.

Neumunz Inc., 117 Fort Lee Road, PO Box 287, Leonia, NJ 07605 USA. (46). Neumunz will exhibit bulletins, flowsheets and pictures of recent installations of Neumunz-designed vegetable oil and food processing plants. A "Uni-Bloc" refinery and latest developments in peanut butter and nut roasting will be featured.

N. Hunt Moore & Assoc. Inc., 3951 Senator St., Memphis, TN 38118 USA (45). The exhibit will consist of displays and literature about the Schroder Kombinator, a scraped-surface heat exchanger used for manufacturing margarine, shortening, mayonnaise, fat-sugar mixtures and salad dressings. Also, there will be displays and literature about the Escher Wyss fluid bed drying system for soybean preparation. Engineers and executives from both Schroder and Escher Wyss will be present to discuss this equipment and its applications.

Novo Laboratories, 59 Danbury Rd., Wilton, CT 06897 USA (24 and 25). 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.

Oil Skimmers Inc., PO Box 33092, Cleveland, OH 44133 USA (47 and 48). The Oil Skimmer was designed to be a unique, maintenance-free solution to mineral and vegetable oils, animal fats, oil froth and other contaminant removal. A working unit will demonstrate the floating tube skimmer. The firm's equipment is currently in use at vegetable oil refineries and various processing plants.

Oregon Meadowfoam Growers Assoc., 2140 Turner Rd. SE, Salem, OR 97302 USA (42). The exhibit will feature

photos, materials and printed handouts to acquaint attendees with meadowfoam oil, a unique triglyceride that is comprised almost completely of fatty acids with chain lengths of 20 and 22 carbon atoms. Samples will be available.

POS, 118 Veterinary Rd., Saskatoon, Saskatchewan S7N 2R4 Canada (5). POS provides pilot-scale processing equipment on a fee-for-use basis. The institute specializes in handling oilseeds, cereal grains and legumes, but has the flexibility and creativity to handle other materials also. POS also offers custom processing, analytical services and project coordination.

Palm Oil Research Institute of Malaysia, PO Box 10620, 50720 Kuala Lumpur, Malaysia (26). PORIM will display samples of processed palm and palm kernel oil products available from Malaysia, including confectionery and other specialty fats and oleochemicals. PORIM literature will be available dealing with analytical characteristics and applications of these products. Technical experts will be in attendance for discussions.

Prater Industries Inc., 1515 S. 55th Court, Chicago, IL 60650 USA (4). Prater Industries specializes in sizing, scalping, screening and flaking of dry products to meet your company's needs.

Süd-Chemie AG, PO Box 20 22 40, 8000 Munich 2, West Germany (1 and 2). Information will be available on the firm's Tonsil bleaching earths used for the adsorptive decolorization and purification of oils, fats and waxes. Data also will be available on the advanced Girdler and NF-20/FS-40 nickel catalysts used in hardening oils such as fish, rapeseed, canola and various fatty acids.

SVO Enterprises Corp., 1550 Old Henderson Rd., Columbus, OH 43220 USA (15). SVO Enterprises will be introducing Trisun, a high-oleic sunflower oil derived from genetically modified sunflowers. The oil may be used in various applications and products. Trisun contains more than 80% oleic and less than 10% linoleic acid. Improved stability from higher oleic content makes Trisun a potentially preferred feedstock for food processors, food additives producers and oleochemical manufacturers.

Tekmar Co., PO Box 371856, Cincinnati, OH 45222-1856 USA (3). Tekmar will exhibit its Model 4000 Dynamic Headspace Concentrators, Model 4100 Heated Sampler Module, Model 1000 Capillary Interface for Cryogenic Trapping, Model 5000 Automatic Desorber for absorbent traps, and fully automatic Karl Fischer Titrators.

The Tintometer Co., 309A McLaws Circle, Williamsburg, VA 23185 USA (61 and 62). Featured will be color grading and measuring instruments for edible oils, fats and tallows, including the Lovibond Color Scale Tintometer, FAC Scale, Gardner Scale, Model E Tintometer, the new American Oil Tintometer for Lovibond and AOCS Scale with options for chlorophyll and carotene, including printer and computer interface.

UOP Inc., Biological and Food Products, Algonquin & Mt. Prospect Rds., PO Box 5017, Des Plaines, IL 60017-5017 USA (31). UOP will display its Sustane line of food-grade antioxidants, including BHA, BHT, TBHQ, propylgallate and a variety of liquid antioxidant blends. Color product brochures, specification sheets and other technical literature will be available. Sample requests will be accepted.

Wurster & Sanger Inc., 222 W. Adams St., Suite 1529, Chicago, IL 60606 USA (27). Catalogs, technical literature, plant photographs and process flowsheets reflecting the complete capabilities of Wurster & Sanger as process engineers for the fats and oils industries will be exhibited. Wurster & Sanger custom-builds plants for oilseeds, glyceride fats and oils, fatty acids, glycerine and related by-products. Samples of products from Wurster & Sanger processes will be displayed.

Zone Devices Inc., 1825 Lincoln Ave., Suite 112, San Rafael, CA 94901 USA (13). Zone Devices will show a number of products primarily involved in the sampling of bulk liquids. The best known is the stainless steel liquid zone sampler, approved for use in AOCS Method C 1-47 (sampling of oils and fats). Various other types of liquid sampling equipment will be exhibited.

Book exhibit

The following publications will be displayed in the AOCS book exhibit during the annual meeting in Honolulu. 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.

From the American Association of Cereal Chemists (AACC), 3340 Pilot Knob Rd., St. Paul, MN 55121 USA:

Approved Methods of the AACC, 8th edition, $240.

Digestibility and Amino Acid Availability in Oilseeds, by John W. Finley and Daniel T. Hopkins, 1985, $58.

Moisture Sorption: Practical Aspects of Isotherm Measurement and Use, by T.P. Labuza, 1984, $33.

Principles of Cereal Science and Technology, by R.C. Hoseney, 1985, $46.95.

Rheology of Wheat Products, by Hamed Faridi, 1985, $41.

From The American Institute of Chemical Engineers, 345 E. 47th St., New York, NY 10017 USA:

Biotechnology Progress, edited by M.L. Shuler, December 1985, $14 for AIChE members, $50 to others.

From the Association of Official Analytical Chemists (AOAC), 1111 N. 19th St., Suite 210, Arlington, VA 22209:

Official Methods of Analysis of the AOAC, 14th edition, edited by Sidney Williams, 1984, $148.50 in the U.S., $151.50 elsewhere.
Journal of the AOAC, published six times a year; one-year subscription is $89.75 in the U.S., $99.75 elsewhere.

From AVI Publishing Co., 250 Post Rd. E., PO Box 831, Westport, CT 06881 USA:

Batter and Breading Technology, by Suderman and Cunningham, 1983, $37.50 (AVI Stock #417).
Carbohydrate Biochemistry and Metabolism, by Roehrig, 1984, $45 (#423).
Chemistry and Biochemistry of Marine Food Products, by Martin et al., 1982, $49.50 (#393).
Chemical Changes in Food During Processing, by Richardson and Finley, 1985, $59 (#504).
Chocolate, Cocoa and Confectionery, 2nd edition, by Minifie, 1980, $65 (#310).
Coconuts: Production, Processing, Products, 2nd edition, by Woodroof, 1979, $37.50 (# 290).
Food Carbohydrates (IFT Symposium), by Lineback and Inglett, 1982, $59 (#384).
Food Oils and Their Uses, 2nd edition, by Weiss, 1982, $37.50 (#401).
Modern Methods of Food Analysis (IFT Symposium), by Stewart and Whitaker, 1984, $59 (#441).
Snack Food Technology, 2nd edition, by Matz, 1984, $49.50 (#437).
Standards for Fats and Oils, minor foodservice series volume 5, by Lawson, 1985, $22.50 (#445).
Statistical Methods for Food and Agriculture, by Bender et al., 1982, $31.50 (#A378).

From Gulf Publishing Co., PO Box 2608, Houston, TX 77001 USA:

Encyclopedia of Fluid Mechanics, Vol. 1: Flow Phenomena & Measurement, edited by N.P. Cheremisinoff, 1985, $165.
Encyclopedia of Fluid Mechanics, Vol. 2: Dynamics of Single-Fluids Flows and Mixing, edited by N.P. Cheremisinoff, 1986, $165.

From ISTA Mielke GmbH, PO Box 90 08 03, 2100 Hamburg 90, West Germany:

Oil World, published weekly, edited by S. Mielke, DM823.80 a year (includes air mail postage to the U.S.).
Oil World—The Past 25 Years and the Prospects for the Next 25 in the Markets for Oilseeds, Oils, Fats & Meals, edited by S. Mielke, 1983, DM 169.

From Plenum Publishing Co., 233 Spring St., New York, NY 10013:

Dietary Fiber: Basic and Clinical Aspects, by Vahouny and Kritchevsky, 1986, 586 pp., $79.50.
Drugs Affecting Lipids Metabolism VIII, by Kritchevsky, Holmes and Paoletti, 1985, 516 pp., $72.50.
Lipids: Chemistry, Biochemistry and Nutrition, by Mead, Alfin-Slater, Howton and Popjak, 1986, 473 pp., $69.50.
The Physical Chemistry of Lipids, (Handbook of Lipid Research, Vol. 4), by Small, 1986, 692 pp., $89.50.

From Rockefeller University Press, 1230 York Ave., New York, NY 10021:

Taste, Olfaction and the Central Nervous System, edited by D.W. Pfaff, 1985, $29.95.

Technical Paper Abstracts

Session A Thursday morning Analysis of Lipids I

1

EVALUATION OF THERMAL OXIDATION STABILITY FOR FATTY ACIDS. Kenichi Uemera, JOCS Fatty Acids Committee, The Japan Oil Chemists' Society, 3-13-11, Nihonbashi, Chuo-ku, Tokyo 103, Japan, and Haruo Hirayama, Tateo Murui, Yasuhiko Kubota, Jun Kurokawa, Hiroyasu Sakurada, Masanori Sonehara and Kazuro Tsuzi, JOCS Fatty Acids Committee.

Thermal Oxidation Stability, heat stability, oxidation stability and reagent stability are being used as test methods for stability of fatty acids. Color determination and chemical tests like anisidine value and peroxide value are done as evaluation method for the stability. Color determination is common to all the tests and thus regarded as the most important method. APHA and Gardner color methods are most commonly used. A few methods of evaluating color for the thermal oxidation stability of fatty acids were studied collaboratively. Since APHA and Gardner color methods often gave different results among individuals as well as laboratories, we focused on studying the spectrophotometric method. As a result, it was shown that photometric index (P I) calculated by the following equation was useful for the color determination of fatty acids. $P\ I = 100 \log 100/A$, where $A = 0.0002\ T_{400} + 0.0093\ T_{440} + 0.0842\ T_{490} + 0.3491\ T_{530} + 0.4029\ T_{550} + 0.1543\ T_{620}$; $*T_{400}$: transmittance at 400nm. Moreover, good relationship was found between P I and APHA standard. Namely, P I X 100 was identical with APHA standard color solution number.

2

STUDY ON EVALUATION OF OXIDATIVE STABILITY OF FATS AND OILS. Takashi Ikeda, Shiseido Laboratory, 1050 Nippa-cho, Kohoku-ku, Yokohama-shi, Kanagawa-ken, Japan, and Keiichi Uehara and Kenichi Tomita, Shiseido Laboratory.

Recently the HPLC method has been applied to measure the peroxide value (POV) of fatty acids and their esters. But it is difficult to apply this method formeasuring POV of fats and oils containing many components such as edible oil. We report a simple and quick HPLC method to determine POV of fats and oils. As a simple model of fats and oils, triolein was autoxidized and the polar parts separated by silica gel column chromatography followed by HPLC analysis (column, ODS; eluent, 30% THF/MeCN; detector, UV 215nm).A linear relationship between the peak area and POV was obtained. Thus, it is possible to determine POV by simply measuring the peak area in the chromatogram of triolein within a certain length of retention time. We are investigating the application to other fats and oils and the evaluation of some antioxidants to triolein by this handy method.

3

POTENTIOMETRIC DETERMINATION OF LIPID PEROXIDES IN VIVO. Setsuko Hara, Faculty of Engineering, Seikei University, 3-1 Kichijoji-kitamachi 3, Musashino-shi, Tokyo 180, Japan, and Yoichiro Totani, Faculty of Engineering, Seikei University.

For determination of low peroxide values (POV) of lipids in vivo, the JOCS method was modified by the use of a potentiometer instead of starch as an indicator. As a result, this method required only 0.02 g of lipids to determine POV when POV was higher than 1 meq/kg, and 0.1 g of lipids when it was lower than 1 meq/kg. In the case of reducing the required amount of saturated potassium iodide aq solution used for iodometry, the time for titration was less and the end point of titration clearer, which gave more reliable results. These modifications made it possible to detect (3-5) X 10^{-2} μeq peroxides with high accuracy and rapidity. Using an automatic titrator for the present method was effective to reduce the time for POV measurement. It was possible to detect peroxides in a few ml of human serum by this modified method. Moreover, by the thiobarbituric acid (TBA) test for measuring peroxides indirectly, detection of peroxides in human serum was possible only at levels of less than 20% of the value by the present method. The present method makes possible the direct determination of a small amount of hydroperoxides in human serum lipid and thus makes it easier to understand the influence of lipid peroxides in vivo on cancer, hypertension, arteriosclerosis, the aging process, etc.

4

HPLC ANALYSIS OF LIPID PEROXIDES. Yoichiro Totani, Faculty of Engineering, Seikei University, 3-1 Kichijoji-kitamachi 3, Musashino-shi, Tokyo 180, Japan, and Setsuko Hara, Faculty of Engineering, Seikei University.

High performance liquid chromatography (HPLC) with RI or UV detector was used to separate and determine hydroperoxides of autoxidized lipids such as autoxidized fatty acid esters, plant oils and cholesterol esters. In the case of reversed phase HPLC analysis using acetonitrile:water:THF(5:2:1) as an eluent for autoxidized fatty acid esters, the hydroperoxides were separated according to the number of their double bonds and hydroperoxy groups and to the configuration of the conjugated double bonds, but not on the basis of the difference in the location of hydroperoxy groups. On plant oils such as olive, safflower and linseed oils, autoxidized oils could be separated in a few peaks of hydroperoxides and one peak of unoxidized oil under the ordinary phase HPLC condition using hexane:2-propanol:methanol(98:1:1) as an eluent. Cholesterol oleate hydroperoxide or linoleate hydroperoxides showed a few peaks, respectively, on the chromatogram by reversed phase HPLC using acetonitrile:2-propanol(1:1) as an eluent. When autoxidized lipids were subjected to HPLC analysis directly, good linear relationships were observed between the ratio of peak area of hydroperoxides to those of all peaks on the chromatogram and POV of the sample lipid. POV determined by this procedure agreed quite well with those measured by the official titration method.

5

APPLICATION OF A TLC-FID CHROMAROD SYSTEM FOR DETERMINATION OF FAT AND OIL PEROXIDES. Toshihiro Itoh, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa 228, Japan; Kiroshi Kaneko, Kitasato University, and Osamu Katoh, Masamichi Tanaka and Jiyukichi Ishii, Iatron Laboratories.

The general application of and various improvements in the TLC-FID Chromarod system (Iatron) were discussed at the symposium of the 75th AOCS annual meeting. The usefulness of this system in the quantitative analysis of lipids and related compounds also has been reported in several of our papers on the boric acid impregnated rod method and argentation method. In this paper, the application of this system to the analysis of the peroxides of fats and oils is also discussed. Methyl linoleate, purified by silicic acid column chromatography, had air bubbled through it at 60 C in a glass flask. An aliquot of the solution was transferred to a small test tube and dissolved in a chloroform methanol mixture to which had been added a small amount of squalene as the internal standard. The sample solution was applied onto a Chromarod S-II with an auto sample applicator. The rods were initially developed 8 cm from the origin with n-hexane-diethyl ether (8:2), dried in vaccuo for 5 min, and developed further

12 cm from the origin with n-hexane. The relative detector response of squalene to methyl linoleate was almost 1.0. When the POV value of the sample was higher than 10 meq/kg, the amount of methyllinoleate peroxide could be directly determined by the Iatroscan system. The profiles of the peroxide formation of methyl linoleate determined by the TLC-FID system were quite close to those obtained by POV analysis. This system was also used to determine the peroxide contents of commercial food oils.

6

THERMAL STABILITY OF EPOXY TRIGLYCERIDES. Shu-pei Chang, Northern Regional Research Center, USDA/ARS, 1815 N. University St., Peoria, IL 61604, and Kenneth D. Carlson and John A. Rothfus, Northern Regional Research Center, Peoria, IL.

During heating at a fixed rate to high temperatures, epoxy triglycerides (TG's) undergo epoxy-ring opening reactions, which generate species that rapidly polymerize and liberate heat readily detected by differential scanning calorimetry (DSC) techniques. Based on this sudden enthalpic change, we devised a DSC method to determine the thermal behavior of several epoxy TG's and to determine the temperature of this irreversible thermal event. TG's with similar chain lengths, but different oxirane oxygen contents, were represented by olive oil (control) and epoxidized soybean, linseed, *Lithospermum tenuiflorum*, and *Vernonia galamensis* seed oils. Mono-, di- and trivernoyl TG's were isolated from vernonia oil, and tetra-, penta- and hexaepoxy TG's were isolated from expoxidized vernonia oil to serve as representative materilas having essentially 1 to 6 epoxy moieties per TG molecule. Thermal stability was determined by heating samples at 10 K/min to high temperatures in 50 K increments above 350 K. After each succeedingly higher 50 K increment (e.g., 400, 450, 500 K), low temperature (100-350 K) thermal behavior was found to be identical to the initial reference scans until an abrupt exothermic change occurred. The low temperature scan then differed markedly from that of the original sample. This "temperature of irreversible change" is designated T_{ic}.

Session B Thursday morning Symposium on Phytolipids I

7

INHIBITORS OF LIPID BIOSYNTHESIS: POSSIBLE ROLE OF STEROLS IN FATTY ACID DESATURASE ACTIVITY. John D. Weete, Auburn University, Department of Botany, Plant Pathology and Microbiology, Auburn University, AL 36849.

Inhibitors of lipid biosynthesis of current interest and their sites of action will be briefly reviewed. Particular emphasis will be placed on the mode of action of the triazoles and allylamines, and their value in determining the possible role of sterols in modulating fatty acid desaturase activity in fungi. In *Taphrina deformans* and other fungi, propiconazole blocks the biosynthesis of brassicasterol (functionally equivalent to ergosterol) by inhibiting C-14 demethylation of lanosterol. In addition to the accululation of 24-methylene dihydrolanosterol in membranes of propiconazole-treated cells, a shift in the phospholipid-fatty acid composition in favor of a higher degree of unsaturation ($C_{18:1} \rightarrow C_{18:2} + C_{18:3}$) also occurs. The degree of response appears to be negatively correlated with brassicasterol content, but the effect of the 14-methyl sterols on desaturase activity is not known. When naftifine, an inhibitor of squalene epoxide cyclase, is used, the accumulation of 14-methyl sterols is avoided and yet essentially the same relationship between brassicasterol content and phospholipid-fatty acid unsaturation is observed. These findings will be discussed in the context of the role of sterols in the regulation of a membrane-fatty acid metabolism in animal, mycoplasma and fungal systems.

8

ROLE OF STEROLS IN THE DEVELOPMENTAL REGULATION OF FUNGI. W. David Nes, Plant and Fungal Lipid Group, Plant Development and Productivity Research Unit, U. S. Department of Agriculture, Albany, CA 94710.

Evidence is presented which documents that sterols may act as the primary modulator of developmental events in the life cycle of fungi. The fitness (structural and quantitative) of the sterol in the regulatory process differs as a function of the growth phase. In order to initiate the cell cycle (mitotic index), physiologically monitored by observing temporal changes in growth, the sterol (non-metabolic role) need only be at trace or vitamin levels. The precise molecular details for the induction depends on the individual specificities selected for by each fungal group. Reproduction, and hence control of the meiotic index (monitored by counting the number of sex organs and spores produced), is also quantitatively regulated by sterols prior to growth arrest. Interestingly, steroid hormones may achieve the same reproductive end-response as certain non-metabolized sterols. While sterols may be sparred (replaced) by or combined (synergism) with sterol-like molecules, e.g. pentacyclec triterpenoids, to effect growth, for production of viable spores the fungus must synthesize or accumulate from the environment (sterol auxotrophs) specific sterols. How the Oomycetes have maintained their phylogenetic homogeneity even though some members have lost a complete sterol pathway serves as a paradigm for the other fungi. It is suggested (and discussed) that environmentally-induced alterations in sterol structure and composition during fungal development may be one evolutionary mechanism which leads to the selection of new populations. This implies that structure-function changes rather than random mutations during development may be the causative agent in the evolution of some fungal groups.

9

STEROLS AND THE PHYLOGENY OF HIGHER PLANTS. G. W. Patterson, Department of Botany, University of Maryland, College Park, MD 20742; T. S. Salt and S. Xu, University of Maryland and J. H. Adler, Department of Biological Science, Michigan Technical University, Houghton, MI.

Beginning with the first systematic review of the sterol composition of higher plants by Bergmann, it was apparent that most plants contained sterols characterized by the presence of the 5(6)-double bond. These plants are sometimes referrred to as "main line" plants due to their composition of the "standard" higher plant sterols, campesterol, stigmasterol and sitosterol. To this date no hgiher plant has been shown to contain as its principal sterol one with the 5,7 conjugated double bond, although such sterols are common in fungi. A few plants are known to contain primarily delta-7-sterols, but these are few in number and are located primarily inteh Cucurbitacae, with only scattered "delta-7-species" among other plant families. Recently, however, detailed and numerous examinations of plants in the Order Caryophyllales have shown that many of them contain delta-7-sterols alone or in a mixture with the usual delta-5-sterols. Delta-7-sterols (primarily spinasterol and 22-dihydrospinasterol) are common in the Phytolaccaceae, Amaranthaceae, Chenopodiaceae and Caryophyllaceae and occur in other less studied families of the Caryophyllales. In some families (ex. Amaranthaceae), delta-7-sterols comprise the great majority of the total sterol composition. In the Chenopodiaceae, delta-5 and delta-7 sterols are mixed and in Cactaceae most species contain only delta-5 sterols. Further studies on sterol composition here should be of value in the taxonomy of this difficult group of plants.

10

THE PRESENT STATUS OF STEROLS IN MEMBRANES. William R. Nes, Drexel University, Department of Bioscience & Biotechnology, Philadelphia, PA 19104.

In early ideas about the nature of membranes of phospholipid bilayer which contains protein but no sterol was envisaged, and sterols were thought to find their meaning simply as precursors to hormones. By 1974, however, sufficient information was available on the structures, organismic distribution and subcellular localization of sterols to postulate [W. R. Nes, *Lipids 9*, 596 (1974)] that actually it is in the membranes that the principal role of these materials is to be found. During the intervening years much additional work has been done on this subject. Both direct and indirect evidence have been brought to bear on the manner in which sterols fit into and are distributed within and among membranes, on the importance of dimensions, on the significance and limitations of the concept of fluidity and other functional properties, on variations in the structure of natural sterols, and on chemotaxonomic and evolutionary correlations. The salient aspects of these and related topics will be summarized and integrated with questions which still remain unanswered.

Session C Thursday morning Symposium on Oilseed Proteins-Nutrition, Health and Disease

11

COMPARISON OF SPECIFIC NUTRITIONAL AND METABOLIC PROPERTIES OF COTTONSEED PROTEIN TO OTHER PLANT AND ANIMAL PROTEINS. Mary Anne Sullivan-Gorman, Texas Christian University, Department of Nutrition and Dietetics, Box 32869, TCU, Fort Worth, TX 76129, and George U. Liepa, Texas Woman's University, Denton, Texas.

Oilseed byproducts have been used widely for some time as a source of crude protein in animal feeds. With the rapid expansion in the usage of cottonseed as a human food ingredient, it has become necessary to critically examine the nutritional and physiological properties of cottonseed proteins (CSP) in order to compare CSP to other plants and animal proteins. Cottonseed protein has not been used historically as a human food source, because most cottonseeds contain pigment glands which are toxic to man and other monogastric animals. The development of the liquid cyclone process of removing gossypol pigment glands and the large scale breeding of glandless cotton plants has greatly extended the potential use of CSP as a human food. Proteins of plant origin tend to be lower in the nutritive value than animal proteins, because most plants proteins are limited in ore or more essential amino acids; however, CSP has a PER which approaches that of casein. In the past 20 years a great deal has been learned about the physiological effects of plant proteins. Consumption of plant proteins and certain dietary amino acids appear to be related to decreased atherosclerotic risk. Americans who consume plant protein diets are known to have a lower risk of developing cardiovascular disease than the typical American. Animal proteins have been associated with hypercholesterolemia while vegetable proteins are found to protect against this condition. Based on clinical and epidemiological data it appears that diet plays and important role in the development of gallstone disease although the mechanisms are not clearly defined. Since supersaturation of bile with cholesterol is the cause of gallstone formation, recent research has focused on a reduction or preventation of gallstone formation via the feeding of dietary vegetable proteins. Several studies using hamsters as the experimental model have shown increased production of gallstones and hypercholesterolemia in animals fed casein diets as compared to soy and cottonseed protein diets. The apparent nutritive and physiological value of CSP makes it useful as a food product. The use of glandless CSP products for human consumption has been classified in three main areas: additives, fillers and protein supplements. Certain qualities imparted to food by cottonseed protein have useful potential for extending the use of cottonseed protein in various food products.

12

THE DEVELOPMENT OF AN ANIMAL MODEL FOR DETERMINING THE EFFECTS OF DIETARY PROTEIN ON CHOLELITHIASIS. Jane M. Anderson, Texas Woman's University/Dept. of Nutrition, P.O. Box 24134, TWU Station, Denton, Texas 76204, and Nancy M. DiMarco, Jim Johnson and George U. Liepa, Texas Woman's University, and Mary Anne Gorman, Texas Christian University.

The purpose of this study was to develop an animal model for determining the effects of various dietary proteins on dietary cholesterol and cholic acid induced cholelithiasis. One hundred and fifty mice were randomly assigned to one of eight dietary groups. Each group contained various levels of cholesterol and/or cholic acid as well as ~ 30.0% cornstarch (percentage of cornstarch varied for each diet, to insure the dietary components equalled 100%), 22.5% protein (casein), 12% butter, 11.1% sucrose, 7.5% dextrin, 6% cellulose, 5.5% vitamins and minerals, and 4.5% cottonseed oil. Each group contained the following levels of cholesterol (C) and Na Cholate (NaCh): Group 1, 1% C and 0.5% NaCh; Group 2, 1% C and 0.25% NaCh; Group 3, 0.25% C and 0.5% NaCh; Group 4, 0.5% C and 0.5% NaCh; Group 4, 0% C and 0.5% NaCh; Group 6, 1%C and 0% NaCh; Group 7, 2% C and 0% NaCh; Group 8, 4% C and 0% NaCh. The specific objective of the study was to determine the optimal dietary conditions for inducing cholelithiasis while using the smallest amount of dietary cholesterol and/or cholic acid. Animals fed experimental diets showed different levels of stone formation (0 to 77%, respectively). Diets containing only cholesterol as a variable (1, 2 or 4% of the diet) showed no stones. The diet which contained cholic acid as the sole variable showed only a 5% gallstone incidence. Of the remaining diets which induced gallstones, the group containing the combination of 0.25% dietary cholesterol and 0.5% cholic acid was chosen as the optimal dietary condition for gallstone formation in protein studies since dietary cholesterol levels are far lower than those used previously with the mouse model. Effects of various dietary proteins (cottonseed protein, casein and soybean) on gallstone formation will be discussed.

13

CASEIN-INDUCED HYPERCHOLESTEROLEMIA: POSSIBLE UNDERLYING MECHANISMS. A.C. Beynen, Dept. of Lab. Animal Science at State University, P.O. Box 80.166, 3508 TD, Utrecht, The Netherlands; R. van der Meer, Department of Nutrition, Netherlands Institute for Dairy Research and C.E. West, Department of Human Nutrition, Agricultural University, Wageningen (Netherlands).

Essentially cholesterol-free, semipurified diets containing casein induce high levels of serum cholesterol in young, growing rabbits, whereas serum cholesterol levels remain low on diets containing soy protein. The hypercholesterolemic response to casein can be affected by non-protein components of the diet. In rats the same phenomenon can be observed but the experimental conditions are more crucial because sex and strain of the rats as well as background components of the diet determine the susceptibility to casein. The mechanisms underlying the differential cholesterolemic effects of dietary casein and soy protein have not yet been fully unravelled. We propose that after replacement of soy protein by casein there is an increased flux of cholesterol and bile acids from the gut to the liver. This causes an increase in the concentration of cholesterol in the liver because the hepatic uptake of cholesterol from chylomicron remnants is increased and the conversion of cholesterol to bile acids is depressed. The liver responds by increasing cholesterol output and/or by decreasing the number of low density lipoproteins (LDL) receptors, which accounts for the increase in serum cholesterol. The liver also responds by depressing its synthesis of cholesterol. The concentration in serum of LDL increases until a new level is reached at which the rate of catabolism of LDL again equals its production. At this new steady state, cholesterol synthesis is depressed and intestinal uptake of cholesterol and bile acids is stimulated. Thus, the enhanced absorption from the gut of cholesterol and of bile acids may be the key to explaining the effect of casein on the level of cholesterol in serum. It is not yet completely clear how this effect is brought about. However, there is some evidence that it may be related to the relatively high degree of phosphorylation of casein. Future work should concentrate on unravelling the effects of dietary proteins on the enterohepatic circulation of cholesterol and bile acids.

14

DIETARY FIBERS AND PROTEINS AND THEIR EFFECTS ON CHOLELITHIASIS IN HAMSTERS. Nancy DiMarco, Texas Woman's University, Dept. NFS, P.O. Box 24134, TWU Station, Denton, TX 76204, and M.J. Liu, P. Lively, M.A. Sullivan, J. Johnson and G. Liepa, Texas Woman's University.

Recent studies have shown that addition of both soluble and insoluble fibers to the diet have prevented saturation and nucleation of bile fluid with cholesterol. Functional effects of dietary fiber on serum and biliary constituents have been related to the specific physiochemical properties of the fiber being tested. Information regarding metabolic effects of fiber are limited. The objectives of our study were to focus on the physiological effects of various fibers on the metabolism of biliary lipids and cholelithiasis in hamsters. Two hundred sixteen (216) male hamsters (60 ± 5 g) were fed Purina hamster chow for 7 days and were then fed modified Dam diets (containing either 20% casein (C) or cottonseed (SC) protein, 74.3% sucrose, and adequate vitamins and minerals), plus 0%, 2%, or 5% pectin, oat bran or in addition to the C group, Metamucil, were added in place of sucrose. Animals were maintained on the experimental diets for 28 days. Among the C-fed animals, presence of oat bran at the 2% level decreased the incidence of gallstones from 66.7% to 46.7%. At levels either higher or lower, the incidence was similar. Addition of pectin to the C-diet appeared to reduce gallstone incidence as the percentage of pectin increased. Metamucil, added at any of the 3 levels studied, appeared to have no effect on reducing gallstone incidence. Among the CS-fed animals, addition of 2% pectin reduced gallstone incidence, relative to those fed no fiber, but at higher and lower amounts of pectin, the incidence of gallstones was approximately one-half the value without any fiber added. One percent (1%) oat bran added to the CS-protein diet decreased the incidence of gallstones about 50% but had no effect at higher concentrations. The effects of dietary fibers and proteins on serum and biliary components will be discussed. We feel that the addition of plant fibers had a positive effect on the incidence of gallstones in the hamster fed casein and cottonseed proteins.

15

IRON BINDING BY COTTONSEED AND SOY PROTEIN. Marilynn Schnepf, Texas Woman's University, Department of Nutrition and Food Sciences, P.O. Box 24134, Denton, TX 76204, and Patricia Johnson, Florence Edokpayi and Nancy DiMarco, Texas Woman's University.

Plant proteins have been implicated in decreased iron absorption in both in vivo and in vitro studies. The objectives of this research were to further investigate and to begin to characterize iron binding by soy and cottonseed proteins using a hemoglobin repletion assay with rats and the in vitro techniques of ultrafiltration and gel filtration. A pH 3.5 buffered solution containing either soy isolated or cottonseed flour was incubated with radiolabeled Iron-59 and ultrafiltered with stepwise increases of pH to 7.5. Soy protein bound 88.9% of the iron. As pH increases, iron forms insoluble hydroxide polymers. When samples were repeatedly ultrafiltered at pH 5 the protein-iron complex remained intact. The addition of ascorbic acid and EDTA to the iron-protein complex was able to remove most of the iron from the complex. When the iron-protein complex was partially digested with trypsin and the peptides separated by gel filtration chromotography, the iron was gradually released from the complex. Some of the iron

appears to be bound on the surface of the complex by ionic bonds and is removed by disrupting those bonds. Other iron which is more difficult to remove may be held within the complex by protein-iron-protein bonds.

16

SEPARATION OF SOY TRYPSIN INHIBITORS USING ULTRAFILTRATION. Eugene C. Baker, USDA, Northern Regional Research Center, 1815 N. University St., Peoria, IL 61604.

Aqueous extracts of defatted soy flour were processed on ultrafiltration (UF) membranes having molecular weight cutoffs (MWCO) from 1000 to 100000 daltons. Soy trypsin inhibitors (MW 8000–21500 daltons), in the presence of non-inhibitor proteins, were rejected by 100000 MWCO membranes. When non+inhibiting proteins were removed by isoelectric precipitation prior to the UP, however, TI appeared in the permeate as expected. Thus, TI apparently associates with non-inhibitor proteins; this association may be involved in the inactivation mechanism of TI. These techniques were used to obtain soy TI concentrations with purities of 40–50% compared to 25% purity reported in previous work at this Center. Solutions of purified soy TI concentrates were ultrafiltered on various membranes and their rejection ratios calculated. Rejection of TI concentrates by the membrane was lower at low pH (1.5–2.5) than at values above 8, suggesting the occurrence of self-association of the TI at higher pH values. Addition of sodium chloride (0.5–1.0N) had little effect on the separation.

Session D Thursday morning Surfactants and Detergents I Surface Chemistry I

17

SURFACE CHEMICAL PROCESSES FOR REMOVAL OF SOLID SEBUM SOIL. Michael F. Cox, Dewey L. Smith and Geoffrey L. Russell, Vista Chemical Company, Research & Development, P.O. Box 500, Ponca City, OK 74602.

The surface chemical removal of solid sebum soil appears to involve a 2-step process. The first step consists of penetration of surfactant (and associated water molecules) into the soil. Under most conditions, penetration results in soil liquefaction which prepares the soil for other secondary processes which actually remove soil. These include both surface chemical (soil roll-up, emulsification/solubilization) and mechanical (agitation) processes. Surfactant penetration can also result in removal of the soil as a semi-solid by reducing soil-substrate adhesion. The effects of surfactant molecular structure (surfactant-type, HLB, hydrophobe size, etc.), soil composition (unsaturation, water content, etc.), and wash conditions (wash temperature, etc.) on penetration and soil removal are discussed.

18

SOLUBILIZATION OF N-ALCOHOLS IN MIXED MICELLES. Cuong M. Nguyen, John F. Scamehorn and Sherril D. Christian, University of Oklahoma, Chemical Engineering and Materials Science, 202 West Boyd, Room 23, Norman, OK 73019.

There have been few studies concerning solubilization of organic materials in aqueous surfactant solutions containing surfactant mixtures, despite the fact that almost all commercial surfactant formulations used include many surfactant components. In this work, the solubilization of n-alcohols into solutions containing a mixture of an anionic surfactant and a nonionic surfactant is systematically studied. The solubilization constant is measured over the range of surfactant compositions from pure anionic to pure nonionic. It is also measured over a wide range of alcohol activities, not just at unit activity as is often done in solubilization work. Micellar-enhanced ultrafiltration is the analytical method used to make the measurements. In this recently developed technique, the micellar solution is forced through ultrafiltration membranes with pore sizes small enough to block micelles from passing. The resulting permeate contains the alcohol at its unsolubilized concentration. The accuracy of the results is confirmed with semi-equilibrium dialysis, another recently developed method to measure solubilization. The results provide insight into solubilization mechanisms, as well as mixed micelle structure. The anionic surfactant is sodium dodecylsulfate and the nonionic is a nonylphenol polyethoxylate.

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THE EFFECT OF SURFACTANT STRUCTURE ON THE RATE OF OIL SOLUBILIZATION INTO AQUEOUS SURFACTANT SOLUTIONS. T.A.B.M. Bolsman and F.T.G. Veltmaat, Koninklijke/Shell-Laboratorium, Amsterdam (Shell Research B.V.), Badhuisweg 3, 1031 CM Amsterdam, The Netherlands.

Reduced washing temperatures decrease the rate of the various processes in a laundry cleaning cycle. This implies that fast acting detergents are needed if the present washing performance is to be maintained within a realistic period of time. An important factor is the rate of oily soil removal which, among other things, is a function of the molecular structure of the surfactants used in the detergent composition. To support the selection of proper surfactants we have established relationships between chemical structure and rate of oil solubilization for a series of alkylarenesulphonates with various alkyl chain lengths, points of attachment of the phenyl group at the alkyl chain, and aromatic substitution patterns. It is shown that oil solubilization kinetics are very sensitive to the geometry of the surfactant structure: for a set of isomeric alkylarenesulphonates the rate of oil solubilization can be made to vary by more than an order of magnitude by changing the substitution pattern around the aromatic ring. These effects of surfactant structure on performance will be related to the properties of the micelles formed in water solution, in particular their aggregate size and diffusion coefficient. The results offer a predictive tool for the design of molecules with the proper surface activity under a wide set of experimental conditions.

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A STUDY ON THE ADSORPTION OF DIALKYLDIMETHYL AMMONIUM CHLORIDE (PART 2) THE ADSORPTION BEHAVIOR OF DIALKYLDIMETHYL AMMONIUM CHLORIDE NONIONIC SURFACTANT SYSTEM. Kenji Yokoi, Hisami Susaki and Osamu Okumura, Lion Corp., Development Laboratory II, No.13-12,7-chome, Hirai, Edogawa-ku, Tokyo 132, Japan.

In a preceding paper (Okumura, O., K. Ohbu, K. Yokoi, K. Yamada and D. Saika, J. Am. Oil Chem. Soc.), the authors reported the results of structural analysis and adsorption behavior toward fabrics of an aqueous dispersion of Arquad 2HT (2HT), or di(hydrogenated tallow-dimethyl ammonium chloride, the most widely used softener base. Following this report, a comparative study was conducted to elucidate the adsorption behavior and structure of binary systems consisting of 2HT C_{12} fatty alcohols, their ethylene oxide ("E") adducts or polyoxyethylene (POE") nonyl phenyl ether with respect to the hydrocarbon chain length and the number of EO units per molecule ("EOP") of the latter which are important auxiliary agents for softeners. By HPLC measurements following Soxhlet extraction of the treated fabrics, it was found that POE C_{16} or $_{18}$ alkyl ethers showed approximately equal adsorption ratio to that of 2HT regardless of EOP in sharp contrast to POE nonyl phenyl ehters whose adsorption ratio had apparent dependence upon EOP, whereas that of 2HT was almost constant in every system depending upon alcohols and their EO adducts had, the

higher adsorption ratio they showed, if EOP of the latter was constant. In order to explain the above-mentioned adsorption behavior and to estimate the adsorption structure of the binary systems, the structural analyses of the aqueous dispersions were done by Differential Scanning Calorimetry, X-ray diffraction and ESR spin label (or probe) methods.

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NEW AMINO ACID DERIVED SURFACTANTS. Kouichiro Sagawa and Masahiro Takehara, Ajinomoto Co., Inc., 1-1 Suzuki-Cho, Kawasaki-Ku, Kawasaki, 210 Japan.

New surface active agents derived from L-Lysine were synthesized and their physicochemical properties investigated. Nε-Acyllysine (AL) was obtained by thermal dehydration of L-Lysine and fatty acid. AL has both hydrophilic and hydrophobic groups in it and is structurally an amphoteric surfactant. However, AL was insoluble in both polar and nonpolar solvents, because of a formation of intermolecular salts and of its strong intermolecular hydrogen bond by amino and amide groups. Consequently, AL didn't show ordinary surface active properties such as foaming power and emulsification power. However, it was found that AL had an extremely low coefficient of friction and excellent lubricating power because of a flat leaflet crystalline form and was easily fractured along cleavage planes. further, AL showed a high water repellency, a good antioxidant ability and an excellent activity as a heavy metal chelating agent. Nα,α-Dimethyl*Nε-acyllysine (DMAL) and Nα,α,α*Nε-acylly sine (TMAL) which were obtained by methylation of the α-amino group of AL, turned out to be water soluble, and showed the surface active properties of amphoteric surfactants. these surface active properties such as cmc values, Krafft points and foaming powers, were measured.

22

SOLUTION PROPERTIES OF MIXED SURFACTANT SYSTEMS. Keizo Ogino, Toshiaki Kakihara, Hirotaka Uchiyama and Masahiko Abe, Department of Industrial and Engineering Chemistry, Faculty of Science and Technology, Science University of Tokyo, 2461, Yamazaki, Noda, Chiba, Japan 278.

Solution properties of mixed anionic-nonionic surfactant systems in aqueous solutions have been studied in terms of surface tensions, electrical conductivities and dielectric constants. These systems were sodium dodecyl sulfate (SDS) alkyl polyoxyethylene ethers ($C_{16}POE_n$; 10, 20, 30, and 40). The molecular interaction parameter($-\beta$) increased with a decrease in the number of oxyethylene groups increased, the electrical conductivities of the mixed surfactant solutions decreased, in spite of decreased activation energy for conduction. The radius of the mixed micelle with the electric double layer was larger for a nonionic surfactant including a shorter polyoxyethylene chain length than for one having a long chain. This may be attributed to the fact that mixed micelles are formed more easily by a nonionic surfactant including shorter polyoxyethylene chain length than by one having long chain lengths. In this paper we will also discuss the effect of alkyl chain lengths in the nonionic surfactant on the mixed micelle formation. We will consider that mixed micelles are formed more easily by a nonionic surfactant including long alkyl chain length than by one having shorter alkyl chain length.

23

STUDIES ON DERIVATIVES OF α,ω DIBASIC ACIDS. I. HOST-GUEST EMULSIONS BY BISGLYCERYL ESTER OF LONG-CHAIN α,ω-DIBASIC ACIDS. Mitsumasa Takasago and Kazuo Horikawa, Osaka Municipal Technical Research Institute, 1-6-50, Morinomiya, Joto-ku, Osaka 536 Japan, and Shinroku Masuyama, Tezukayama Junior College.

We carried out the study of bisglyceryl esters as a series in the research development on derivatives of α,ω dibasic acids. Dodecane, hexadecane and icosane-dioic acids bisglyceryl esters were prepared by the direct esterification of 3-chloro-1,2-propanediol with potassium salts of those acids. The esters were purified by repeated recrystallization from benzene. The structures were determined by means of TLC-FID, NMR and elemental analysis. Vegetable oils or minerals oils and water were emulsified into O/W type high viscosity emulsion by using these esters. The range of observed particle diameter of these emulsions as from 0.2 mm to 0.4 mm. It is found that the size of the particles is very much larger than the particles of ordinary emulsion (from $5X10^{-4}$ mm to $5X10^{-2}$). The emulsifying power, the relationship between viscosity and composition, and the stability of the emulsion under the different conditions are discussed. The results of these measurements revealed that these esters function as a special emulsifier different from ordinary emulsifiers. We have, in consequence, given a presumption of cream configuration composed of "host-guest phenomena."

Session E Thursday morning Symposium on New Oils with Industrial Potential

24

THE STATUS OF MEADOWFOAM (*LIMNANTHES*) DEVELOPMENT OF A NEW INDUSTRIAL OILSEED CROP. Gary D. Jolliff, Oregon State University, Corvallis, OR 97331-3002.

Meadowfoam was domesticated and commercially produced in the Willamette Valley of Oregon. Local interest was precipitated by the need for alternatives to grass seed field burning. A meadowfoam growers association was formed in 1984 to organize producer interest and to aid in minimizing the "boom/bust" production cycles which often hinder new-crop development. The first cash sale of oil was made in 1985 to a Japanese firm for use in the cosmetic industry. Meadowfoam development is at a delicate stage. Interest level is closely associated with apparent profit potential, yet relatively little research has been done to increase seed yield and to define high-value applications of the unique composition of the oil. A stable, long-term commitment to development is needed.

25

PRESENT STATUS OF DOMESTIC HIGH-ERUCIC OILSEED CROPS. E. Charles Leonard, Humko Chemical, P.O. Box 125, Memphis, TN 38101; Ron L. Haaland, Sun Rise, Inc., Auburn, AL, and Durwood Beatty, Murray State University, Murray, KY.

High $C_{22:1}$ triglyceride oil is used to produce a variety of specialty chemicals. Almost all of the vegetable oil used currently to produce these chemicals is imported into the U.S. from Canada

Errata

The 1986 annual meeting registration form has three errors. Correct price for Friday luau is $25. Correct price for golf tournament is $60. Section cocktail parties are on Thursday, May 15.

and Eastern Europe. This paper describes efforts to commercialize these oilseed crops in the Southeastern U.S.—work applied to both rapeseed and to crambe. Particular emphasis is placed on the key to successful implementation of new commercial crops—the integration of agronomic, logistical, processing and marketing strategic planning into a simultaneous coordinated effort. Emphasis will be placed also on the concept of a world market for oleochemical feedstocks and the pricing cycles of these materials.

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THE COMMERCIALIZATION OF JOJOBA: NEARING THE HORIZON. Carole Anne Whittaker, Hyder Jojoba, Inc., 3320 E. Shea Blvd. #290, Phoenix, AZ 85028.

To the extent that jojoba can be commercialized as an agro-industry, the jojoba oil can provide a direct, renewable source of high-molecular weight, unsaturated wax esters and monounsaturated fatty alcohols and fatty acids. The double bond and ester linkage of the wax ester molecule provide sites for synthesizing a number of potentially interesting derivatives. There are approximately 45,000 acres of jojoba under cultivation in the Sonoran Desert of Mexico and the southwestern U.S. and 2,000 acres in Israel. Approximately 6,000 acres are expected to be commercially harvested in 1986. Seed yield from these plantations alone is expected to increase from 1,000 tons in 1986 to 25,000 tons per year by 1995. In recent years, jojoba oil obtained from harvesting native plants has been sold at prices ranging from $4 per pound up to $30 per pound in quantities of 200 tons per year. As plantations come into production, prices are expected to stabilize quickly in the range of $5-6 per pound and to decrease eventually to $2 per pound as harvesting and processing efficiency are improved and higher yielding cultivars are developed. In anticipation of increased supplies and stabilized prices available from plantation-produced jojoba oil, several large companies have initiated research and development programs aimed at utilizing jojoba. New products containing jojoba oil have been developed in cosmetics, lubricants and low-calorie foods. Jojoba oil is being evaluated in industrial processes including the production of penicillin, magnetic storage media and synthetic fibers. The final success of jojoba as an agro-industry will depend critically upon the degree of cooperation that can be achieved between the producer and user industries, government and research institutions in matching the supply/demand/price requirements of the user industries with those of jojoba producers.

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VERNONIA GALAMENSIS, A PROMISING NEW CROP FOR SEMI-ARID AREAS AND THE TROPICS AND SUBTROPICS. R.E. Perdue Jr., Plant Exploration & Taxonomy Lab, ARS, USDA, BARC-East, Bldg. 003, 03-1 Beltsville, MD 20705.

An Agricultural Research Service program identified *Vernonia anthelmintica* (L.) Willd. (India) as a promising natural source of seed oil rich in epoxy acid. Chemists conducted research on the oil to establish markets. Agronomists developed improved lines, with 25% oil in the seed and up to 75% epoxy acid in the oil, for cultivation in the U.S. Yields were limited by poor seed retention. *V. galamensis* (Cass.) Less. (Senegal and Guinea to Ethiopia, south to Zimbabwe and Mozambique) is a superior source. Seed from Ethiopia contained 40% oil and 75% epoxy acid in the oil. Under cultivation in Kenya, this unimproved germplasm produced an impressive yield of seed with up to 42% oil and 80% epoxy acid. *V. galamensis* has good natural seed retention. Small preliminary trial plantings in Zimbabwe in 1984 provided seed yields up to 2,000 kg/ha. A large (0.77 ha.) 1985 planting yielded 2,600 kg/ha.

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CHINESE TALLOW AS A SPECIALTY OIL CROP: PROGRESS IN DEVELOPMENT. H.W. Scheld, Phyto Resource Research, Inc., 707 Texas Avenue, Suite 202D, College Station, TX 77840.

The Chinese tallow tree has been studied sporadically in the U.S. for over 60 years. The primary emphasis, during this time, has been upon oil chemistry. In the last six years, a more concerted effort has emphasized accumulation of a data base to support development of the tree as a modern specialty oilseed crop in the U.S. and other parts of the world. Current work is focused upon relatively basic aspects which govern the approach taken in configuration of the modern crop and the definition of product emphasis. Several strains have been selected and tissue microculture studies are underway with this selected material aimed at understanding the genetic potential of the tree and at development of methodology for rapid, large-scale multiplication of strains having desirable physiological and oil chemistry characteristics. Field and laboratory studies of tree biochemistry have focused upon the biochemical ecology in insect resistance and upon the special problems of the phorbol ester content of the tree parts, the genetic relationships of phorbol esters and the potential hazard posed by this class of chemical constituents. Physiological mechanisms governing environmental adaptation and productivity are being studied in terms a capability of the root systems for utilization of mineral nutrients from deep soil horizons and the control of carbon allocation in growth and seed production.

29

CURRENT RESEARCH ON CUPHEA, A NEW MEDIUM-CHAIN FATTY ACID SOURCE. Anson E. Thompson, U.S. Water Conservation Lab, ARS, USDA, 4331 E. Broadway Road, Phoenix, AZ 85040.

A unique 3-way, equally funded program for the development of cuphea as a new industrial oilseed crop by USDA/ARS, the Oregon State AES and member companies of the Soap and Detergent Association is in progress. Cuphea seed oils contain high levels of lauric ($C_{12:0}$) and other medium-chain fatty acids. Major constraints of domestication and production are excessive seed shattering, seed dormancy, indeterminate flowering and seed production, and sticky resins from glandular hairs. Germplasm is being evaluated to determine agronomic potential and adaptability in 11 locations throughout the U.S. Interspecific hybridization; mutation breeding; tissue, anther and protoplast culture and the possibility of somaclonal variation are being utilized to generate, recombine and develop new sources of genetic variability to remove constraints to the successful development of this potentially useful new crop.

Session F Thursday morning Symposium on Hazard Identification in Manufacturing

30

AN OVERVIEW OF HAZARDS ANALYSIS. Paul Baybutt, Battelle's Columbus Division, 505 King Ave., Columbus, OH 43201.

The accidents in Bhopal, India and Mexico City last year have caused a great deal of attention to be focused on the risks posed by chemical process industries. Many companies have initiated efforts to appraise their safety programs: the Chemical Manufacturers Association has organized meetings on hazards analysis; the American Institute of Chemical Engineers has established a Center for Chemical Process Safety, and the National Association of Manufacturers has organized a meeting on gas dispersion. There is great concern in industry that new government regulations may be established to control the use of hazardous materials by industry. The various efforts by industry are, in part, intended to forestall such developments. However, regulatory agencies such as EPA and OSHA already have instituted projects to examine this subject, and

some form of regulation is quite possible. These events parallel developments in Europe during the 1970s, where major accidents at Flixborough, UK, and Seveso, Italy, focused attention on chemical process industry risks. This lead to the establishment of major hazards legislation in a number of European countries. Battelle has recently completed the preparation of a set of guidelines for hazards analysis for AIChE's Center for Chemical Plant Safety. These guidelines are intended to help chemical process industries identify hazards and thus provide the basis for their control. These developments are described in detail in this paper, and their implications for U.S. industry are discussed. An overview is provided of methods for hazard identification and control, and an approach to risk management is described.

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HAZARD AND OPERABILITY ANALYSIS EXPERIENCE. S.J. Schechter, Process Hazard Analysis Manager, Rohm and Haas Co., Box 584, Bristol, PA 19007.

A description will be given of the approach used for HAZOP studies at a specialty chemicals manufacturing company. Types of results generated will be provided along with a case study involving a flammable solvent.

32

DOW'S FIRE AND EXPLOSION INDEX. D.V. Gagliardi and Thomas O. Gibson, The Dow Chemical Company, 2030 Willard H. Dow Center, Midland, MI 48674.

The Dow Fire and Explosion Index first evolved from the 1964 Factory Mutual Chemical Occupancy Guide. The Dow Index is a number from which the degree of risk can be evaluated along with the dollar value for property damage and business interruption. This systematic approach does correlate with some engineering data and, therefore, has a great deal of reliability in risk management application. The system does look at material response and risk exposure along with the protective schemes installed. This F & EI can help to evaluate the impact of protective schemes on days outage and property damage.

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HAZARD CONTROL FOR OILSEED PROCESSING AND REFINING. Harold J. Sandvig, vice president and Corporate Safety Director, Cargill, Box 9300, Minneapolis, MN 55440.

The development of an effective hazard identification program begins with hazard recognition. Industry cannot implement a successful safety and loss control program without first properly identifying hazards. This paper deals with the methodology of hazard identification used in the preparation, solvent extraction and refining of oilseeds and begins with education and training. It addresses the use of simple mechanical devices as well as computer controlled systems for hazard monitoring and concludes with the control of the hazardous situation or recognized hazard.

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HAZARD IDENTIFICATION AND CONTROL IN THE OLEOCHEMICAL INDUSTRY. R.C. Slagel, Chemical Products Division, Union Camp Corp., P.O. Box 2668, Savannah, GA 31402.

Accidents affect lives and profits dramatically. On top of the personal anguish due to injury or death, the cost of lost time accidents has tripled over a decade ago. In 1983 the average cost of a lost workday case was nearly $18,000. The cost of facility repair or replacement can easily exceed that figure. Identification of hazards prior to facility installation or process implementation is a necessary first step to prevent accidents. Hazard control is accomplished through equipment design, equipment maintenance, employee training and, above all, management commitment. Procedures for the above will be reviewed with special emphasis on peer review and special hazards such as solvent, hydrogenation, high pressure, high vacuum, toxic chemicals and dust explosions.

35

SAFETY IN HYDROGEN MANUFACTURING AND HYDROGENATION PROCESSES. S.N. Milazzo, President, S.N. Milazzo Associates, Inc., P.O. Box 5147, Greenville, SC 29606.

New innovations in plant design have led to unique safety problems necessitating changes in methods of handling liquids and gases. The identification of potential hazards is detailed, with emphasis on preventive maintenance. A review of widely used hydrogenation process parameters will be outlined with detailed designs to afford safe, trouble-free plant operation.

Session G Thursday morning Symposium on Biological Oxidation of Lipids I

36

DAMAGE TO DNA, ENZYMES AND PROTEINS BY LIPID PEROXIDATION AND OTHER OXIDANT REACTIONS. Al L. Tappel, University of California, Davis, Department of Food Science and Technology, 1480 Chemistry Annex, Davis, CA 95616.

Peroxidative damage to DNA was studied in rats fed either a diet with 10% tocopherol-stripped corn oil and 30 IU DL-α-tocopheryl acetate/kg (+E), the same diet without vitamin E (−E), or a diet with 24% corn oil without vitamin E (UF-E). During 14 mo of feeding −E and UF-E, some body parameters showed adverse effects, and lipid-soluble fluorophores in testes increased. +E and −E had higher hepatic DNA template activities at 9 and 14 mo than UF-E. +E had higher testicular DNA template activities than −E and UF-E at 6, 9 and 14 mo. Hepatic DNA template activity decreased from 6 to 14 mo. DNA-bound tryptophan and DNA crosslinking were inversely related to DNA template activities. Thus, DNA damage correlated with biochemical and physiological changes characteristic of cellular impairment in vitamin E deficiencies. A study of dietary vitamin E effects on methyl ethyl ketone peroxide (MEKP) damage to cytochrome P-450 and its enzymatic activity was done. In vivo, MEKP damaged liver cytochrome P-450 and P-450-mediated peroxidases in vitamin E-deficient rats. Dietary vitamin E protected microsomal enzymes from peroxide damage. In vitro, MEKP damage to enzymes was highest in vitamin E-deficient microsomes, and addition of MEKP induced more thiobarbituric acid-reacting substances in liver microsomes from vitamin E-deficient than from supplemented rats. Adequate vitamin E, NADH and NADPH are necessary to protect the endoplasmic reticulum during metabolism of toxic organic peroxides in vivo.

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MEMBRANE DAMAGE IN ERYTHROCYTES AFFECTED BY RADICALS AND THE EFFECT OF TOCOPHEROL AS A RADICAL SCAVENGER. Makoto Mino, Masayuki Miki, Hiroshi Tamai and Hiroshi Yasuda, Department of Pediatrics, Osaka Medical College, 2-7, Daigakucho, Takatsuki-City, 569, Japan.

In this study, two different radical initiators were used to determine membrane damage in erythrocytes (RBCs), one was hypoxanthine-xanthine oxidase (HX-XOD) system and the other an autodegradation system of 2,2′-azobis(2-amidinopropane) hydrochloride[AAPH]. Hemolysis was induced by HX-XOD system in vitamin E deficient RBCs. Prior to the hemolysis, an increase in chemiluminescent values occurred that was followed by the increase in TBA-reacting substances. In the hemolysis, a decrease in phospholipids and arachidonic acid in the RBCs was observed, ac-

companying a constant consumption of oxygen. Fluorescent substances (Ex:355nm, Em:450nm) also increased. The tocopherol concentration of 150 μg/100 ml packed cells was the critical level to inhibit the hemolysis, and tocopherol concentrations in excess of the critical level could inhibit hemolysis without any consumption of the tocopherol involved. With the AAPH reaction, on the other hand, the large amount of tocopherol in RBCs decreased constantly and hemolysis developed when tocopherol was consumed up to the critical level. However, the lipid changes observed in HX-XOD reaction were not marked in the AAPH reaction even when hemolysis developed. Catalase inhibited the hemolysis induced by HX-XOD, while no marked inhibition was observed in the AAPH. These findings indicate membrane damages occurring in different manner between the two systems. Tocopherol in RBCs inhibited the above lipid changes in both systems.

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PEROXIDE TONE IN REGULATING EICOSANOID FORMATION. William E.M. Lands, Department of Biological Chemistry, University of Illinois at Chicago, 1853 W. Polk St., Chicago, IL 60612.

The initial committed step in the biosynthesis of prostaglandins and leukotrienes is catalyzed by fatty acid oxygenases. These enzymes selectively convert polyunsaturated fatty acids into lipid hydroperoxides and also require a lipid hydroperoxide to catalyze rapid oxygenation. Thus, the oxygenases provide an intracellular amplification of triggering amounts of lipid hydroperoxides which raises the ambient hydroperoxide abundance. Production of peroxides and hydrogen peroxide by cellular responses to environmental challenge can trigger further increases in lipid hydroperoxides and thus enhance eicosanoid biosynthesis in adjacent cells. The oxygenation reaction may be suppressed physiologically by constraining the accessibility of the oxygenase to optimal concentrations of either substrate fatty acid or hydroperoxide activator. The local steady-state concentration of lipid hydroperoxide (peroxide "tone") thus represents an important factor regulating the action of lipoxygenases and cyclooxygenase in forming leukotrienes and prostaglandins. Cellular peroxidases function to keep a low abundance of intracellular hydroperoxides, removing the peroxides generated by the oxygenases. Inhibitors of oxygenase action may also tend to keep the abundance of hydroperoxides from increasing excessively. The ambient levels of hydroperoxide required by n-3 fatty acids reacting with cyclooxygenase are much higher than those for n-6 fatty acids. Thus the n-3 acids are poor amplifiers in this system and can antagonize the rapid conversion of n-6 acids into prostaglandins. This antagonism may underly the apparent ability of dietary seafoods to diminish the occurrence of certain disease processes.

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FREE RADICAL LIPID PEROXIDATION IN STRESS-INDUCED GASTRIC MUCOSAL LESIONS IN RATS. Toshikazu Yoshikawa, Haruo Miyagawa, Norimasa Yoshida and Motoharu Kondo, First Department of Medicine, Kyoto Prefectural University of Medicine, Kamikyo-ku Kyoto 602, Japan.

This study was designed to determine whether free radical lipid peroxidation plays a role in the pathogenesis of gastric mucosal lesions by water immersion restraint stress and burn shock in rats. Rats were restrained in a wire cage and immersed in 23 C water to the depth of the xiphoid for 3 or 6 hr producing water immersion stress, or immersed in 80 C water for 10 sec producing a 60% body surface area burn. Local blood flow in the gastric wall was determined by the hydrogen clearance method. Thiobarbituric acid (TBA) reactants were measured in serum and gastric mucosa. The serum α-tocopherol level was assayed using high performance liquid chromatography. After induction of stress, the blood flow in the gastric wall was significantly decreased, and TBA reactants in serum and gastric mucosa were significantly increased. Serum α-tocopherol levels were immediately increased after the stress and then decreased. Superoxide dismutase and d-α-tocopherol significantly protected against stress-induced gastric lesions. However, catalase had no effect. These findings suggest that free radical lipid peroxidation plays an important role in the formation of gastric lesion produced by stress.

Session H Thursday afternoon Symposium on Oilseed Proteins-Texturization

40

THE APPLICATION OF NEW EXTRUSION PROCESSING METHODS OF TEXTURED VEGETABLE PROTEIN PRODUCTION. Joseph P. Kearns, Wenger International, Inc., One Crown Center, Suite 510, 2400 Pershing Rd., Kansas City, MO 64108.

Textured vegetable proteins have been a commercial success for many years; this resulted from the development of machinery that was capable of continuously producing these products efficiently and uniformly. Over the years many of the variables have been studied that relate to production of textured vegetable proteins in an effort to improve the quality and usage of these products. These variables include ingredient specifications, machinery and processing conditions and how these affect the end products. This paper will review production of textured vegetable proteins as they are presently produced as well as new methods. These processing methods will be related to production by single and twin screw extrusion systems. Also discussed will be the changes in raw material selections and specifications, the range of final products that can be produced as well as their functional properties.

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STUDIES ON THE PROCESSABILITY OF SOY PROTEIN AND ITS RHEOLOGICAL PROPERTIES. Isao Hayakawa and Nobuyuki Hayashi, Department of Food Science and Technology, Faculty of Agriculture, Kyushu University, Hakozaki, Higashi-ku, Fukuoka City, Fukuoka, 812 Japan.

The extrusion method is different from the spinning method during processing, but rheological properties of materials inside the running barrel are almost the same as those of the spinning dope. The mechanism of both texturizations is considered to be one of expression of relaxation phenomena. Measurement of the flow properties of soy protein under high temperature and high pressure is conducted with a flow tester, and those of the dope were measured by a rheometer. Shear rate was most effective on the denaturation of soy protein. Especially the viscosity reduction (less than 1000 poise) was very large when the soy protein was treated with a faster shear rate than about 3000/second under the above conditions. Spinnability of dope was influenced by velocity gradient of dynamic storage modulus at the terminal zone when the dope indicated good spinnability. This phenomena is also brought about by the decrease of the molecular weight of the protein dope by alkaline hydrolysis. Those symptoms are those of the relaxation phenomena, and were easily changed by experimental conditions such as solids content and processing temperature. These delicate changes of rheological properties were caused by the decrease of viscosity and elasticity components of soy protein due to the decrease of molecular weight and the molecular orientation during processing.

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EXTRUSION COOKING IN HIGH MOISTURE: MEAT ANALOGUE FROM DEFATTED SOY FLOUR WITH TWIN SCREW EXTRUDER. Akinori Noguchi, National Food Research Institute, 2-1-2, Kannondai, Yatabe, Tsukuba, Ibaraki 305 Japan; Lim Jae-Kag, Korea University, Korea, and Sukeyoshi Wakamiya, Mitsubishi Heavy Industries, Ltd.

A method of extrusion cooking in high moisture was developed

for defatted soy flour (DSF) using a twin screw extruder. This method produced a dense meat analogue with high moisture and layered fibers, showing significant lengthwise strength more than three times that of the crosswise strength. The twin screw extruder used in the study was Creusot-Loire BC 45, equipped with a breaker plate and a cooling slit die. In the optimum process, 14 kg/hr of DSF (NSI ca. 30) with 60% moisture was adjusted to pH 7 and extruded at 180 C. The extrudate retained its original moisture content and was in the form of thin strips. The destruction of extrudate was observed by reducing the slit width at the outlet or middle position of the die, which indicated that the final structure of extrudate was achieved just after the breaker plate. Light and scanning electron microscopic analysis revealed that the extrudate was composed of well oriented fine protein strings and that the structure was disturbed by oil addition, resulting in the remarkable strength reduction. SDS-PAGE showed no significant change in soluble protein fractions. The extrudate strength and soluble protein fractions were not affected by monoiodo acetate. These results suggest that the covalent bonds except —S—S— bond contributed most to the protein texturization.

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MICROSTRUCTURAL INVESTIGATION ON TEXTURIZED SOY PROTEIN PRODUCT. Stanislaw Gwiazda, Agricultural University of Warsaw, Bernardniska 3/30 02904, Warsaw, Poland, and Akinori Noguchi and Kyoko Saio, National Food Research Institute.

The present paper deals with microstructural changes of soybean flour in a twin screw extruder (Creusot-Loire BC-45) attached to a long cooling die. The following two experiments were carried out: (1) effects of addition of oil to soybean flour on the microstructure, and (2) observation of microstructure of the inside materials at several positions of the extruder under normal operation. Small pieces of the extrudates and soybean flour, coagulated in lukewarm agar, were respectively fixed by glutaraldehyde and osmium tetraoxide solutions, dehydrated and embedded in Epon resin. The block obtained was sliced into sections and observed by light and/or transmission microscopy. The result of (1) showed that when higher concentration of oil was added, weaker fibrous structures resulted and the orientation of fiber changed from lengthwise to crosswise. The results of (2) showed that microstructure clearly changed from feeding part to die end of the extruder and that the use of long cooling slit die was effective to get well oriented structure.

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RESEARCH ON EXTRUSION COOKING OF TOFU BY USE OF TWIN SCREW EXTRUDER. Yoshinobu Akiyama, Sakashi Yoshihashi and Iwao Sakauchi, Kyodo Milk Industry Co., Ltd., 1-4-18 Shin-Machi, Hoya-City, Tokyo, Japan 202.

A method for the preparation of tofu-meat analogues by use of twin screw extruder and evaluation of the quality of the extrudate is described. In this experiment, pressed and dried tofu was subjected to extrusion cooking, using a twin screw extruder (Creusot-Loire BC-45). The evaluation of the quality of extrudate was carried out by the following techniques: (i) fine structure by scanning electron microscope (SEM); (ii) investigation on heat-denaturated proteins by SDS PAGE, and (iii) digestive characteristics in the artificial stomach. SEM revealed that the extrudate has a fine structure similar to that of other texturized vegetable products (TVP). Clear differences were not found between tofu-meat analogues and TVP in the electrophoretic patterns. The digestive characteristics of tofu-meat analogues were almost the same as another processed tofu such as aburage. These results suggest the soy protein in tofu is texturized in the same way as TVP. The odor of the tofu-meat analogue was desirable and requires no deodorization. The tofu-meat analogue just after extrusion was edible without any additional processing.

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THE APPLICATION OF TEXTURED PROTEINS IN VEGETARIAN FOOD SYSTEMS. Richard S. Leiss, Worthington Foods Inc., 900 Proprietors Rd., Worthington, OH 43085.

The use of textured proteins in vegetarian food systems is reviewed and includes a brief history of the development of vegetarian entrees in the United States. The unmodified cereal protein wheat gluten was used in early entree development because of unique physical properties which impart the desired textural attributes to the finished product. Currently, textured soybean protein products are used in commercial vegetarian foods alone and in combination with wheat gluten. The manufacture of vegetarian entrees will be discussed.

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TEXTURED WHOLE SOYBEAN PRODUCT: ITS PROPERTIES AND APPLICATION. Tokuya Harada, Hideaki Shiga and Kazuko Ohata, Kobe Women's University, Aoyama, Suma-ku, Kobe-shi, Japan 654, and Megumi Mukaiyama and Shigehiko Sato, Asahimatsu Food Inc.

Using an apparatus with a structure similar to a stone mill in principle, we succeeded in manufacturing a new type of textured whole soybean product. In the process, steamed soybeans are introduced between a stationary and a rotating disk separated by a small gap. After passing through the gap, the soybean emerges in the form of fibers, flakes or granules depending upon operating conditions. Extending or (and) folding the bean by the apparatus was conducted under steaming. The process was performed without separating oil, under appropriate conditions such as water content of soybean, rotation speed of disk and gap distance between the two disks of the mill. TWSP thus obtained, shows remarkably less beany flavor than conventional vegetable protein products which are made from defatted soy flours. Besides, TWSP is suitable to mix with a variety of other food materials, so this has many applications as a new food material for processed food and home dishes. Nutritional values of TWSP were compared with those of casein or of granular type of vegetable protein product by digestibility by mice and enzymatic hydrolysis. All the samples tested gave almost the same values by both experiments. Electron micrography of the samples may explain the suitable properties of TWSP for its uses to food. Our product is stable to storage without significant loss as a food material.

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EFFECT OF SOY PROTEIN SOLUBILITY ON EMULSION CURD CONFORMATION. Hideyuki Tenmyo, Teruo Gomi, Toshihiro Tsuchiya and Keiko Baba, Ajinomoto Co., Inc., 1-1, Suzuki-cho, Kawasaki, 210 Japan.

We prepared emulsion curd using soy protein isolate of different solubility, soybean oil and water, and changing the ratio of protein-oil-water. We investigated the relation between the structure-texture of emulsion curd and the solubility of soy protein. We evaluated emulsion curds by various methods. The textural properties were measured by texturometer and rheometer. The viscoelastic properties were measured by creepmeter. The surface structures were observed by scanning electron microscopy. From the results so far obtained, it is clear that in consequence of increasing solubility of soy protein, high oil emulsion curd could be prepared easily, and its hardness is stronger and its surface structure smoother at the same time.

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PRODUCTION OF EXPANDED FOODS FROM SOY PROTEIN EMULSION. Masahiko Terashima, Kazuto Mashima, Toshiyuki Nagata, Tsutomu Katayama and Hitoshi Taniguchi, Fuji Oil Co. Ltd., 1-Sumiyoshi-cho, Izumisano City, Osaka, Japan.

"Aburage" and "Ganmodoki" are Japanese traditional fried soy-

bean foods and are made from "Tofu." Therefore long experience is necessary to produce these foods. The production processes of "Tofu" and soy protein isolate (SPI) are similar, but until recently no one has researched the production of "Aburage" and "Ganmodoki"-like foods from SPI. Therefore we investigated the production of them from SPI emulsion and considered their expansion mechanisms. Food grade SPI (Fujipro-R), soybean oil and water were mixed and emulsified using the Stepan Universal Machine. The emulsion of SPI was formed in a flat square (45 × 45 × 5 mm) for "Aburage" and a disk (55 × 12 mm) for "Ganmodoki" and fried. The frying processes consisted of pre-heat, low temperature and high temperature; standard conditions were 70 C for 7 min, 110 C for 3.5 min. and 170 C for 3 min, respectively. The expansion ratio was calculated from the surface areas of the raw materials and final products of the SPI emulsion. The added water for optimum expansion ratio was about 3 times the water content of SPI. When the added water was increased more than 3 times or decreased less than 3 times, the expansion ratio was reduced. The amount of added oil influenced the expansion ratio negatively. When oxidants such as hydrogen peroxide were added into the SPI emulsion, its expansion was good but the expanded final products shrank when cooled. On the other hand, reductants like sodium bisulfite reduced its expansion. Preheat treatment was necessary and heating at 60–70 C for 5–10 min was suitable for good expansion. However, a long period of preheating induced shrinking of the expanded final products when cooled and a pre-heating higher than 85 C ballooned the SPI emulsion during frying.

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PROPERTIES OF MILK ANALOGUES WITH SOYBEAN PROTEINS AND CHEESE WHEY. V.H. Holsinger, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.

Milk analogues prepared with plant proteins, especially soybean, have been developed and evaluated in many areas where milk and other high quality protein sources are scarce. Problems of flavor, acceptability, texture, product stability and cost have been encountered. Because soy proteins are low in sulfur amino acids, nutritive value can be increased by combination with cheese whey proteins which contain significant amounts of methionine and cystine. Protein efficiency ratio of a spray dried beverage mix containing soybean flour and cheese whey was 2.1 compared to reference casein at 2.5 and soybean flour at 1.8. Processing factors were related to redispersibility of this product in water. Rehydration properties measured included dispersibility, free fat content of the powder, soluble nitrogen (NSI), average equivalent spherical diameter (d_e) of the dispersed particles and phase separation on standing after reconstitution. Examination by light and scanning electron microscopy after rehydration revealed that many rehydrated particles were proteinaceous material coated with fat droplets. The hydrophobic surface inhibits aggregate dispersal during rehydration, enhancing phase separation after reconstitution.

Session I Thursday afternoon Symposium on Phytolipids II

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CURRENT CONCEPTS ON FATTY ACID BIOSYNTHESIS. P.K. Stumpf, University of California, Department of Biochemistry and Biophysics, Davis, CA 95616.

Much is now known about the mechanism of fatty acid biosynthesis in procaryotic and eucaryotic (animal) systems. In most procaryotic systems the enzymes responsible for fatty acid biosynthesis are discrete separate proteins which make up a non-associative system. In eucaryotic (animal) systems, all the reactions required for synthesis are catalyzed by domains located on two separate protein subunits. In yeast the subunits are dissimilar, but in animal cells the subunits are identical. In all higher plants, the molecular structure of the fatty acid synthase (FAS) is almost identical to the procaryotic non-associative system. In addition, the separate enzymes are compartmented; in leaf tissues the FAS enzymes are localized in the chloroplast stroma; in the seed and mesocarp tissue they are localized in the proplastid. The Δ^9 desaturase is also associated with the same compartment. However, the Δ^{12} desaturase is found in endoplasmic reticuli particles. With these data at hand, a number of correlations and predictions can now be made.

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CHLOROPLAST LIPID METABOLISM. J.B. Mudd, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568.

The synthesis of fatty acids in higher plants is entirely dependent on enzymes localized in the plastid. Although the membranes of the chloroplast (envelope and thylakoids) comprise more than 70% of the membrane structure of the higher plant cell, a large fraction of the intermediates is obligatorily metabolized in the cytoplasmic compartment. Fatty acids are exported to the cytoplasm where they are incorporated into diacylglycerol (DAG) moieties which serve as precursors for the glycolipids of the chloroplast. Although the galactosylation reactions forming the galactolipids take place in the plastid envelope, the head group donor (UDP-galactose) is fabricated in the cytoplasm. On the other hand the head group donor for the synthesis of sulfoquinovosyldiacylglycerol (SQDG) is probably made in the plastid. The phosphatidylglycerol (PG) of the plastid is unique in that it is synthesized entirely in the plastid. The first acylation of glycerol-3-phosphate (G-3-P) takes place in the stroma. The acylation is specific for the sn-1 position. Positional analysis of the fatty acids of PG indicates that in some plants there is specificity for oleic acid (18:1) but in other plants there is no selectivity. Acylation at the sn-2 position takes place in the plastid envelope and is highly specific for palmitic acid (16:0). Further intermediates in the synthesis of PG are cytidinediphosphatediacylglycerol (CDP-DAG) and phosphatidylglycerol phosphate (PGP). Both of these intermediates are synthesized in the inner membrane of the plastid envelope. The selectivity for head group transfer to molecular species of DAG is not yet understood. The pool of DAG giving rise to monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and SQDG is probably the same, and yet there are marked differences in the fatty acid composition of these three lipids. There is also evidence that the formation of CDP-DAG is specific for molecular species of phosphatidic acid (PA). The molecular species of lipids synthesized in plastids may have important consequences for membrane function.

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REGULATION OF PHOSPHOLIPID SYNTHESIS. Thomas S. Moore Jr., Louisiana State University, Department of Botany, Baton Rouge, LA 70803.

The phospholipid composition of plant cells, with the primary exception of the chloroplast, is similar in many respects to that of other eukaryotes. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) generally are the most abundant phospholipids, followed by phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol (PG) and bisphosphatidylglycerol (BPG). Our current information on regulation of synthesis of these phospholipids in terms of compartmentalization, enzyme requirements, etc. will be discussed, with emphasis on the synthesis of PC. Phosphatidylcholine can be synthesized by at least three pathways in plants. The final steps can be summarized as: (i) CDPcholine + Diacylglycerol → PC + CMP; (ii) PE + 3 ($-CH_3$) → PC, and (iii) PE + choline → PC + ethanolamine. The first pathway generally is the most active, with the second being next. These two will be discussed in most detail. All these reactions derive precursors from water-soluble substrates, and it has been suggested that the production of CDPcholine is the regulatory step for reaction (i). The compartmentalization in plant cells of the enzyme responsible for this step has variously been reported as soluble, ER-bound, Golgi-bound, or a combination of these. In the castor bean endosperm it appears to be completely

membrane-bound, probably entirely in the ER, but at a site different from that of the final step in PC synthesis (reaction (i). The levels of precursors and products suggest it is not the regulatory enzyme in PC synthesis in this tissue. These data and additional information on the regulation of PC synthesis, including the reconstitution of detergent-solubilized cholinephosphotransferase, will be presented.

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LIPIDS AND PLANT DISEASE RESISTANCE. Joseph Kuć, University of Kentucky, Department of Plant Pathology, Lexington, KY 40546.

Plants have evolved different mechanisms to defend themselves against disease. Of these mechanisms, phytoalexin accumulation has received the most attention. Phytoalexins are low molecular weight antimicrobial compounds which are either absent or found in low levels in healthy plants. They are synthesized by the host plant after infection and accumulate around the site of infection. The phytoalexins produced by the members of a plant family usually belong to the same structural class of compounds. The members of the Leguminosae produce isoflavonoid phytoalexins while members of the Solanaceae produce sesquiterpenoids and polyacetylenes. Despite their structural diversity, phytoalexins are generally lipophylic. Compounds that cause plants to produce phytoalexins are called "elicitors." Two lipid elicitors, arachidonic acid and eicosapentaenoic acid, have been isolated from the late blight fungus *Phytophthora infestans*. They elicit accumulation of sesquiterpenoid phytoalexins in potato. Recent evidence from our laboratory indicates that β-glucans from the fungus and CA^{2+} mobilization are involved in the elicitation.

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MOLECULAR MECHANISMS OF LIPID COMPOSITIONAL CHANGE DURING THE ACCLIMATION OF PLANTS TO CHILLING TEMPERATURES. Guy A. Thompson Jr., University of Texas, Department of Botany, Austin, TX 78713.

It is well known that the qualitative composition of many plant oils is significantly affected by growth temperature. Yet the mechanisms underlying these changes are still understood only very superficially. The most frequently reported effect of temperature is upon the content of polyunsaturated fatty acids, with lower temperatures favoring elevated levels of these components. This type of change is now widely thought to be part of a natural response by which plants become acclimated to grow optimally at a given set of environmental conditions by modifying the lipids of their cellular membranes. The structural features of lipids are the principal determining factors of membrane fluidity, which in turn controls a multitude of membrane-associated physiological functions. Recent advances in lipid analytical techniques and plant cell fractionation have led to the realization that fatty acid unsaturation is not the only compositional parameter that changes as a result of environmental stress. The proportions of different lipid classes present and the distribution of glycerolipid molecular species, i.e. the types of fatty acids paired together on the same glycerol moiety, are but two of several other parameters subject to change. There are subtle but important differences in the rates of change and in the intracellular sites where the diverse lipid modifications are carried out. An understanding of the internally programmed pattern of compositional change triggered by temperature stress of varying severity may be useful in the development of improved products.

Session J Thursday afternoon Quality Aspects in the Use of Fats

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SENSORY AND GAS CHROMATOGRAPHY TECHNIQUES TO EVALUATE THE STABILITY OF OILS. Patricia L. Pinkowski, Calreco, 8015 Van Nuys Blvd., Van Nuys, CA 91412; K.B. Witherly, C.D. Harvey and V.A. Tadjalli.

The storage stability of several fats and oils was determined by sensory methodology and dynamic headspace gas chromatography (GC). The sensory evaluation was performed by incorporating the fat or oil into a milk emulsion. The emulsion was scored by trained judges on a 1-5 scale (1—acceptable, 5—unacceptable) and correlated to the total peak area of the GC chromatogram. The correlation coefficient between the two methods was .95. The compounds developing in storage were identified by mass spectrometry, and some were then added into the emulsion to determine their sensory impact. Using the methods described above, the stabilities of corn, canola and soy oil were determined under various experimental conditions, storage temperatures, hydrogenation levels, initial oxygen concentrations and added antioxidants. Antioxidants evaluated included TBHQ, BHA, BHT, propyl gallate, ascorbyl palmitate and dl-α-tocopherol. TBHQ and propyl gallate were the most effective antioxidants, and synergism was seen with propyl gallate/BHA and BHA/BHT. Using statistical analysis the interaction between the experimental variables was determined. The results from the study were further applied to a production environment.

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DETERMINATION OF SOYBEAN OIL VOLATILES BY PURGE-AND-TRAP/CAPILLARY GAS CHROMATOGRAPHY. Mark S. Fraser, Beatrice Grocery Group, 1645 West Valencia Dr., Fullerton, CA 92634.

A purge-and-trap/gas chromatographic method has been developed for the measurement of volatile components present in commercially deodorized, unhydrogenated soybean oil. Volatiles evolved from heated (150 C) oil are swept with nitrogen to a Tenax GC trap, desorbed onto a cool (30 C) capillary gas chromatographic column and separated by temperature programming. Determination of the identity and response factor of individual compounds was achieved by analysis of oils containing added quantities of authentic compounds. Freshly deodorized (PV = 0) oils contain apparent amounts of C_5-C_{11} aldehydes in the 0-100 ppb range; oxidized oils contain much larger quantities. The relevance of these and other oil volatiles to oil flavor and oxidation renders the method a potentially valuable tool as a means of assessing oxidative changes occurring during storage and for estimating flavor scores. Analysis of peroxide-containing oils to which triphenylphosphine is added suggests that thermal decomposition of precursors, presumably peroxides, during the volatiles collection step is partially responsible for the observed quantities of individual volatiles.

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VOLATILE COMPONENT ANALYSIS OF FATS AND OILS. E.G. Perkins, Department of Food Science, University of Illinois, 1208 W. Pennsylvania Avenue, Urbana, IL 61801; V. Ducruet, INRA, Dijon, France; M. Jablonsky, Chemistry Department, University of Wisconsin, Madison, WI, and D. Hendren, Department of Food Science, University of Illinois, Urbana, IL.

Methodology to characterize volatile flavor/odor components of fats and oils has evolved into very sophisticated techniques involving automated collection of volatile components and subsequent separation. Problems remain in determination of the optimal conditions for such work as well as standardization of methodology. In the present study we have investigated the effects of a cryofocusing type sample concentrator and gass chromatograph parameters on the collection of volatile components from small samples (<200 mgm) of fats with very low peroxide values (PV = <2). The equilibration of the sampie at the analysis temperature prior to volatile collection greatly influenced the reproducibility of the results. Parameters such as purge and desorber times and temperatures influence recovery and yields of volatile components. Restrictions placed upon the analytical system by the effects of capillary column diameter on flow rates also affect a column capacity and resolution. With these considerations, an automated programmable cryofocus-

ing sample concentrator was connected to a gas chromatograph via a 50-m, 5% phenylmethyl silicone column. The flame detector or Hewlett Packard 5985B mass spectrometer was used to monitor the separations obtained. Dynamic headspace analysis yielded a volatile profile from both soybean oil and edible beef tallow containing over 80 components. The mass spectrum of each component was evaluated; the compounds formed were a mixture of saturated and unsaturated aldehydes and ketones, hydrocarbons and alcohols.

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NEW METHODOLOGY TO EVALUATE QUALITY OF SOYBEANS STORED AT DIFFERENT MOISTURE LEVELS. Edwin N. Frankel, Northern Regional Research Center, ARS-USDA, 1815 N. University St., Peoria, IL 61604; A.M. Nash, J.M. Snyder and L.T. Black, Northern Regional Research Center, ARS-USDA.

Investigations of the effect of storage on quality of soybeans have been limited by lack of reliable, sensitive and quantitative methods to evaluate oxidative and hydrolytic deterioration. The quality of soybeans and oil extracted from seeds stored at 13, 16 and 20% moisture content (MC) was determined by headspace gas chromatography (HS-GC), fluorescence measurements, silicic acid chromatography and near infrared (NIR) spectrometry. HS-GC run on both ground beans and crude oils provided a sensitive measure of oxidative deterioration based on pentanal, 2-heptenal, hexanal and total volatiles. Fluorescence measurements (excitation: 364 nm, emission: 437 nm) on chloroform-methanol extracts were much less sensitive and showed a significant increase only in the most damaged samples stored at 20% MC. Silicic acid chromatography of crude oils showed a significant decrease of polar lipids (methanol eluate) with storage of beans, in agreement with the decreased phosphorus observed. Less polar lipids (diethyl ether eluate) increased significantly with storage at the levels of moisture tested. NIR analyses at 2260 nm showed a correlation coefficient of 0.864 with concentrations of free fatty acids ranging from 0.3 to 1.0 at 13% MC, from 0.5 to 2.0% at 16% MC, and from 0.6 to 2.3% at 20% MC. Other NIR wavelengths contributed by volatiles, phosphorus and peroxide compounds produced less satisfactory correlations. Among the new methods developed, HS-GC is most useful to evaluate oxidative deterioration and NIR analysis is most suitable and rapid to evaluate hydrolytic deterioration in stored soybeans. This new methodology provides a better basis than traditional methods (e.g. free fatty acids, peroxide values and phosphorus) for evaluating factors affecting food quality of soybeans for domestic and foreign markets.

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EFFECTS OF THERMAL TEMPERING, STORAGE DAMAGE AND LOW LIPOXYGENASE (L-1) ACTIVITY ON QUALITY OF CRUDE SOYBEAN OIL. K. Warner, Northern Regional Research Center, ARS-USDA, 1815 N. University St., Peoria, IL 61604; and E.N. Frankel and K.J. Moulton, Northern Regional Research Center, ARS-USDA.

Reliable evaluations of quality differences of crude vegetable oils are needed to assist processors to improve flavor quality of finished products. The quality of crude oils from soybeans of different origins and treatments was determined by sensory evaluation and capillary gas chromatographic (GC) analysis of volatiles. Taste panelists were specially trained to evaluate crude oils diluted with and compared to deodorized oils. Crude oils from untempered soybeans were rated significantly lower in quality than oils from beans tempered at 104 C for 4 min. Capillary GC analyses confirmed these observations by monitoring total volatiles and 2,4-decadienal. Crude oils extracted from beans stored at 45 C and 13% moisture received flavor scores decreasing from 5.7 to 3.6 with increasing storage time from 0 to 35 days. These flavor data correlated highly with capillary GC analyses of oils. Hexanal content and total volatile content increased with storage time. Crude oils extracted from low L-1 lipoxygenase (5% activity) soybeans (University of Illinois) and from normal beans showed no significant differences in flavor quality, total volatiles and 2,4-decadienal. Therefore, factors other than lipoxygenase (L-1) appear to affect the food quality of soybeans. Sensory evaluation and GC-volatile analysis of crude oils provide sensitive and reliable screening methods to assist in improving the quality of finished soybean oils by controlling soybean storage and processing and by providing information important to breeders.

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LOCALIZATION OF LIPOXYGENASE IN GERMINATING SOYBEAN (*Glycine max.* [L]). Mark H. Love, Young Sun Song, P.A. Murphy and D. Resurreccion, Food and Nutrition Department, Iowa State University, Ames, IA 50011.

Lipoxygenase (LOX) (E.C.1.13.11.12) which catalyzes the oxidation of polyunsaturated fatty acids such as linoleic and linolenic acid. Products of this reaction give rise to numerous flavor and aroma compounds, many of which are thought to be obnoxious when they carry through to finished, further processed forms of soy. Investigations into the subcellular localization of LOX should provide information relating to its physiological action. Sucrose density gradient separations revealed that isozymes with pH activity optima at 6.0 (LOX I) and 9.0 (LOX II) were associated with the supernatant fraction and not an organelle. To provide more specific localization, specific immunocyto chemical methods were employed. LOX I and LOX II isozymes were separated by conventional enzyme purification steps and checked for purity by PAGE. Antibodies to each were grown in female goats and the immunoglobulins were precipitated by caprylic acid and kept frozen at −40 C until used. This presentation will compare the use of immunofluorescent isothrocyanate microscopy and protein A-colloidal gold TEM as tools to monitor LOX I and II localization in two varieties of germinated soybeans.

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ISOMERIC LINOLENIC ACIDS IN PARTIALLY HYDROGENATED SOYBEAN OIL. E.G. Perkins, Department of Food Science, University of Illinois, 1208 W. Pennsylvania Ave., Urbana, IL 61801, and Cathysue Smick, Land O'Lakes Inc., Minneapolis, MN.

A combination of techniques, preparative high performance liquid chromatography, hydrazine reduction and capillary gas chromatography, was used to concentrate, isolate and characterize the isomeric octadecatrienoic (18:3) fatty acids present in partially hydrogenated soybean oil (PHSBO). The results obtained indicated that the 18:3 fatty acids present in PHSBO with an iodine value of 109 consisted of four isomers. The naturally occurring linolenic acid (*cis* 9, *cis* 12, *cis* 15-octadecatrienoic acid) was present at 2.7% in the oil, and composed 68.60% of all of those isomeric 18:3 acids present in PHSBO. The other three isomers which comprise 1.2% of the total fatty acids of PHSBO were identified as the *trans* 9, *trans* 12, *cis* 15-; the *trans* 9, *cis* 12, *cis* 15-, and the *cis* 9, *cis* 12, *trans* 15-isomers of 18:3. The relative concentrations of the isomers are 15.44%, 10.10% and 4.65%, respectively. Such results both support and can be predicted from current hydrogenation theory as applied to heterogenous nickel catalysts.

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THE QUALITATIVE EFFECTS OF PHOSPHOLIPIDS ON THE FLAVOR STABILITY OF SOYBEAN OIL. Suk Hoo Yoon and David B. Min, Ohio State University, Department of Food Science and Nutrition, 122 Vivian Hall, 2121 Fyffe Rd., Columbus, OH 43210.

The effects of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylglycerol (PG), glycerophosphorylcholine (GPC) and cadiolipin (CL) on the flavor stability of purified soybean oil. Purified soybean oil which was obtained from soybean oil by silicic acid chromatography does not contain measurable iron, tocopherols and phospholipids. The 300 ppm of PC, PE, PI, PA, PG, GPC, or CL was added to the purified soybean oil with and without 1 ppm added

ferrous iron. The flavor stabilities of the sample, which was stored at 60 C for 10 days under dark, were determined by a combination of volatile compounds formation and molecular oxygen disappearance in the headspace of air-tight serum bottles every 48-hr. Results showed that, in general, phospholipids worked as prooxidant in the purified soybean oil containing no added ferrous iron and as antioxidants in the purified soybean oil containing 1 ppm added ferrous iron. The results also suggest that phospholipids work as prooxidants by increasing the solubility of oxygen on the surface of oil and work as antioxidant in the oil containing 1 ppm ferrous iron by chelating iron. The results showed that PE and PA are better antioxidants than PC and PG. CL, GPC and PI showed the least antioxidant activities in the oil containing 1 ppm added ferrous iron.

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VOLATILE OXIDATION PRODUCTS IN OILS AND FATS TO BE USED IN POULTRY FEED. A PARAMETER TO PREDICT "OFF-FLAVORS"? A.T.G. Steverink, COVP Het Spelderholt, Spelderholt 9, 7361 DA Beekbergen, The Netherlands, and H. Steunenberg.

Many studies have shown that the presence of certain amounts of fatty acids like linolenic, erucic, 4,7,10,13,16,19-dodecahexaenoic acid in poultry diets may lead to "off-flavors" in poultry meat. In their investigations, Crawford and Kretsch (1976) found that not the fatty acids but oxidation products of (polyunsaturated) fatty acids may cause the mentioned effect. Shortage of animal fat, high prices of vegetable oils on one side and the need of fat/oil as a source of energy in poultry diets on the other, have contributed to the origin of a market for all kinds of blended fats, including acid oil (from chemical refining), bleach earth oil (from physical refining), distillation residues (from fatty acid production) and spent frying oils (from restaurants, etc.). During the different processes the original fats and oils are maltreated, resulting in oxidation and polymerization products. In this preliminary study the profiles of fat/oil volatiles and the flavor profiles of poultry meat of animals raised on feed with the corresponding oil/fat are compared. The profiles were obtained with the help of a purge and trap/thermal desorption injector combined with capillary gas chromatography.

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METHOD TO PREDICT THE OXIDATIVE STORAGE STABILITY OF WHOLE MILK POWDER. A.R. Keen, New Zealand Dairy Research Institute, Private Bag, Palmerston North, New Zealand.

Whole Milk Powder (WMP) contains 26–28% milkfat which can undergo oxidative deterioration. If the deterioration is sufficient the resultant oxidized flavors will cause organoleptic rejection and consequent economic loss to the producer. A method to predict oxidative storage stability of WMP based on the accelerated oxidation of WMP samples followed by an analysis of their extent of oxidation has been developed. In the method a sample of WMP (0.03 g) in a disposable glass tube undergoes accelerated oxidation with ultraviolet light. The peroxides formed are decomposed in the tube in a controlled manner in the heated injector port of a glc to form various products including pentane. The amount of pentane released following 1 hr of irradiation was found to be well correlated with the oxidative storage stability (measured organoleptically) of a number of freshly prepared WMP samples. This method, which has subsequently been adapted for use with fats and oils, has two major advantages over analogous predictive tests. These are: (i) a very short period of accelerated oxidation, and (ii) simple equipment requirements.

Session K Thursday afternoon Surfactants and Detergents II, Surface Chemistry II

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NATURE OF THE INTERFACE IN CONTACT WITH A NON-WETTING SOLUTION. Arthur W. Adamson and R. Massoudi, University of Southern California, Department of Chemistry, University Park, Los Angeles, CA 90089-1062.

There has been a strong tendency to assume that if a liquid does not wet a given surface, that is, if the contact angle is non-zero, then no vapor adsorption occurs if the surface is exposed to the vapor of the liquid. Ellipsometrically determined adsorption isotherms of water and of various organic liquids on smooth surfaces of polyethylene and polytetrafluoroetylene show that this is not the case. There is a significant film pressure of adsorbed vapor present as the adsorbate pressure approaches P°, the saturation pressure. Scanning electron microscope examination of such systems, however, show that the adsorbed material is actually present in the form of scattered microdroplets. The bulk liquid-solid interface must in most cases be quite near interactive. In other instances, there is good evidence that liquids which should not wet a given polymer surface will in fact do so, and the indication is that local surface restructuring occurs.

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ENHANCEMENT OF HARDNESS AND SALINITY TOLERANCE IN ANIONIC SURFACTANT SOLUTIONS BY ADDITION OF NONIONIC SURFACTANTS. Kevin L. Stellner and John F. Scamehorn, University of Oklahoma, 202 West Boyd, Room 23, Norman, OK 73019.

The addition of nonionic surfactant to solutions of anionic surfactants results in an increase of hardness tolerance and salinity tolerance. In this work, precipitation phase boundaries for these mixed surfactant systems are presented with added calcium and added sodium as model counterions. Formation of the precipitate can be described by a simple solubility product expression between the anionic surfactant monomer and the counterion. The observed increase in salinity/hardness tolerances can be explained by the formation of nonideal mixed micelles. A negative deviation from ideal mixing for the micelles in this system results in reduced anionic surfactant monomer concentration, so that a higher counterion concentration is required to cause precipitate formation. Electrostatic models are used to describe the monomer-micelle equilibrium and counterion binding. When combined with the solubility product relationship and material balances, an a priori model results that can predict salinity and hardness tolerances for the mixed surfactant system. This model is shown to agree well with experimental precipitation phase boundaries. Practical consequences in detergent formulation for applications in high hardness/salinity environments are discussed.

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THE STUDY OF EFFECTS ON MICELLES OF HOMOGENEOUS NONIONIC SURFACTANTS. Kenjiro Meguro and Minoru Ueno, Science University of Tokyo, Kagurazaka, Shinjuku-ku, Tokyo, 162 Japan.

The values of cmc, area per molecule and equilibrium surface tension at the cmc of a homologous series of alkyl octakis (oxyethylene) ethers (C_nE_8: n = 10 to 15) were determined from the concentration dependence of surface tension in the temperature range 15.0 C to 40.0 C. The areas per molecule and equilibrium surface tension values at the cmc decreased with increasing carbon number and they showed zigzag curves by the difference in even and odd carbon numbers. These findings may be attributed to the difference in the molecular orientation between the molecules with even carbon number and ones

with odd carbon number on the air-water interface. On the other hand, the plots of log cmc vs. the number of carbon atoms (N) in the alkyl chain exhibited a linear relationship, and the difference between even and odd carbon numbers was not observed on the bulk properties. The free energy change relating to micelle formation, ΔG_m, was calculated from the temperature dependence of the cmc. ΔH_m and ΔS_m values were estimated from the slopes and the intercepts of the log cmc versus the reciprocal of temperature plots, respectively. This result indicates that ΔS_m dominates over the micellization. Furthermore, the values of $\Delta G_m(-CH_2-)$, $\Delta H_m(-CH_2-)$, $\Delta S_m(-CH_2-)$ and $T\Delta S_m(-CH_2-)$ per methylene group calculated from the slope of each thermodynamic parameter against the carbon number were −0.68 kcal/mol, −0.33 kcal/mol, 1.16 cal/mol·deg), and 0.35 kcal/mol, respectively. It was concluded that the summation of $\Delta H_m(-CH_2-)$ and $T\Delta S_m(-CH_2-)$ contributed to the micellization.

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ANIONIC POLYMERIC DISPERSANTS. Joseph R. Wechsler, Stepan Co., Edens & Winnetka Roads, Northfield, IL 60093, and Jayne Tacke and Marty O'Reilly.

Dispersants are electrolytes that have the ability to impart stability to suspensions of solid particles in a liquid in which they are not soluble. This paper attempts to classify dispersing agents according to chemical nature and application, then focuses on one of the classes of such compounds and discusses the chemistry involved in making them and in making them work. There follows a brief presentation of some work done with one particular type of polyelectrolyte, namely naphthalene sulfonate/formaldehyde condensate, as an illustration of the problems involved, and to contribute a few ideas for solving some of them.

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COLLOIDAL GAS AND LIQUID APHRONS—A ROLE IN CLEANING AND SCOURING? Donald L. Michelsen, Felix Sebba and David A. Wallis, Department of Chemical Engineering, Virginia Tech, Blacksburg, VA 24061.

Colloidal Gas Aphrons are a 60 to 70% dispersion of 25 to 50 micron bubbles encapsulated in a "stabilized" soap film. The bubbles are tenacious and do not coalesce when pressed together. The CGA can be generated, pumped and used in various applications with little degradation within a 5- to 15-min test period. CGA's are effective in scouring gasoline from sand in one dimensional plug flow tests. Following water flushing, 50 to 70% of the remaining gasoline could be scoured from sand using CGA's generated from a nonionic surfactant solution containing less than 1 g/l of surfactant. From a practical standpoint pressure drop is a concern. Glassware cleaning also is enhanced. Oil Core Aphrons (predispersed solvents) can be made as a 95% dispersion of less than 1 up to 50 micron solvent droplets in a 5% continuous water phase. The polyaphrons upon dilution are maintained as droplets and because of the large surface area provide effective cleaning (extraction) of dissolved organics from contaminated water. Extractions with solvent/feed ratios of 1/100 to 1/500 are possible. These predispersed solvents have further shown improved removal of bitumen from tar sands as compared to straight solvent treatment. Soils can be removed from textiles in a similar way so prudent scientists keep them around the laboratory for use as a spot remover. Preparation, properties and applications will be discussed.

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NONIONIC SURFACTANT/POLYMER INTERACTIONS. A REVIEW. Shuji Saito, Momotani Juntenkan Ltd., Ichioka 2, Minatoku, Osaka 552, Japan.

Unlike anionic surfactants, the PEO-type nonionic surfactants have no interaction with nonionic polymers such as polyoxyethylene, polyvinyl pyrrolidone and polyvinyl alcohol in water, but they interact strongly with polymeric acid such as polyacrylic acid (PAA) and polymethacrylic acid (PMA) by multiple hydrogen bonding between ether oxygens and hydroxyls and by hydrophobic interaction. The binding or complex formation causes changes in every property of both components such as viscosity, pH, solubilization, surface tension and so on. The water-soluble complexes are precipitated by lowering pH or addition of neutral salts, especially of polyvalent cations. In the case of a water-dispersible polymeric acid like Carbopol, the nonionic surfactants are insolubilized without addition of acid or salt. By the same mechanism as described above, the nonionic surfactants are adsorbed by silica and solid acid resins. In the system of PAA and polyoxyethylene octylphenyl ether, the most insoluble complex at low pH is composed of about 0.8 in the ratio of ether oxygen to hydroxyl, but they are dispersed or water-soluble again in excess addition of nonionic surfactants. The binding of nonionic surfactant to a polymeric acid occurs above a certain nonionic surfactant concentration, and this suggests the cooperative nature of the binding. In addition to pH and salts, the interaction is affected by the presence of ionic surfactants and nonionic polymers. Emulsions and suspensions stabilized with the nonionic surfactants are easily flocculated by addition of polymeric acid at low pH or with salts.

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SORPTION OF CATIONIC SURFACTANTS ON VARIOUS TEXTILES. Manfred Wentz, University of North Carolina-Greensboro, 242 Stone Building, Greensboro, NC 27412, and Chantelle Gradert and Lynne Olson, University of Wisconsin at Madison.

The first part of this study focused on the kinetic and thermodynamic aspects of sorption of stearyl trimethyl ammonium bromide (STMAB) and distearyl bromide (DSDMAB) on three types of fabrics. Cotton, polyester and a blend of 50% cotton/50% polyester fabrics were selected. Two finishes, dimethylol dihydroxyethylene urea (DMDHEU), a durable press finish, and poly (acrylic acid), a soil release finish, were applied to the fabrics. Sorption of the surfactants from three concentration levels generally followed first order kinetics, and the rates increased with temperature. Equilibrium sorption was obtained after about 120 min. At low surfactant concentrations (0.0034% wt/v) all fabrics except 100% polyester exhausted the bath completely. Surfactant sorptions on the fabrics ranged from 0.088% to 0.101% wt/wt. At higher concentration levels, differentiations of cationic surfactant uptake due to fabric type and finish became apparent. For example, 0.02% wt/v solutions of DSMAB resulted in sorption of 0.319% wt/wt on cotton with no finish and 0.659% wt/wt on cotton with poly (acrylic acid) finish. A direct linear relationship between the amount of cationic surfactant sorption and the acid number of the fabrics was established. This suggests that ionic interaction forces play an important part in the sorption process. Subjective sensory evaluation was selected to assess fabric softness modifications of the test fabrics due to surfactant sorption. In general, cationic surfactant treatments improved the perceived softness of the fabrics. Differences in softness due to textile finishes were statistically significant. Electrical resistivities and electrostatic clinging time of all test fabrics were reduced after the surfactant treatments.

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FORMATION OF W/O/W EMULSIONS DURING THE SIMPLE AGITATION OF OIL-WATER MIXTURES. Sachio Matsumoto, Hiroshi Makino and Asako Ueno, Department of Agricultural Chemistry, College of Agriculture, The University of Osaka Prefecture, Sakai-shi, Osaka 591, Japan.

Water-in-oil-in-water (W/O/W) type dispersions developed together with the O/W emulsions due to the mechanical agitation of a mixture of oil- (liquid paraffin or olive oil) and water-containing hydrophobic and hydrophilic emulsifiers in each liquid phase. This phenomenon could be observed with a variety of emulsifiers such as Span 80, polyglyceryl fatty acid esters, Tween 80, SDS, etc., when the concentration of hydrophobic emulsifier in the oil phase was

relatively higher than that of hydrophilic one in the aqueous phase. The mechanical agitation of the oil-water mixture could be made by any type of high-speed mixers or homogenizers, while the procedure was completed by the agitation of the mixture for a few minutes at room temperature. The above appearance in the ordinary procedure of emulsification suggests a possibility to develop a simple technique for preparing W/O/W emulsions. It is also possible to interpret partly the complexity of O/W emulsion viscosity in reference to the phase volume of water entrapped in the oil vesicles of the W/O/W-type dispersion.

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ACID-BASE CATALYZED HYDROLYSIS REACTIONS ON SODIUM DODECYL SULFATE MICELLES. Masayuki Nakagaki, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan, and Shoko Yokoyama, Kyoto University.

In order to examine the effects of micellization and of the composition of mixed micelles on the acid-base catalyzed hydrolysis reactions of esters, the acid catalyzed hydrolysis of sodium dodecyl sulfate (SDS) and base catalyzed hydrolysis of acetylcholine chloride (Ach) in the presence of SDS micelles were followed by measuring the formation of HSO_4^- using a pH-stat or the decrease of Ach concentration using a spectrometer. The distribution of Ach to the SDS micelle was determined by ultrafiltration. The rate of acid-hydrolysis of SDS increased about 50 times by the micellization, because of the high H^+ concentration on the micellar surface due to the electrostatic attraction from the high surface charge density $|\sigma|$. By addition of 1-dodecanol (DOH), the rate decreased at first by the reduction of $|\sigma|$, and then increased to reach a constant value at above 0.5 of the mole ratio, DOH/SDS, suggesting high reaction rate of a complex, $(DOH)\cdot(SDS)_2$. The rate of base-hydrolysis of Ach decreased with increasing concentration of SDS. This indicates that the reaction rate is small on the micellar surface because the OH^- concentration on the surface is decreased by the electrostatic repulsion from $|\sigma|$, as is well expected from the Gouy-Chapman theory. It may therefore be concluded that the effect of σ on the surface pH (that is surface concentration of H^+ or OH^-) is substantially important for the acid-base catalyzed hydrolysis reaction on micellar surface, although the reaction rate is modified by the formation of a complex or by the distribution of substrate onto micelles.

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A STUDY ON THE CHEMISTRY OF DODECYLAMINE HYDROCHLORIDE AQUEOUS SOLUTIONS USING THE AMMINIUM ION SELECTIVE ELECTRODE. S.H. Castro and J.S. Laskowski, Department of Mining & Mineral Process Engineering, University of British Columbia, 6350 Store Rd., Vancouver, BC V6T1W5.

Primary alkylamines, weak electrolytes, hydrolyse in aqueous solutions and both neutral molecules and ionized species are present under alkaline conditions. The association of dodecylamine molecule and dodecylaminium ion determines the properties of dodecylamine acqueous solution in an alkaline pH range. The chemistry of dodecylamine hydrochloride aqueous solution is studied with the use of an ion surfactant selective electrode which utilizes an active complex, $C_{12}H_{25}NH_3^+C_{12}H_{25}SO_4^-$, and is responsive to the dodecylaminium ion concentration. The electrode can be used to measure amine concentration at constant pH and to determine the critical micelle concentration. This technique is supplemented in our work with the tensiometric and transmittance measurements. Such measurements, if carried out at a constant amine concentration but varying pH, indicate the pH range where the electrode potential value sharply decreases and a miniumum appears on both, the surface tension and the transmittance vs pH curves. With further increase in the pH, the surface tension and the transmittance values raise back almost to the original level, while the concentration of dodecylaminium ion is so low that it is not measurable with the use of the surfactant selective electrode. The results are interpreted in terms of the formation of neutral amine associations, their interaction with aminium ions and precipitation of colloidal particles. Increase in the transmittance at the higher pH values indicates redispersion of the colloidal precipitate caused by charge reversal of colloidal particles confirmed in our microelectrophoretic tests.

Session L Thursday afternoon Analysis of Lipids II

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FOURIER TRANSFORM INFRARED INVESTIGATIONS OF IMMOBILIZED ANTIMICROBIAL AGENTS. J. Blitz, Colorado State University, Department of Chemistry, Fort Collins, CO 80525, and R.S.S. Murthy and D.E. Leyden, Colorado State University.

Silica surfaces chemically modified by silylation reactions find wide use in science and technology. Examples are chromatographic substrates, catalysts, metal complexing materials and immobilized enzymes, antibodies and antimicrobial agents. Silylation reactions are easy to perform and a variety of natural and synthetic substrates may be used. Chemical groups used for modification may be as simple as a short chain alkane, or as complex as an enzyme. Quaternary amine compounds containing various long chain alkyl groups display antimicrobial properties. These properties are retained when one alkyl group contains an alkoxysilane which is used to bond the compound to the surface of a substrate such as silica gel or controlled pore glass. The materials have been demonstrated to be effective antimicrobial agents. However, little is known about the structure of the material on the surface of a substrate. Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) has been demonstrated repeatedly to be a powerful qualitative tool for the investigation of powdered materials. Recent research in our laboratory has demonstrated the potential for quantitative measurements as well. DRIFTS results will be presented to show the nature of the bonding of the quaternary amines to silica surfaces. Variable temperature DRIFTS is used as a technique to follow reactions dynamically. Hydrolysis of the alkoxy groups and the formation of Si-O-Si bonds between the substrate and silane will be discussed.

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COMPARATIVE PHOSPHORESCENCE AND OPTICALLY DETECTED MAGNETIC RESONANCE STUDIES OF SERUM ALBUMIN BINDING TO FATTY ACIDS. Su-Yau Mao and August H. Maki, Department of Chemistry, University of California, Davis, Davis, Ca 95616.

Fatty acid transport is serum albumin's most important physiological function. We have investigated the binding of bovine serum albumin (BSA) and human serum albumin (HSA) to fatty acids with various chain lengths using optical detection of triplet state magnetic resonance (ODMR) spectroscopy. The tryptophan (Trp) residues, Trp-134 and Trp-212 in BSA, Trp-214 in HSA, serve as strategically located intrinsic luminescent probes which lie in different protein domains. We have found that oleic acid perturbs the excited triplet state of Trp-134 (subdomain 1-C), but not that of Trp-212 (subdomain 2-AB) in BSA. The assignment is made by comparing the BSA results with those obtained from oleic acid binding to HSA. The differences in binding between octanoic and oleic acid to both serum albumins also will be presented.

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COMPOSITION OF MIXED OCTADECADIENOATES VIA OZONOLYSIS, CHROMATOGRAPHY AND SOLUTION OF LINEAR EQUATIONS. H.J. Dutton, The Hormel Institute, University of Minnesota, 801 16th Ave. N.E., Austin, MN 55912, and S.B. Johnson, F.J. Pusch, R.T. Holman, A.C. Beckwith, M.S.F. Lie Ken Jie and F.D. Gunstone, The Hormel Institute.

An approach to the analysis of 55 nonconjugated positional isomers of octadecadienoic acids in mixtures is described and tested with mixtures of synthetic individual methyl esters. In the first example, by ozonolysis, a seven-component mixture consisting of *cis,cis* 5,12-, 6,10-, 6,11-, 6,12-, 7,12-, 8,12- and 9,12-octadecadienoates was converted to aldehydes, aldehyde-esters and dialdehydes. These oxidized fragments were separated on a 50 m × 0.2 mm FFAP vitreous silica capillary column. Equations for an arbitrarily restricted 12 × 15 matrix of linear simultaneous equations and a computer solution of the matrix provided the composition of the initial methyl octadecadienoate mixture. The power of this method becomes apparent with the observation that only two of the seven isomers in the known and analyzed mixture were resolved as single peaks by state-of-the-art capillary gas chromatography, but all seven were analyzed by the ozonolysis-capillary gas chromatography-computer procedure. In a generalized approach to the analysis of the 55 nonconjugated isomers, a program, BRAIN, writes the appropriate matrix of linear simultaneous equations, based on the aldehyde data supplied by the analyst. To illustrate applicability at this stage of development, the method has been used to analyze the diene products of the hydrazine reduction of γ-linolenic acid and the diene products from the biological desaturation of isomeric monoenes.

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ANALYSIS OF SERUM LIPOPROTEIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. Ichiro Hara, Scientific Instrument Division, Toyo Soda Mfg. Co., Hayakawa, Ayase-shi, Kanagawa Prefecture, Japan 252, and Mitsuyo Okazaki, Akira Tanaka, Kiyomaro Shima, Yaeko Nakajo and Noriyuki Komoriya, Tokyo Medical and Dental University.

HPLC (high performance gel permeation liquid chromatography) by monitoring cholesterol (TC), triglyceride (TG) and phospholipid (PL) is one of the most useful methods in the analysis of serum lipoprotein. We compare the particle size of each lipoprotein fraction (VLDL, LDL, HDL) between diabetic and normal sera by elution volume of HPLC (ml), because the elution volume is reversely proportional in the molecular sieve procedure. Diabetic and normal sera are divided into two groups, that is a hyperlipidemic group containing more than 220 mg/dl for TC, 150 mg/dl for TC and a normolipidemic group, respectively. These sera are applied on HPLC apparatus with a TSK G 5000PW column and monitored by TG resulting in the following data on particle size of lipoprotein fractions in diabetic and normal sera— Normal sera: normolipidemic (Kn 87), 28.67 ± 0.35, 31.25 ± 0.14, 35.88 ± 0.44; hyperlipidemic (n 24), 28.49 ± 0.34, 31.39 ± 0.29, 36.23 ± 0.60. Diabetic: normolipidemic (n 26), 28.69 ± 0.65, 30.89 ± 0.62, 35.40 ± 0.75; hyperlipidemic (n 41), 28.59 ± 0.33, 31.04 ± 0.45, 35.39 ± 0.72. In the VLDL fraction no significant difference is observed in both groups, but the LDL fraction of diabetic class, both hyperlipidemic and normolipidemic, shows the peak shifted toward larger particle size. In the case of HDL, the same results are observed. These changes in HPLC pattern may be attributed to the changes in metabolic pathway of lipoprotein fractions.

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POTENTIOMETRIC TITRATION ON LONG CHAIN QUATERNARY AMMONIUM COMPOUNDS USING SODIUM TETRAPHENYL BORATE. C.N. Wang, Akzo Chemie America, 8401 W. 47th St., McCook, IL 60525, and J.J. Donkerbroek, Akzo Chemie, and L.D. Metcalfe, Akzo Chemie America.

Determinations of long chain quaternary ammonium compounds presently are based on partition or colorimetric methods. The disadvantages of these methods lie in differing partitioning coefficients and/or fading end points. Some potentiometric titration methods were reported in the literature; however, back titration as well as complicated electrode systems are generally involved. A new potentiometric system is presented which uses sodium tetraphenyl borate (TPB) as a titrant and a platinum-platinum electrode system to detect the end point. Standard potentiometric titration instruments are suitable. The cell design consists of one platinum electrode in the titration vessel and one platinum electrode in the titrant stream. When the concentration of TPB is in excess in the vessel, a potential change is observed. A proposed mechanism for electron transfer at the electrode involving a TPB complex will be presented. The results obtained from both visual and potentiometric methods indicate the potentiometric method to be superior with respect to precision and accuracy.

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QUANTITATIVE ANALYSIS BY HPLC OF AMINOPHOSPHOLIPIDS USING A PRECOLUMN FLUORESCENT LABELING METHOD. Yoshiaki Machida, Central Research Laboratory, Showa Sangyo Co., Ltd., 2-20-2, Hinode, Funabashi-shi, Chiba, 273 Japan, and Yoshio Ota, Toshiyuki Kaneko and Haruo Watanabe.

The application of NBD-Cl(7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) and NBD-F(7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole) as precolumn fluorescent labeling reagents for the analysis of amino group-containing phospholipids, such as phosphatidylethanolamine, phosphatidylserine, lysophosphatidylethanolamine and lysophosphatidylserine is described. The highly fluorescent NBD derivatives of aminophospholipids are separated on a silica gel column with gradient elution and detected with excitation at 465 nm and emission at 520 nm. The fluorescent response of the NBD derivatives of various phosphatidylethanolamines is discussed. NBD-F was superior to NBD-Cl as regard to reactivity for aminophospholipids, but both labeling reagents could be applicable to the rapid and sensitive quantitative analysis of aminophospholipids at the pmol level.

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IMPROVED ANALYSIS OF TOCOPHEROLS IN USED FRYING FATS AND OILS. Phillip R. Bross, Procter & Gamble Company, 6071 Center Hill Rd., Cincinnati, OH 45224, and Michael P. Purdon, Procter & Gamble Company.

The separation of α-, β-, γ- and δ-tocopherols as well as their corresponding tocotrienols provides important information when assessing the vitamin E nutritional (antioxidant) value of fresh and heat-abused frying fats and oils. The separation has been achieved by high performance lipid chromatography on an amino-bonded column using a hexane/isopropanol gradient elution mobile phase. The method requires no sample clean-up or sample preparation other than dilution and is applicable to fresh and highly oxidized used frying fats and oils. The complete gradient elution separation from 1% to 10.5% isopropanol polar modifier in n-hexane is achieved in less than 10 minutes. The analysis is done on a 25 cm × 4.6 mm Supelcosil LC-NH2 5 μm particle diameter analytical column with a 3 cm × 2.1 mm Peliguard LC-NH2 40 μm particle diameter guard column. The limit-of-detection is approximately 8 ppm with a 1 cm path length UV absorption detector operated at 295 nm.

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CARBON-13 NMR STUDY OF 1,3-BENZODIOXOL-5-OL (SESAMOL), A NATURALLY OCCURRING ANTIOXIDANT. D.A. Rosie and P.A. Hailey, Department of Applied Biology & Food Science, South Bank Polytechnic, Borough Road, London SE1 OAA, England.

During the course of a study into the oxidation products of 1,3-Benzodioxol-5-ol (sesamol), a naturally occurring antioxidant, it was necessary to obtain comprehensive spectral data of the compound. The ^{13}C NMR spectrum has previously been reported, but despite the apparent relative simplicity of the compound, the spectral assignment of the non-protonated carbons has still to be resolved. This paper describes the unequivocal assignment of all spectral peaks employing $^{13}C[^1H]$ BB (broad band) decoupling; $^{13}C[^1H]$ SFORD (single frequency off-resonance decoupling); $^{13}C[^1H]$ SFSD (single frequency selective decoupling) in conjunction with T_1 (spin-lattice) relaxation measurements employing the IRFT (inversion-recovery

Fourier transform)[2] and FIRFT (fast-inversion recovery Fourier transform([3] pulse sequences, N.O.E. (nuclear overhauser enhancement) measurements using gated decoupling and measurement of the long range ^{13}C-^{1}H coupling constants.

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HIGH-SPEED LIQUID CHROMATOGRAPHY AS APPLIED TO OILS AND FATS ANALYSIS. Vijai K.S. Shukla, Research Laboratories, Aarhus Oliefabrik a/s, P.O. Box 50, DK 8100 Aarhus-C, Denmark.

Very recently a new generation of high-speed liquid chromatography (HSLC) has emerged based on the use of short columns packed with very small particles together with significantly improved instrumentation to reduce extra-column band broadening effects. HSLC permits faster separations, improved resolution, increased mass sensitivity and reduced solvent consumption. Application of HSLC for analyzing tocopherols and antioxidants such as tertiary butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) isomers in oils and fats will be presented. Examples of the simultaneous separations of different antioxidants will be shown. The application of HSLC will be extended for the separation of triglycerides in various natural oils and fats.

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METHODS TO ANALYZE DIFFERENT TYPES OF POLYMERS. A.T.G. Steverink, COVP Het Spelderholt, Spelderholt 9, 7361 DA Beekbergen, The Netherlands, and J.K. Waltmann.

By-products of oil and fat processing like acid oil, bleach earth oil and distillation residues as well as used frying oils frequently are used as blends in poultry feed. The quality of fat as an ingredient in poultry diets, in terms of feeding value and digestibility, is determined by the fatty acid composition. In comparison to e.g. vegetable oils, the mentioned blends contain (because of the processing) several unnatural components like oxidation and polymerization products. Especially the polymers are suspected to decrease fat digestibility and to influence animal health, although the results of different investigators do not agree. This might be explained by the fact that different types of polymers are involved, which might show different effects. The polymers can be divided into several groups: cyclic/acyclic, thermal/oxy- and polymeric fatty acids/polymeric triglycerides. In order to be able to study the effects of the different types of polymers, (a) reliable analysis method(s) is(are) necessary. Preliminary investigations show it is not possible to distinguish between all the different types of polymers with one simple method, but that with a combination of LC and GC methods very acceptable results can be obtained. In this paper some results will be given.

Session M Thursday afternoon Processed Oils—Methods and Applications

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NEW APPLICATIONS OF ACETYLATED MONOGLYCERIDES. Kiyoto Murakawa and Toshinori Kubozuka, Riken Vitamin Co., Ltd., 8-10, 3-Chome, Nishikanda Chiyoda-ku, Tokyo, Japan.

Acetylated Monoglyceride (A.M.), described in the Code of Federal Register (C.F.R.), is recognized as a nontoxic substance and is used mainly in the food industry. However, we haven't put its properties to practical use, because of the Reichert Meissl Value (R.M.V.), 75-150, specified in the C.F.R. We reevaluated the properties and the effects of various A.M., hoping to expand new applications. Materials studied: A.M. consisted of capric, lauric, palmitic, stearic, oleic and linoleic monoglycerides and their mono-aceto type and di-aceto type. We evaluated properties and effects as plasticizers, coating agents, chewing gum softeners, etc. As the results of these studies, we found the possibility of new applications, especially as plasticizer for synthetic resins.

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MODIFICATION OF FATS AND OILS FOR ALTERNATIVE FUELS AND CHEMICALS. Kenneth D. Carlson, Northern Regional Research Center, USDA/ARS, 1815 N. University St., Peoria, IL 61604.

Because of the worldwide surplus of vegetable oils and depressed prices, it is essential that new markets be found. These need not necessarily be very large markets, because even small increases (several % of the whole) will help maintain market activities and price structures. Recent research to alter the physical/chemical properties of soybean oil, soy fatty acids and esters, and other vegetable oils and esters aims at extending the range of their utility and at developing new markets in non-food uses. Such new markets may be in lubricants, plastics, fuels and cosmetics. New catalysts and novel catalyst systems make it possible to dramatically alter vegetable oils and esters from their traditional compositions and permit reactions to be carried out more selectively, at lower cost, and with fewer associated by-product problems. Isomerization of double bonds is readily achieved with heterogeneous catalysts, and other examples of catalyst systems that yield novel modified products from a variety of vegetable oil substrates will be discussed. Products were characterized by IR, GC, MS and NMR.

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SOME ASPECTS OF POLYVINYL CHLORIDE HEAT STABILIZER BY COPOLYMER OF ISOCYANATE AND EPOXY FISH OIL CONTAINING HYDROXYL GROUP. Masanori Sonehara and Hirohisa Tawada, Miyoshi Oil & Fat Co., Ltd., Kobe Factory, 1-48, 7-Chome, Karumo-Dori, Nagata-Ku, Kobe, Japan, 653.

Epoxy oils are used mainly as heat stabilizers as well as plasticizers for flexible type polyvinyl chloride. On the other hand, they are rarely used for rigid type polymers because they lower the softening point. Generally, the epoxy group in oil converts to hydroxyl groups in the epoxydizing process and the hydroxyl groups cause decreasing heat stability of PVC. Epoxy oils derived from soybean oil and linseed oil having moderate unsaturation have good performance for this purpose. We synthesized a copolymer of hexamethylene diisocyanate and epoxy fish oil containing hydroxyl groups and examined the effect of physico-chemical properties on PVC when this copolymer was used as the heat stabilizer, then compared its performance with that of epoxy soybean oil which is used as conventional heat stabilizer of PVC. The results are: (i) In the case of rigid type PVC, the heat stability is almost the same. Heating loss and tensile strength are quite improved. The softening point rises. (ii) With flexible type PVC, this copolymer does not improve the performance.

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THERMAL STUDIES ON POLYMORPHIC BEHAVIORS OF UNSATURATED FATTY ACIDS—PALMITOLEIC, ERUCIC, LINOLEIC AND LINOLENIC ACIDS. Masao Suzuki, Oils and Fats Research Laboratory, Nippon Oil and Fats Co., Ltd., 1-56 Ohama, Amagasaki, 660, Japan, and Kiyotaka Sato, Faculty of Applied Biological Science, Hiroshima University, Fukuyama, Japan.

Differential Scanning Calorimetric studies have been carried out on the polymorphic behaviors of a series of unsaturated fatty acids: palmitoleic acid (C_{16}:1ω7), erucic acid (C_{22}:1ω9), linoleic acid (C_{18}:2ω6,9) and linolenic acid (C_{18}:3ω3,6,9). The polymorphs obtained could be divided into two groups by taking into account their thermal behaviors; the most stable forms (β) whose melting points are the highest due to their lowest free energies, and the unstable

polymorphs (α, γ and δ) among which reversible transformations occur in the solid state. The main characteristics of the above whole polymorphs can be summarized in comparison to those of oleic acid (C_{18}:1ω9) in what follows: (a) melting points and enthalpies of fusion for α, which have the second highest melting points, increase with increasing carbon number in a monoenoic acid series. Meanwhile those values decrease with increasing numbers of double bonds in acids with the same carbon number (C_{18}). (b) The differences in the phase transformation temperatures of δ-γ, γ-α and α-melt increase with increasing number of double bonds for C_{18}-acids.

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EFFECTS OF PHOSPHOLIPID CONTENT ON REFINED SOYBEAN OIL QUALITY. C.B. Ungermann, Harshaw/Filtrol Partnership, Kaiser Center for Technology, P.O. Box 877, Pleasanton, CA 94566, and D.R. Taylor, Harshaw/Filtrol Partnership.

A comparison is made between physical and caustic refining of soybean oil, focusing primarily on the effects that phospholipid content has on refined oil characteristics and processing parameters. Additionally, the effects of including a phosphoric acid pretreatment step in the physical refining sequence are measured. The role of bleaching clay adsorption efficiency is also analyzed. Refined oil color and oxidative stability (as measured under mild heating conditions) generally have been found to increase with increasing phospholipid content. Under mild conditions color reversion is present, regardless of the phospholipid content. Typically, the relative phospholipid adsorption capacities of the bleaching clays studied show a trend similar to that for pigment removal. Oil properties measured in the study include color, peroxide and anisidine values, and phospholipid, free fatty acid, iron and tocopherol contents.

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PHOSPHORUS AND TRACE METAL REMOVAL WITH A NOVEL REFINING MATERIAL. William A. Welsh and James M. Bogdanor, W.R. Grace & Co., Davison Chemical Division, 7379 Route 32, Columbia, MD 21044.

Laboratory tests with soybean, rapeseed and corn oils, and pilot plant and commercial scale tests with soybean oils indicate that a unique adsorbent can be successfully employed by the industry in conjunction with, or in place of, bleaching clay to refine glyceride oils. In most cases little or no modification of existing equipment would be required. Observed benefits include improved finished oil quality (color, flavor, trace metals), reduction in total solids usage, improved filtration while eliminating use of filter aid, reduced oil losses, less solid waste, and less risk of autoignitioin. These benefits, which can dramatically reduce a refiner's operating costs while allowing the refiner to make a better product, can be obtained in both physical and caustic refining applications, but the benefits are more dramatic in the case of physical refining. In addition to laboratory and pilot plant work, some commercial scale test results will be discussed. The major function of the adsorbent is to adsorb molecules containing P, Ca, Mg and Fe. Laboratory data on P removal indicate typical phospholipid loadings of 60 wt% for soybean oils and up to 35 wt% for rapeseed oils, based on adsorbent weight.

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A NEW OIL REFINING PROCESS. Haruo Watanabe, Kiichi Aonuki, Takeshi Mizuno, Mutsuhito Watanabe and Takao Arima, Showa Sangyo Co., Ltd., 2-20-2 Hinode Funabashi Chiba, Japan, 273.

Alkali refining is the process generally used for edible fats and oils refining. However, this process produces a large amount of waste water from the oil-washing step, which is a disadvantage from the viewpoint of environmental pollution as well as an economic aspect. A new, anti-pollution oil refining system has been developed and a new oil refining plant based on this system is now in operation. In this system, the oil separated from soapstock but containing residual soap is treated with a small amount of acid instead of hot water washing. Various acids, organic or inorganic, are used for this purpose, and all of them give a good result in the removal of residual soap from the oil. The refined product of this system has a good quality which is equal to that produced by the usual process. This new, no waste water generating system has been in practice since 1982, and is advantageous in energy saving.

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BLEACHING CLAYS. Aurelia Maza, Best Foods, 1120 Commerce Ave., Union, NJ 07083.

The selection of bleaching clays during the last several years increased considerably on the U.S. and world markets. A systematic evaluation of the major brands was necessary to establish a comparative data base for the bleaching efficiency and cost of various adsorbents. Focused mostly on soybean and corn oil, the investigation revealed the best clay choices, based on oil quality and economics. Adsorbtion isotherms for several brands of bleaching clays were evaluated with respect to red pigments, pheophytin, soap and phosphorus removal. The experimental work directed toward refining of high pheophytin oils indicated that bleaching itself is not sufficient as a corrective measure for obtaining good quality oil. However, proper refining in conjunction with efficient bleaching will result in satisfactory quality product. The large variety of bleaching clays/adsorbtive agents available today gives more flexibility to the refining operation. Considering that process economics depend on several factors (cost of bleaching agent, oil loss, spent clay disposal), the oil refiner has an opportunity to select the best clay for each oil at the right price.

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IDENTIFICATION AND QUANTITATION OF ANTIOXIDANTS IN LUBRICANTS BY TLC, UV SPECTROPHOTOMETRY AND HPLC. Takao Sangai, Tetsumi Saito and Kiyoshi Musha, Asahi Denkakogyo K.K., 4-1 8-Chome Higashi-Ogu, Arakawa-Ku, Tokyo, 116 Japan.

A simple and rapid method was established for identification and quantitation of forty-four typical antioxidants in lubricants by thin layer chromatography (TLC), ultraviolet spectrophotometry (UV) and high performance liquid chromatography (HPLC). Antioxidants were separated by TLC on silica-gel plates with a solvent system consisting of chloroform-benzene (1:1, v/v). 2,6-Dichloroguinone-chloroimide and six other reagents were used as detection reagents. Identification of antioxidants was achieved by observing the coloration and Rf value. Most antioxidants have spectra in the UV region (210–360 nm). Identified antioxidants were determined by UV spectrophotometry. The absorbance of lubricants dissolved in ethanol was measured and antioxidants were determined using a calibration curve. The determination of antioxidants in lubricants by HPLC was especially effectife for lubricants containing several antioxidants. The analytical conditions were as follows: column: Hitachi #3056 4 mm × 150 mm, eluent: methanol-water (9:1, v/v) or 0.05 mol/1-sodium acetate in methanol-water (9:1, v/v), detector: ultraviolet detector (254 nm). Good recovery and reproducibility were obtained from the sample of standard lubricants containing known amounts of antioxidants. Antioxidants in some commercial lubricants were determined by the proposed method with no interference.

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IGNITION PRECURSORS IN DIESEL TYPE INJECTION SPRAYS OF VEGETABLE OILS. Thomas W. Ryan III, Southwest Research Institute, 6220 Culebra Rd., San Antonio, TX 78284, and T.J. Callahan, Southwest Research Institute, and M.O. Bagby, USDA, Northern Agricultural Research Center.

The use of vegetable oils as fuels for diesel engines has been hampered by durability problems resulting from deposit formation in the engine and contamination of the engine lubricant. These prob-

lems have been attributed to the physical and chemical differences between the vegetable oils and petroleum derived diesel fuel. Previous work, reported by the authors, indicated that the injection and atomization characteristics of the vegetable oils are different than those of an equivalent (same viscosity) petroleum-derived material. In another study, the authors also reported that the differences in the physical characteristics of the sprays were due to very rapid chemical changes occurring in the vegetable oils during the injection event. The objective of the work reported in this paper is to define the specific nature of these chemical changes by injecting (diesel engine injection system) various vegetable oils and vegetable oil derivatives (triolein, trilinolein, trilinolenin) into environments of high-pressure, high-temperature nitrogen and air. Samples were collected from the sprays during the injection event and analyzed using GC-MS techniques. Very dramatic chemical changes were observed to occur in the very short residence times for the oils ($\sim$200 μs). The reactions in the nitrogen environments tended toward reduced molecular weight hydrocarbons. In the air environments more oxygenated products were obviously present. The results are discussed in terms of the types of fatty acid present and in terms of interactions between the various fatty acids. Conclusions are presented regarding the impact of the experimental findings on the selection of the most appropriate types of vegetable oils for use as fuels for diesel engines.

Session N Thursday afternoon Symposium on Biological Oxidation of Lipids II

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LIPID PEROXIDATION IN PLANTS—PRODUCTS AND PHYSIOLOGICAL ROLES. Don C. Zimmerman and Brady A. Vick, ARS, U.S. Department of Agriculture, Box 5674, University Station, Fargo, ND 58105.

Most plants contain lipoxygenase, an enzyme which incorporates molecular oxygen into linoleic and linolenic acids. The fatty acid hydroperoxide products serve as substrates for other enzymes that form several different compounds. Three enzymes which have been identified are hydroperoxide lyase, hydroperoxide cyclase, and hydroperoxide isomerase. The products of these enzymes are a 6-carbon aldehyde and 12-carbon oxo-acid, an 18-carbon, cyclic oxo-acid, and alpha and gamma ketols, respectively. These enzymes are widely distributed in plant tissues, and their activity varies with the stage of growth. The products of these enzymes contain chemical structures which can react with sulfhydryl and amino groups of other biochemicals and thereby alter metabolism. The product of hydroperoxide lyase has been identified as a wound hormone. The 18-carbon, cyclic oxo-acid is the precursor to jasmonic acid. Physiological roles for the products of these enzymes will be discussed.

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GC-MS METHODS FOR MEASUREMENT OF PHOSPHOLIPID AND TRIGLYCERIDE PEROXIDATION PRODUCTS IN TISSUES: EXAMPLES FROM RETINA AND OTHER TISSUES. Erik van Kuijk, Biochemistry Department, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands, and Edward A. Dratz, Department of Chemistry, University of California, Santa Cruz.

Phospholipid peroxidation has been suggested as a causal factor in many types of tissue degeneration such as aging, cancer, heart disease and retinal degeneration, but the evidence has been rather indirect. We are developing high-sensitivity GC-MS methods aimed at determining the chemical identity and amounts of lipid peroxidation products to seek more direct evidence for or against involvement of lipid peroxidation in tissue degeneration. Phospholipid hydroperoxides were synthesized by photooxidation and reduced, transesterified to form fatty acid methyl esters or fatty acid pentafluorobenzyl (PFB) esters, and trimethylsilyl (TMS) or tert-butyldimethylsilyl (TBMS) derivatives were formed of the hydroxy groups. EI GC-MS yields characteristic fragmentation patterns with 10 ng sensitivity while negative ion chemical ionization (NICI) GC-MS yields molecular ions with about 10 pg sensitivity for 22:6, 20:4, 18:2 and 18:1 products. We also developed methods to determine primary decomposition products, such as 4-hydroxy alkenals, since phospholipid peroxides are reactive and unstable. For example, 4-hydroxy nonenal (a gift from Prof. Esterbauer, Univ. of Graz) was reacted with PFB hydroxylamine, to form the PFB oxime derivative. This derivative provides pg sensitivity using NICI GC-MS. Aldehydes are covalently bound to abundant amino groups as aldimine Schiff bases in tissue samples, but are released quantitatively with the PFB hydroxylamine reagent. Analysis of retinas, exposed to oxidative stress will be presented, as well as results of analysis from other tissues in vitamin E-deficient and supplement animals.

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CYTOCHROME P-450 AND THE OXYGENATED METABOLISM OF ARACHIDONIC ACID. Jorge Capdevila, Department of Biochemistry, University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235, and G.D. Snyder and J.R. Falck, Departments of Physiology and Molecular Genetics, University of Texas Health Science Center at Dallas.

Microsomal cytochrome P-450 actively catalyzes the oxygenated metabolism of arachidonic acid by a combination of (1) allylic oxidation to form 6 isomeric hydroxyeicosatetraenoic acids (HETEs); (2) ω-oxidation to form the 19- and 20-hydroxyeicosatetraenoic acids, and (3) olefin epoxidation (the Arachidonic Acid Epoxygenase Reaction) to form four isomeric *cis*-epoxyeicosatrienoic acids (EETs). The EETs are potent in vitro stimuli for the release of several peptide hormones such as somatostatin from the median eminence, anterior and posterior pituitary hormones and the pancreatic hormones insulin and glucagon. Changes in cell Ca^{++} homeostasis as well as further oxidation of the EETs appear to be linked to the stimulated hormonal releases. The presence of EETs in samples extracted from rat liver, rabbit kidney and human urine has been demonstrated utilizing gas chromatography/mass spectral analysis. These results establish the Epoxygenase Reaction as a new member of the arachidonate cascade and suggest a role for the EETs in cell and organ homeostasis.

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FREE RADICALS INDUCE LIPID PEROXIDATION AND PROTEIN DEGRADATION BY INDEPENDENT MECHANISMS. Kelvin J.A. Davies, University of Southern California, Institute of Toxicology and Department of Biochemistry, 1985 Zonal Ave., Los Angeles, CA 90033.

Exposure of cells and cell membranes to free radicals has long been known to induce radical chain reactions which result in lipid peroxidation. Recent work from this laboratory has demonstrated that cellular proteins are also subject to oxidative damage, when exposed to free radicals, and that the oxidized proteins are highly susceptible to degradation by intracellular proteolytic systems. It appeared possible that proteins might be directly damaged by certain free radicals, thus leading to degradation. Alternatively, however, evidence exists in the literature to suggest that lipid peroxyl radicals and/or lipid hydroperoxides might be the proximal damaging species. In an attempt to resolve these possibilities red blood cells (RBC) were exposed to oxygen radicals generated by xanthine or acetaldehyde + xanthine oxidase, ascorbic acid + ferric iron, hydrogen peroxide + ferrous iron, or ^{60}Co radiation under nitrous oxide. Hemoglobin oxidation to met-hemoglobin, choleglobin, verdoglobin, and other derivatives was rapid and extensive. Similarly, protein degradation to free amino acids was increased 15- to 30-fold by oxygen radical exposure, and occurred immediately (i.e. with no

lag-phase). In contrast, lipid peroxidation (conjugated dienes, lipid hydroperoxides, malonyldialdehyde) were not evident prior to extensive loss of vitamin E and reduced glutathione. Lipophilic antioxidants significantly decreased lipid peroxidation without affecting hemoglobin damage or degradation, whereas membrane permeant hydrophilic antioxidants decreased hemoglobin damage and degradation without affecting lipid peroxidation. All experiments were repeated with dialyzed extracts of RBC which were lipid-free. The RBC extracts exhibited both damage and degradation of hemoglobin, thus demonstrating the independence of these processes from lipid peroxidation. These results (and others to be presented) indicate that lipid peroxidation and protein damage and degradation are induced by different mechanisms following exposure to free radicals.

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PEROXIDATIVE ALTERATION OF MEMBRANE STRUCTURE AND INCREASED PHOSPHOLIPASE A_2 HYDROLYSIS. Alex Sevanian, Institute for Toxicology, University of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90033.

The peroxidative deterioration of membrane lipids is widely viewed as a likely means by which disturbances in membrane structure, and consequent membrane related functions, may be precipitated by means of free radical reactions. Progressive formation of lipid hydroperoxides and subsequent decomposition products can be initiated using a variety of radical generating systems. Accumulation of oxidized products in membranes has been shown to alter membrane structure, and studies will be presented showing an increase in membrane order (as determined by fluorescence polarization) following lipid peroxidation in artificial membranes (constituted as unilamellar liposomes). Large increases in membrane order are observed after relatively mild lipid peroxidation where such increases are otherwise produced by significant alterations in the composition of phospholipids (in this case the proportions of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Mild peroxidation of liposomes consisting of a 4:1 ratio of liver PC and PE produces increases in fluorescence anisotropy equivalent to membranes comprised of a 2:1 ratio of these phospholipids. Under these conditions a marked increase in phospholipase A_2 hydrolysis of the phospholipids occurs with a preferred release of fatty acid peroxides. PE is found to be more readily hydrolyzed. Furthermore, significant hydrolysis of intact fatty acids also occurs. The relative extent of hydrolysis correlates approximately with the degree of unsaturation, where arachidonic and docosahexanenoic acids are most readily cleaved from peroxidized liposomes. Alteration of membrane structure appears to occur very readily as a consequence of lipid peroxidation, and our findings show that phospholipase A_2 activity is remarkably sensitive to these structural changes. It appears that transitions from predominantly lamellar to nonlamellar (intermediate) states predispose the membrane to hydrolysis.

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SPECTROPHOTOFLUOROMETRIC AND MICROSCOPIC STUDY OF AGE-RELATED FLUORESCENT SUBSTANCE (LIPOFUSCIN) IN RAT TISSUES. Hiroyuki Shimasaki and Nobuo Ueta, Department of Biochemistry, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi, Tokyo 173, Japan.

An autofluorescent pigment (lipofuscin or ceroid) which accumulates during senescence in mammalian tissues has been widely regarded as an end product of in vivo lipid peroxidation. The fluorescent substance associated with the age pigment is soluble, in part, in organic solvents. The autofluorescent pigment in various organs and tissues of rats ranging in age from 2 to 29 months was investigated by spectrophotofluorometric and fluorescent microscopic techniques. Quantitative analysis of this substance in various organs showed that it increased with age, but the amount of the substance in the hepatic cells and skeletal muscle was relatively little even in old rats. The substance in the spleen appeared in the younger age of rats and increased linearly with age until 11 months. Formation of the age-related fluorescent substance in rat organs will be discussed.

Session O Friday morning
Surfactants and Detergents III. Nonionics—Versatile Surfactants for Household and Industry

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NONIONIC SURFACTANTS IN U.S. LAUNDRY DETERGENTS. Jesse L. Lynn Jr., Lever Brothers Company.

This paper will provide a general introduction to the use of nonionic surfactants in U.S. laundry detergents. Emphasis will be placed on alkyl ethoxylates in comparison with classical anionic surfactants, their benefits including oily soil removal, reduced calcium sensitivity and protection of anionic actives against precipitation as calcium salts. Problems inherent in the use of nonionic surfactants including powder processing and properties will also be covered, as well as solutions to these problems. The relatively recent introduction of ethoxylates with narrow range ethylene exide distribution will also be discussed, as well as their use for processing and performance benefits.

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ALKYL ETHOXYLATES: AN ASSESSMENT OF THEIR ORAL SAFETY ALONE AND IN MIXTURES. T.B. Zerkle, The Procter & Gamble Co., Ivorydale Technical Center, 5299 Spring Grove Ave., Cincinnati, OH 45217, and J.F. Ross and B.E. Domeyer, The Procter & Gamble Co.

Certain alkyl ethoxylates (AEs), nonionic surfactants which are widely used in industry and household cleaning products, have been demonstrated to produce acute neuropharmacologic effects when sufficient systemic exposure is achieved. An investigative program was undertaken to characterize these effects and to evaluate the neuropharmacologic potential of some AEs, both commercially available materials and a pure compound, following oral exposure. Results show that AEs can be safely used in household cleaning products. Intravenous and intraperitoneal administration of aqueous solutions of AEs to rats resulted in a progressive and reversible syndrome of early central nervous system (CNS) excitation followed by CNS depression (ataxia, loss of righting) that was characterized as resembling the introduction of and recovery from general anesthesia. Oral administration to rats and mice of aqueous solutions of commercial materials at concentrations that approach or exceed the maximum expected in household products (10–25%) rarely induced general anesthesia, and then only at very high dose volumes (10 ml/kg and above). A pure alkyl exthoxylate, noneathylene glycol mono-n-tridecyl ether ($C_{13}E_9$), did induce general anesthesia when administered orally to rats at 10 ml/kg as a 25% (w/v) aqueous solution. Plasma levels of $C_{13}E_9$ were shown to correlate with specific neuropharmacologic signs (e.g. ataxia) associated with the induction of anesthesia. Subsequent oral administration to rats of aqueous mixtures containing $C_{13}E_9$, one of two anionic surfactants and ethanol at 10 ml/kg did not result in either observable AE-induced general anesthesia or plasma levels of $C_{13}E_9$ which have been shown to correlate previously with specific neuropharmacologic signs. Based on these results and the fact that products containing AEs usually produce emesis in humans, it is concluded that: (1) AE-induced general anesthesia is not predicted to occur in humans following oral exposure to products containing both alkyl ethoxylates and anionic surfactants, and (2) general anesthetic effects in humans following oral exposure to products containing fairly high levels

of AEs as the sole nonionic surfactant would be expected to occur only in cases of extremely high volumes of ingestion (≥ ml/kg).

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DETERGENCY OF NONIONIC SYSTEMS; IS IT RELATED TO THE PHASE INVERSION TEMPERATURES? H.L. Benson, Shell Development Co., Westhollow Research Center, P.O. Box 1380, Houston, TX 77251.

Studies of phase inversions, well known in emulsion technology and surfactant-enhanced oil recovery, have been extended to household laundry applications. At phase inversion temperatures surfactants solubilized equal amounts of water and oil, and minimum ultralow interfacila tensions are achieved. Detergency systems containing nonionic alcohol ethoxylates also function best, especially at low temperatures, when the surfactants' water solubility is limited, as measured by their cloud points. Recent detergency vs. temperature scans for mineral oil removal from polyester/cotton now indicate that this optimum activity occurs when the temperature is near the phse inversion temperature and about 20-20 C above the cloud point. Comparisons with known phase diagrams reveal that the intital surfactant-water systems are dispersions of lamellar liquid crystals and/or isotropic surfactant-rich phases, i.e., coacervate phases. Additionally, for multicomponent systems partitioning rate studies also indicate that limited oil phase solubility is beneficial for detergency.

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NONIONIC ALKYL POLYGLYCOSIDES UNIQUE SURFACTANTS. N.F. Borys, A.D. Urfer and G.M. Verboon, A.E. Staley Mfg. Co., Decatur, IL.

Alkyl polyglycerides are a unique class of nonionic surfactants. While known as laboratory curiosities for many years, they can now be economically manufactured on a commercial scale. Alkyl polyglycerides are produced by the glycosidation of primary or secondary alcohols. Feedstock flexibility of using either synthetic or natural alcohols combined with a stable carbohydrate source ensures attractive long term economics. The physico-chemical properties of alkyl polyglycerides differ fromconventional nonionic surfactants. Surface and interfacial tensions are very low while acid, caustic and electrolyte stability are excellent. As a result, they can be used in areas historically inaccessible to conventional nonionics. In existing nonionic surfactant applications, alkyl polyglycosides demonstrate additional performance benefits. Physical properties of alkyl polyglycosides which have direct interest to the detergent industry such as wetting and biodegradability will be reviewed.

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A COMPARISON OF BLOCK COPOLYMER SURFACTANT GELS. Irving R. Schmolka, Consultant, 21490 Parke Lane, Grosse Ile, Michigan 48138.

The aqueous gel-forming properties of several different series of block copolymer surface active agents are reviewed and compared. The surfactants include the Pluronic®, Tetronic®, and Butronic® polyols. These are prepared by the addition of ethylene oxide to condensation products of propylene or butylene oxide on a low molecular weight water soluble glycol initiator. Similar surface active products are obtained by the reverse order of addition of the alkylene oxides. The Pluronic® and Tetronic® polymers are similar to each other in that both series exhibit a reverse thermal gelation behavior, which enhances their usefulness. However, the Butronic® series differs in that it follows a normal thermal gelation pattern and exhibits other differences from the Pluronic® and Tetronic® polyols. These differences will be explained on the basis of differences in molecular structures. The advantages of using newly developed aerosol spray techniques, which can be used to convert ambient fluid liquid systems to gels, will be described. These include a more practical method of application, since it elimiates the need for maintaining a refrigerated system and expands the range of their versatility. The utilization of these gels in various application areas will be covered.

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PHYSICAL PROPERTIES OF POLYOXYETHYLENE POLYGLYCERINE ALKYLPHENYLETHERS. Yoshiharu Tanizaki, Akinori Suginaka and Shinichi Akimoto, Nippon Oil & Fats Co., Inc., No. 10-1, Yurakucho, 1-chome, Chiyoda-ky, Tokyo, Japan.

As a new nonionic surfactant, we synthesized Clycidol (GLD) and Ethyleneoxide (EO) adducts to Nonylphenol (NP) and Octylphenol (OP) according to a reaction which will be presented in our paper. Physical properties of these surfactants are examined and compared with conventional nonionic surfactant, NP + EO adducts. These surfactants have higher cloud points than NP + EO adducts, especially in NaOH aqueous solution. Around EO 10 adducts to NP + CLD$_1$ and OP + GLD$_1$, are as superior in the wettability as NP + EO adducts. Regarding foaming properties, around EC $_{20}$ adducts to NP + GLD$_1$ are comparatively favorable, and OP + GLD$_1$ + EO $_{18}$ shows as higher foam of 140 mm/120 mm (immediate/after 5 min) as NP + EO adducts. EO $_4$ adducts to NP + GLD and OP + GLD indicate similar lower surface tension of 30 dyne/cm^2 (0.01%, 25%) to NP + EO adducts. In proportion to EO addition, the surface tension of its adducts became gradually higher. We consider somewhat the relation among GLD and EO additional mole, physical properties and HLB of these adducts. Considering these results, EO $_6 \sim _{10}$ adducts to NP + GLD$_1$ and OP + GLD$_1$ can be available for cleaning agent, especially with alkali and inorganic builders.

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PREPARATION AND PROPERTIES OF ω-(LONG-CHAIN ALKYL)OLIGO(OXYETHYLENE)-OXYACETOHYDROXAMIC ACIDS. Mitsuo Okahara, O. Araki Masuyama and Ko-ichi Akiyama, Osaka University, Faculty of Engineering, Yamadaoka 2-1, Suita, Osaka 565, Japan.

For the purpose of preparing surfactants having an ability to interact with metal ions, hydroxamic acids containing the oxyethylene units ($C_mH_{2m+1}(OCH_2CH_2CONHOH$) were prepared from polyoxyethylen (POE) type nonionic surfactants. Hydroxamic acid consisted of above four oxyethylene units (n≥4) and an octyl or decyl group as a hydrophobic part (m=8,10) freely dissolved in water. It was proved that those water-soluble hydroxamic acids had a micelle-forming ability and showed behavior as POE-type nonionic surfactants under acidic and neutral conditions from the relationship between the surface tension and the concentration of the aqueous solution at various pH values. For example, CMC and γ_{CMC} values at pH 5.5 are as follows: C_8H_{17} $(OCH_2CH_2)_4OCH_2$-CONHOH: 1.65 x 10^{-3} mol/1, 31.5 mN/m; $C_{10}H_{21}$ $(OCH_2CH_2)_4$-$OCH_2CONHOH$: 7.70 x 10^4 mol/1, 13.0 mN/m. Additionally, those compounds were found to form a water-soluble dark red complex with Fe(III) at pH 2, and the aqueous solution of these complexes showed an ability to lower surface tension.

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ENZYMATICALLY MODIFIED PROTEINS AS NEW SURFACTANTS: THEIR PROPERTIES AND FUNCTIONS. Soichi Arai, Department of Agricultural Chemistry, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

The sophisticated methodology currently available to improve the

surface properties and functions of proteins emphasizes the potential of chemical and enzymatic modifications to their structures. The covalent attachment of hydrophobic amino acid esters to hydrophilic proteins with the aid of an unconventional reaction catalyzed by cysteine proteinases, e.g., papain (EC 3.4.22.2), is useful for this purpose. Examples are provided from our work on proteinaceous surfactants produced from hydrophilic proteins by attachment of leucine alkyl esters. In particular, an enzymatically modified gelatin with leucine dodecyl ester (EMG-12) was found to have unique properties and functions. Data are shown of its flow behaviors in dispersion and emulsion systems, phase characteristics, critical micelle concentration, and ability for decreasing the surface tension of water. EMG-12 also functioned as an agent to retard the freezing of water by supercooling even in the presence of added silver iodide crystals as heterogeneous ice-nuclei. This pehnomenon is probably a reflection of the antinucleating property of EMG-12. The significance of designing and producing such a modified protein for use in cryo-preservation of food and biological systems is stressed. The utility of modification of proteins in improving their surfactancy and future research strategies are also discussed.

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STABILIZING EFFECT OF MULTI-BRANCHED NONIONIC SURFACTANTS ON COAL-OIL MIXTURES. Tomoo Sugiyama and Akihiro Naka, Dai-Ichi Kogyo Seiyaku Co., Ltd., 55 Nishi-Shichijo, Higashikubo-Cho, Shimokyo-Ku, Kyoto, Japan.

To put a mixture of coal and fueld oil (COM) into practical use, it is indispensable to prevent its coal particles from sedimenting. Since conventional surfactants were ineffective for such prevention, the authors synthesized multi-branched high molecular weight nonionic surfactants by copolymerizing propylene oxide (PO) and ethylene oxide (EO) to sorbitol. Some of them were found to be effective. The above surfactants were cross-linked between their molecules in order to strengthen the three-dimensional structure of the molecules, and consequently they were remarkably improved in effect. Next, the authors synthesized a polyethylene-polyamine base PO-EO copolymer having several tens of branches. it was found that this surfactant was very effective when it was produced with appropriate values of average molecular weight and of PO/EO ratio. A cross-linked version of the surfactant proved to be able to stabilize COM's under the severe conditions possible to be encountered in the practical utilization of COM.

Session P Friday morning Symposium on the Role of Lipids in Cancer I

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GLYCOSPHINGOLIPIDS OF CULTURED PC12 PHEOCHROMOCYTOMA CELLS. Robert K. Yu, Yale University School of Medicine, Department of Neurology, 333 Cedar St., New Haven, CT 06511; R.K. Margolis, SUNY Downstate Medical Center; T. Ariga, L.J. Macala and M. Saito, Yale University School of Medicine, and L.A. Greene and R.U. Margolis, NYU Medical Center.

PC12 cells are a clonal line of rat adrenal medullary pheochromocytoma cells which display many properties associated with normal adrenal chromaffin cells. They also display remarkable morphological and physiological changes in response to nerve growth factor (NGF). Since glycosphingolipids (GSLs) have been implicated in many cell surface phenomena, several groups of investigators have studied ganglioside composition in PC12 cells, but with conflicting results. We have recently reexamined the ganglioside composition of PC12 cells grown in the presence and absence of NGF. We found that the ganglioside content of the NGF-grown cells was nearly twice that of the cells grown in the absence of NGF (0.34 vs. 0.18 μg lipid-bound sialic acid per mg protein). However, the ganglioside patterns remained the same for the two. The ganglioside fraction consisted of mono- (17%), di- (46%), trip (32%) and tetrasialo- (5%) species. Several of them also contained a fucose residue; one of the monosialogangliosides corresponded to fucosyl-GM1. In addition, a number of gangliosides contained an asialo-GM1 backbone, including the tri- and tetrasialo species. These cells therefore contain many gangliosides with "brain type" oligosaccharide structures. Analysis of the total ganglioside fraction revealed the presence of high amounts of fatty acids with chain-lengths greater than C20 (>50% of total). We have also examined neutral GSLs of these cells and found globoside to be the most abundant species. The significance of these findings in relation to cell differentiation will be discussed.

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ACIDIC GLYCOSPHINGOLIPID, GANGLIOSIDE GM3, CAN INDUCE SPECIFICALLY MONOCYTIC DIFFERENTIATION IN HUMAN MYELOID AND MONOCYTOID LEUKEMIA CELL LINES, HL-60 AND U937. Masaki Saito and Hisao Nojiri, Div. Hemop., Institute of Hematology, Jichi Medical School, 3311-1 Yakushiji, Minami-Kawachi-machi, Kawachi-gun, Tochigi-ken 329-04 Japan.

We recently demonstrated that HL-60 cells expressed distinct glycosphingolipid (GSL) profiles, depending not only on differentiation-stages but also on differentiation-directions (*Blood 64*:534, 1984). During granulocytic differentiation, GSLs having longer sugar moieties characteristically increased with a concomitant decrease of those with shorter sugar chains. In marked contrast to such changes, a remarkable increase of ganglioside GM3, with a concurrent marked decrease of ceramide dihexoside, was observed during macrophage-like cell differentiation. In the present experiment, we have tested whether such increased GSL molecules exhibit any physiological functions in cell differentiation processes. HL-60 cells were subcultured and maintained in serum-free culture. When the cells were treated with exogenous ganglioside GM3 (50 nmol/ml), their morphological and functional maturation along monocytic lineage was observed with a simultaneous growth inhibition and with a marked increase of monocyte-specific surface antigens detectable by monoclonal antibodies OKM1, OKM5 and Mo2. Other gangliosides such as GM1 and GD1a gave no activity on cell differentiation, and instead, showed stimulatory actions on the growth of HL-60 cells in serum-free culture. Similar results were obtained with U937 cells. The present results indicate that the ganglioside which specifically increased during monocytic cell differentiation might play, in turn, an important role as a primary trigger in induction of the cell differentiation, possibly through its acidic sugar-moiety and membranophilic properties.

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TUMOR-ASSOCIATED GLYCOLIPID ANTIGENS WITH SIALIC ACIDS AS IMMUNOGENIC DETERMINANT. Yoshio Hirabayashi, Shizuoka College of Pharmacy, 2-2-1, Oshika, Shizuoka-shi, Shizuoka 422 Japan; Hideyoshi Higashi, Department of Pathology, Research Institute for Microbial Diseases, Osaka University, and Makoto Matsumoto, Shizuoka College of Pharmacy.

We have found the presence of gangliosides containing N-glycolylneuraminic acid (NeuGc) in human colon cancers. The molecular species of the gangliosides were determined to be GM3(NeuGc), GM3(4-0-Ac-NeuGc), NeuGc-nLac$_4$-cer and GM2(NeuGc) by the combined method of 2d-TLC/enzyme-immunostaining with treatment of specific glycosidases or chemical reagent. NeuGc-containing gangliosides were also expressed in human melanoma tissues. Four molecular species could be determined to be GM3(NeuGc), GM2(NeuGc), GD3(NeuAc-NeuGc or NeuGc-NeuAc), by their behaviors on 2d-TLC. NeuGc-containing gangliosides were isolated and characterized from human meconium and fetal intestinal tissues. Three species of antigenic gangliosides

in pooled meconium were identified as GM3(NeuGc), NeuGc-nLcOse$_4$-cer and NeuGc-nLcOse$_6$-cer by their migration on 2d-TLC and endo-β-galactosidase treatment. GM3(NeuGc) was a sole NeuGc-containing ganglioside in one fetal intestinal tissue from three individuals. Since the gangliosides such as GM3(4-0-Ac-NeuGc) and GM2(NeuGc) are foreign substances in human, they are expected to be strong immunogens. We developed a method of TLC/enzyme-immunostaining to measure serum-antibodies against gangliosides. By this sensitive method the antibodies against GM3(NeuGc), GM3(4-0-Ac-NeuGc) and GM2(NeuGc) could be detected and determined simultaneously. We are examining the levels of serum-antibodies against NeuGc-containing gangliosides in cancer patients and normal control.

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MEMBRANE FATTY ACYL MODIFICATION IN TUMOR CELLS: A POTENTIAL THERAPEUTIC APPROACH. Arthur A. Spector and C. Patrick Burns, University of Iowa Department of Biochemistry, Bowen Science Building, Iowa City, IA 52242.

The purpose of this work was to determine whether the fatty acid composition of a tumor can be modified sufficiently to alter its membrane properties. Mice bearing either the Ehrlich ascites carcinoma or L1210 ascites leukemia were fed semi-purified diets to which a 16% fat supplement was added, either sunflower oil containing 71% linoleic acid or coconut oil containing 93% saturated fatty acid. Substantial differences occurred in the fatty acid compositions of the tumor cell plasma membranes, microsomes and nuclear membranes. No differences occurred in the plasma membrane cholesterol content, phospholipid content or phospholipid composition, indicating that the lipid modifications were confined to the fatty acid chains. Analysis by electron spin resonance with 5- and 12-nitroxystearate (NS) spin probes indicated that membrane fluidity was altered. With 5-NS, the order parameter (S) at 37 C was 0.572 for plasma membrane of L1210 cells grown in the mice fed sunflower oil, as opposed to 0.603 in those from the mice fed coconut oil. Likewise, the main transition of the spin parameter, τ_o, was lower in the membranes from the L1210 cells grown in the mice fed sunflower oil, 19.5 as compared with 22.0 C. Several plasma membrane transport systems were affected by these lipid modifications. In L1210 cells, the K_m' for methotrexate uptake at 37 C was reduced from 4.1 to 2.9 μM when the cells were grown in mice fed sunflower oil. Likewise, the K_m' for the Na^+-dependent component of α-aminoisobutyrate uptake was reduced from 2.3 to 0.9 μM when Ehrlich cells were grown in mice fed sunflower oil. However, not all carrier mediated transport systems were affected. For example, there were no changes in the kinetic parameters of phenylalanine transport in Ehrlich cells or melphalan transport in L1210 cells. The insulin binding properties of the Ehrlich cell also were affected. Three times more insulin-binding sites were contained in the cells grown in the mice fed sunflower oil, but the affinity constants were reduced by about 50%. The dietary lipid modifications had no effect on the growth rate of the L1210 cells, but the mice fed coconut oil survived 6% longer, 201 hr after transplantation as compared with 188 hr. Fatty acid compositional changes also occurred in the tissues of the host, suggesting that host factors may be responsible for the differences in survivla. Even though the changes are not specific to the tumor and there is no effect on the rate of tumor growth, the approach may offer some therapeutic potential because certain membrane properties and functions are modified. In this regard L1210 cells enriched with polyunsaturated fatty acids are more sensitive to the cytotoxic effects of adriamycin and to hyperthermia.

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ROLE OF POLYUNSATURATED FATTY ACIDS IN PARENTERAL HYPERALIMENTATION OF CANCER PATIENTS. Yoshiya Mashima, T. Tashiro, H. Yamamori, H. Misawa, K. Hayashida and K. Horibe, The 1st Department of Surgery, Chiba University, School of Medicine, 1-8-1 Inohana Chiba, Japan, and M. Kunimasa, K. Okamoto, T. Takai and T. Wakabayashi, Terumo Co., Tokyo, Japan.

Parenteral hyperalimentation (IVH) is widely used to support cancer patients. Clinical manifestations of essential fatty acid deficiency (EFAD) have been reported to occur during fat-free IVH. To date, 10% soybean fat emulsion to cover about 5% of total calories as linoleate has been proved to prevent or correct EFAD. However, the dose requirement of linolenate or its derivatives (ω-3 series) such as EPA (C20:5 ω-3) or DHA(C22:6ω-3) have been neglected up to now. We therefore studied whether a daily dose of soybean fat emulsion satisfying the linoleate requirement is also enough to supply ω-3 fatty acids. We also made a new soybean fat emulsion enriched with EPA and DHA by adding finely prufified fish oil. During clinical IVH with conventional fat emulsion to cover 10% total calories as fat in 5 patients, Triene/Tetraene ratios (of which above 0.4 is EFAD) of the total serum lipids were less than 0.06. The EPA and DHA fractions, however, decreased from 1.2 ± 0.6% to 0.7 ± 0.2%, and from 4.2 ± 0.8% to 2.6 ± 0.9% respectively, after two weeks, both tending to decrease further. Even when 20% of total calories was given by fat, EPA (0.6%) and DHA (3.8%) were far below normal range (3.8%) were far below normal range (3.3 ± 0.4% for EPA and 6.3 ± 0.3% for DHA). Infusions of fat emulsions on rats in EFAD resulted in improvement of T/T ratio from 0.8 to less than 0.1. However, increases of EPA and DHA to normal range were attained only by giving the fat emulsion fortified with fish oil. These results show that the dose of soybean fat emulsion for linoleate might not be enough for linolenate and its derivatives and that addition of ω-3 derivatives makes the soybean fat emulsion more ideal.

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CHANGES IN THE OXIDATIVE METABOLISM OF FATTY ACIDS IN A PRECANCEROUS STATE. Nirmala K. Menon and James F. Mead, University of California, Los Angeles, Laboratory of Biomedical and Environmental Sciences, 900 Veteran Ave., Los Angeles, CA 90024.

Our earlier work with spontaneous lymphoma-bearing AKR mice showed a significant decrease in the in vitro oxidation of [1-^{14}C]palmitate by liver mitochondria. A subpopulation of asymptomatic controls also had oxidation rates as low as that of the lymphoma-bearing group. This suggested the possibility that the mere initiation of the neoplastic process may lead to defective fatty acid oxidation by the host liver mitochondrial system in a precancerous state. To verify this, newborn AKR mice were injected with the oncogenic virus SL3-3$_C$. At 30 days of age bone marrow cells from these mice were removed and injected intravenously into irradiated ADF$_1$ mice. After this procedure, 80–120 days later on the average, thymic lymphoma is clinically detectable. The β-oxidation of [1-^{14}C]palmitate to $^{14}CO_2$ by the liver mitochondria of these mice was studied during the latent period, as well as after the appearance of lymphoma. Ten days after bone marrow transplantation, in the absence of ATP in the incubation medium, the oxidation rate of the experimental group decreased to 60%, whereas in the controls the rate dropped to only 80% of the complete mixture. This trend continued until the advanced stages of lymphoma, when the oxidation rate decreased to 10%. Our results suggest that lymphomagenesis at a very early stage may be accompanied by an impairment of ATP-generating processes, possibly stemming from the fragility of the mitochondria, which results in an inhibition of fatty acid oxidation to CO_2.

Session Q Friday morning
Symposium on Oilseed Proteins —Valuable By-Products of Oilseeds

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ANTIOXIDANT PROPERTIES OF OILSEEDS. Ki Soon Rhee, Department of Animal Science, Texas A&M University, College Sta-

tion, TX 77843.

Many oilseeds and their products have been shown to have antioxidant properties in simple lipid systems and various foods. Antioxidative compounds in different oilseeds will be discussed. Extensive studies have been conducted in our laboratory on uses of oilseed food ingredients as antioxidants for various meat products. Oilseed ingredients were incorporated into either the meat or non-meat adjuncts such as sauce, gravy or batter/breading. Results of our studies suggest that certain oilseed food ingredients can effectively retard lipid and pigment oxidation in raw meat products and lipid oxidation in cooked meat products, even when used in the meat at levels equal to or lower than 3% (based on the weight of meat).

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ISOLATION OF PURIFIED PHOSPHOLIPIDS FROM COMMERCIAL LECITHIN AND DETERMINATION OF THEIR ANTI-OXIDIZING PROPERTIES. Rüdiger Ziegelitz, Lucas Meyer GmbH & Co., P.O. Box 280 246, D-2000 Hamburg 28, West Germany.

Commercial soybean lecithin contains 62–65% of acetone-insoluble matters, ca. 80% of which are phospholipids and 20% sterols, sterol esters, glycolipids and coloring substances. The deoiling process with acetone does not remove these compounds. A production process is described in which purified phospholipids are isolated from an N-hexane solution by means of an adsorbing agent. Phospholipids thus obtained are free from triglycerides, FFA, sterols and sterol esters as well as glycolipids. The pure phospholipids are examined for their anti-oxidative properties and for the synergism with α-tocopherol or ascorbyl palmitate.

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UTILIZATION OF BY-PRODUCTS FROM THE REFINING OF FATS AND OILS. Karl T. Zilch, Emery Chemicals, 4900 Este Ave., Cincinnati, OH 45232.

Although many methods have been investigated for the refining of fats and oils, only two methods are practiced commercially on a large scale; namely, alkali refining and steam distillation refining. Both methods produce by-products which can generally be described as mixtures of fatty acids, glycerides and unsaponifiable materials of various percentages and composition. The composition of the by-products is related both to the method of refining and the fat or oil being refined. Also, the yield of by-product depends upon the degree of refining as well as the fat or oil being refined. For example, only a minor amount of by-product is obtained from the refining of safflower oil, whereas sizeable quantities are obtained from soybean oil. Refining by-products are used primarily for the manufacture of fatty acids, fatty acid derivatives and animal feeds. The latter represents the principal use. However, some by-products contain sterols which are isolated commercially and converted into pharmaceutical products. The major portion of this talk will cover the commercial aspects of by-product utilization in such end use applications as metalworking fluids, surface coatings, ore recovery processes, paper coatings, bar soaps, lubricating greases and fatty nitrogen derivatives.

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PEANUTS AND PEANUT BY-PRODUCTS AS FOODS. E.J. Conkerton, Southern Regional Research Center, USDA, ARS, P.O. Box 19687, New Orleans, LA 70179.

Peanuts (groundnuts) *Arachis hypogaea* L. are grown in most of the tropical and subtropical areas of the world. About 17 million metric tons (MT) are produced annually, with China, India and the U.S. providing about 10 million MT. The pleasant aroma, flavor and crunchy texture of peanuts, along with their high protein and mineral content make them an appetizing as well as a nutritious food. In addition to being eaten as a whole nut, raw or roasted, various products can be prepared from whole fat, partially defatted or defatted flours. These flours have potential applications in many types of food products, such as bakery items, beverages, soups, desserts and as meat extenders. Some of the uses of peanuts as food and food supplements and their possible application in food products will be discussed.

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GOSSYPOL—A VALUABLE COMMODITY? Robert J. Hron Sr., S.P. Koltun, J. Pominski and G. Abrham, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179.

Decreased demand for domestic cotton fiber has increased surpluses to their highest point in two decades, renewing interest in cottonseed byproducts. With cottonseed oil prices projected to be slightly lower and meal prices at their lowest point in this decade, gossypol, a cottonseed constituent long considered a scourge, may now be of value to a troubled industry. A comprehensive review of the literature on gossypol from 1886 (the date of its isolation) until 1986 will be presented, with emphasis on its potential commercial uses.

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COTTONSEED LINTERS. Jacques J. Hebert, USDA-ARS, P.O. Box 19687, New Orleans, LA 70179.

The cottonseed linter or fuzz fiber is the short cellulosic fiber which is left after the normal length cotton fiber is removed from the seed surface by ginning. Cottonseed normally yields approximately 200 pounds of linters per ton of seed. The linter differs from the cotton lint only in length and to some extent morphology. X-ray evidence illustrates that they are both largely alpha cellulose having almost identical amounts of crystalline fractions. Electron and light microscopic studies reveal the similarities and differences in linter and lint fiber structure.

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OIL SEEDS AS A SOURCE OF FOOD FIBER. Robert Anderson and Melanie Kenyon, Technical Service Department, Grain Processing Corp., 1600 Oregon St., Muscatine, IA 52761.

Even though there is a continuing controversy on the role fiber plays in health and nutrition, fiber is now recognized as an important constituent in the human diet. Consumer interest in high fiber and fiber added products has created a demand for fiber ingredients, but confusion has risen regarding what dietary fiber is, how it is measured and how fiber ingredients differ. Oilseeds and the products from oil extraction are potential sources for fiber ingredients. Processors must investigate the quantity, type, and quality of fiber to determine its fit as an ingredient in new high fiber foods. In this presentation, we will attempt to show the types and qualities of fiber found in the common oilseeds, how fiber from oilseeds is being marketed today, and the potential for new fiber ingreidents.

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ESTABLISHMENT OF A SOYBEAN PROCESSING PLANT DEDICATED TO MAKING FOOD PROTEINS. Frank D. Wende, SKET, 20 Maraienstrasse, Magdeburg, German Democratic Republic.

The latest vegetable food protein facility in Europe was established in the USSR. The plant capacity is about 1,500 tons per year. The paper presents the main problems in soybean processing to make food proteins. New technical solutions occurring for the first time in the oilseed processing industry will be mentioned. This refers especially to an economical process for dehulling and hull separation using electrical fields and some process steps newly introduced in protein production. Additionally, some applications of protein products will be presented in the presentation. The feasibility of construction and running of a food protein processing plant will be discussed using the results of the first years of operation.

Session R Friday morning
Frying Fats

122

CHEMICAL ANALYSIS OF VEGETABLE OILS OBTAINED FROM COMMERCIAL FRYING PROCESSES. J.L. Sebedio, I.N.R.A., Station de Recherches sur la Qualité des Aliments de l'Homme, B.V. 1540, 21034 Dijon Cédex France; C. Septier, J. Prevost and A. Grandgirard, I.N.R.A.

Samples were collected from French restaurants and market vendors. Total polar components were determined by adsorption column chromatography on Kieselgel 60 using the IUPAC method. The total polar components were also determined by the Iatroscan TLC/FID technique using a mixture of hexane:THF:acetic acid as developing solvent system. The response factors were calculated using the polar and the non-polar fractions collected after column chromatography. Methyl heptadecanoate was used as internal standard. The polymer content was measured by gel permeation chromatography. Separation of the polymerized triglycerides was carried out on two μ-styragel columns using THF as solvent with a flow rate of 1.0 ml/min. The oil samples were converted to methyl esters. These were analyzed by gel permeation chromatography under the same conditions as those used for the triglycerides. The cyclic fatty acid contents were determined by gas liquid chromatography on a Silar-10c columns after total hydrogenation on PtO_2 and urea complexation. Correlations between the different methods to assess the quality of a frying fat will be discussed.

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STANDARDIZATION OF TESTS FOR FAT THERMAL OXIDATIVE STABILITY. Goro Kajimoto, JOCS Fat Stability Committee, Japan Oil Chemists' Society, 3-13-11, Nihonbashi, Chuo-ku, Tokyo 103 Japan, and Kazuo Horikawa, Yukio Araki, Tsugio Izumi, Tadashi Satoh, Yotaro Nishino, Joichi Hanaoka, Yoshiaki Hirata and Kyozo Morimoto, JOCS Fat Stability Committee.

After much study of the above subject, we conducted joint research and obtained the following result. Refined oils of soybean, rapeseed, corn and high oleic safflower as well as refined lard were selected for test samples. 20 g of sample oil was heated and maintained at 180 C or 200 C for 12 hr up to 50 hr. Carbonyl value, paranisidine value, acid value and viscosity were tested; however, two test values of carbonyl value and para-anisidine value were mainly used in the study, while those of acid value and viscosity were considered inadequate by reason of so little change of value caused by heating. In the case of the oils other than refined lard, it is considered feasible to evaluate the stability test value for thermal oxidation of oil by comparing how many hours are required for the carbonyl value to reach 15 points by heating and for the para-anisidine value to reach 115 points, respectively.

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NUTRITIONAL STUDIES OF VEGETABLE OIL USED IN A PRESSURE FRYER. J.C. Alexander and B.E. Chanin, Department of Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1, and V.E. Valli, Department of Pathology, University of Guelph.

The fats in this comparative study were corn oil (CO), peanut oil (PO), corn oil and peanut oil heated in the laboratory at 180° for 72 hr with 8 hr aeration per day (HCO and HPO), and peanut oil used in a commercial pressure deep-frying operation to prepare chicken (CPO). Each of the fats was fed to male weanling rats as 15% of a purified diet for 28 d. Weight gain, feed consumption, feed efficiency and coefficient of digestibility of the fats were depressed by the HCO and HPO, which also produced relatively heavier kidneys and livers. Heart weights were unaffected. Hematological evaluations indicated that HCO and HPO caused significantly decreased values for MCV and MCH. None of the dietary fats influenced the proportions of lymphocytes, monocytes, segmented neutrophils, eosinophils, basophils or disintegrated cells. All fats except the CO produced deleterious effects on the thymus gland, but the lesions were most pronounced with the HPO. The testes and epididymal tissue were severely damaged by HPO and CPO with cessation of spermatogenesis in the central tubule and spermatid debris in the lumina. Symptoms of heated fat toxicity such as irritability, seborrhea, hair loss from the crown of the head, and diarrhea were present only in the HCO and HPO groups.

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TOXICOLOGICAL EFFECTS OF CYCLIC FATTY ACID MONOMERS ISOLATED FROM HEATED LINSEED OIL ON RATS DURING REPRODUCTION. J.L. Sebedio, A. Grandgirard, J. Prevost, Ch. Septier and F. Juliard, I.N.R.A., Dijon, France.

A cyclic fatty acid fraction (98% cyclic fatty acid monomers, 2% linoleic acid) was isolated from a heated linseed oil by a combination of esterification, column chromatography on silica gel and urea adduct fractionation. Adult female rats were fed a diet containing 1, 0.1 and 0.01% of cyclic fatty acid methyl esters (CFAM) during gestation and lactation. Each diet contained 10% of lipids including CFAM, straight chain methyl esters and soybean oil. A high mortality (98%) was observed during three days after birth for the pups of the groups of rats receiving 1.0 of CFAM in the diet. The group receiving 0.1% of CFAM also showed a high mortality (40%), while the group at 0.01% did not seem to show any significant difference compared to the control group. Differences in weight were also observed for the weanling rats. These depended on the CFAM levels in the diet.

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FORMATION OF MONOMERIC CYCLIC FATTY ACIDS DURING HEATING OF PARTIALLY HYDROGENATED SOYBEAN OIL. Jose Rojo and Edward G. Perkins, Department of Food Science, University of Illinois, 1208 W. Pennsylvania Ave., Urbana, IL 61801.

The extent of formation of monomeric fatty acids was studied in a model system in which partially hydrogenated soybean oil was intermittently heated during 80 hr of simulated deep fat frying. Fresh and heated oils and their methyl esters were fractionated according to their molecular size using semi-preparative gel permeation chromatography (GPC). Oils as well as GPC fractions were analyzed for cyclic monomers using an improved gas chromatographic (GLC) method that allowed the utilization of a small amount of sample and shorter analysis time. The method developed included: (1) preparation of methyl esters (FAME); (2) microhydrogenation of FAME; (3) urea fractionation of FAME; (4) GLC analysis in high resolution capillary column, and (5) confirmation of cyclic monomer peaks by GC-MS. GC-MS with capillary columns allowed the conclusive identification and structural characterization of 13 cyclic fatty acids (disubstituted cyclohexane and cyclopentane methyl esters). Other non-cyclic and contaminant compounds eluting within the expected retention range of cyclic monomers were also identified and characterized in all the samples and GPC fractions. Under simulated frying conditions the total concentration of cyclic monomers increased from 776 ppm (0.08%) in fresh oil to 1916 ppm (0.19%) in heated oil.

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EFFECTS OF THERMAL OXIDATION ON THE CONSTITUTION OF BUTTERFAT, BUTTERFAT FRACTIONS AND CERTAIN VEGETABLE OILS. D.B. Kupranycz, M.A. Amer and B.E. Baker, Department of Food Science and Agricultural Chemistry, Box 187, Macdonald College of McGill University, Ste. Anne de Bellevue, Quebec H9X 1C0, Canada.

Butter is used extensively in food preparation both as a component and as a frying medium; this is primarily because of its favorable

flavor characteristics. There is, however, little published information on the thermal oxidation of butterfat and the formation of non-volatile decomposition products. In order to gain information in this area of butterfat chemistry and to make comparisons between the behavior of butterfat (summer, winter; solid and liquid fractions) and selected vegetable oils (sunflower, soyabean, Canola, corn), samples of these materials were heated (185 C; 8 and 16 hr) in the presence of air. The gel permeation properties of the intact samples and their methyl esters have been previously reported. The present paper deals with the fatty acid and triglyceride compositions of the non-polar fractions (isolated by column chromatography from the heated samples) in relation to the composition of dimers and higher oligomers. The amounts of polar compounds and cyclic monomers in the thermally oxidized samples will be reported. The thermal oxidative stability of the various fats and oils will be compared according to their DSC curves under dynamic heating conditions.

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EFFECT OF THERMALLY OXIDIZED COMPOUNDS ON THE FLAVOR STABILITY OF SOYBEAN OIL. Suk Hoo Yoon and David B. Min, Ohio State University, Department of Food Science and Nutrition, 122 Vivian Hall, 2121 Fyffe Rd., Columbus, OH 43210.

Purified soybean oil was obtained from refined, bleached and deodorized (RBD) soybean oil by silicic acid column chromatography. Free fatty acids, phosphorus containing materials, tocopherols, and polar lipids in RBD soybean oil were completely removed by silicic acid column chromatography. Purified soybean oil was then thermally oxidized at 180 C for 96 hr in a dark oven. Thermally oxidized compounds thus formed (31.3%) were separated from the purified soybean oil by gradient elution silic acid chromatography using hexane and methanol as solvents. Thermally oxidized compounds were added to RBD soybean oil and purified soybean oil at 1.5, 3.0, 4.5 and 6.0%. The oil samples containing different levels of thermally oxidized compounds in air-tight, serum bottles were stored at 60 C in a dark, air-oven to study their effects on the flavor stability of oils for 10 days. The effects of thermally oxidized compounds on the flavor stability were determined by a combination of volatile compounds formation and molecular oxygen disappearance in the headspace of oils. Results showed that the higher the amounts of thermally oxidized compounds added to RBD soybean oil or purified soybean oil, the larger the amount of volatile compounds formed and the faster the disappearance of oxygen in the headspace of soybean oil. Duncan's Multiple Range Test showed that thermally oxidized compounds had a significant effect on the volatile compounds formation and oxygen disappearance in the headspace of oils. Butane, propane, propanal and hexanal were identified as major volatile compounds in the headspace by GC-Mass Spectrometry. Thermally oxidized compounds which had strong prooxidant effects were rich in hydroxyl groups and *trans*-double bonds according to infrared spectra.

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A UNIQUE PAPER STRIP WHICH MEASURES FREE FATTY ACID LEVELS IN FRYING SHORTENINGS. J.W. Mlinar, 3M, St. Paul, MN 55144.

In the restaurant industry and particularly in the fast food industry, many pounds of different foods are prepared by frying in hot shortenings. During the frying of these foods, the heated shortenings are oxidized and hydrolyzed into by-products that produce off flavors, discolor foods, reduce cooking efficiency, etc. The point at which these by-products reach objectionable levels varies with the temperature and types of food fried and are not readily measurable on site with existing products. Our objective was to produce a simple device that would readily indicate the increase in degradation products so that a consistency in shortening discard could be obtained. For a device to be effective and usable in the restaurant industry, certain requirements have to be met such as: simplicity, temperature and shortening type independence, user safe, non-toxic, unaffected by types of foods prepared and give a quickly discernable reading. The device developed which met these requirements consisted of a paper strip containing independent reaction cells capable of measuring free fatty acids (FFA) in shortenings. These reaction cells give a distinct and fast color change when immersed into a used shortening. Each reaction cell is designed to meter in a constant amount of shortening containing a quantity of FFA that will then react with a predetermined amount of constituents in the cell, and depending on the resultant stoichiometry, will or will not produce a color change. The strip has proven to be very functional in accurately measuring FFA from 0.5% to 7% in vats of shortening whose temperatures ranged from room temperature to 400 F. Triangle and preference taste panel tests were used to determine acceptable levels of shortening degradation products for various deep fat fried food, as measured by FFA concentrations in the shortening. These FFA concentrations were monitored with the paper strips and utilized by a number of fast food units as discard points for their shortenings. The results of a one-year study showed an improved discard schedule and a reduction in shortening costs.

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THE STABILITY OF LOW LINOLENIC ACID CANOLA OIL TO FRYING TEMPERATURES. N.A.M. Eskin, M. Vaisey-Genser, S. Durance and R. Przybylski, Department of Foods and Nutrition, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 Canada.

Canola and soybean oils both exhibit the phenomenon of heated odor after exposure to frying temperatures. This is generally attributed to the presence of linolenic acid (C18:3). The recent introduction of an experimental low C18:3 canola oil (1.6%) provided a unique opportunity to compare this laboratory deodorized oil with high C18:3 canola oil samples, one laboratory deodorized (9.0%) and one commercially deodorized (5.5%). Oil samples were heated for 10 min at 185 ± 5 C in air and under nitrogen. Subsequently, the heated odor was assessed at 50 C by a trained panel against unheated controls for odor intensity and acceptability. Chemical oxidation products measured included peroxide value (PV), thiobarbituric acid value (TBA), free fatty acids (FFA) and carbonyl concentration. A significant reduction in odor intensity was observed for the low C18:3 oil as well as a marked improvement in the overall acceptability of this oil compared to the high C18:3 canola oil samples. These differences were corroborated by the lower levels of PV, TBA, FFA and carbonyl determined in the low C18:3 canola oil sample. Oil samples heated under nitrogen were associated with lower levels of oxidation products, reduced odor intensity and improved acceptability. This study demonstrates the marked improvement of the low C18:3 canola oil with respect to the development of heated odor, thus confirming the important role that C18:3 plays in the development of this phenomenon.

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EFFECT OF ALTERED FATTY ACID COMPOSITION ON STABILITY OF SOYBEAN OIL. T.L. Mounts, K. Warner, G.R. List and R. Kleiman, Northern Regional Research Center, ARS-USDA, 1815 N. University St., Peoria, IL 61604.

Research conducted during the last 15 years using hybridization and induced mutation breeding of soybeans has been successful in producing an altered fatty acid composition in the extracted oil. The objective of this research was to achieve a low linolenic acid soybean oil. Crude oils extracted from the seeds of three such genotypes were processed in laboratory simulations of commercial procedures to finished deodorized oils. Analysis of the fatty acid composition of the oils showed the linolenic acid composition to be: I—2.6%, II—3.3%, III—4.8%. The stability of these finished oils was compared to oils from soybean varieties having linolenic acid contents of 7–10% and commercial hydrogenated soybean oil. Test and control oils were evaluated by a trained sensory panel initially, after accelerated storage at 60 C, and during use at 190 C in room odor tests. Peroxide values were determined at the time of evaluation. Results indicate that there is no significant difference in the flavor

stability during storage between test and control oils. There was no significant difference in peroxide development during accelerated storage. Test oils showed significant improvement in room odor stability compared to control oils. Test oils were rated as having lower overall odor scores and lacked the fishy odors of unhydrogenated soybean oil and the hydrogenated odors of commercial cooking oil.

Session S Friday morning Analysis of Lipids III

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METHODS TO IMPROVE THE QUALITATIVE AND QUANTITATIVE ANALYSIS OF LIPIDS ON IATROSCAN CHROMARODS. J.K.G. Kramer and E.R. Farnworth, Animal Research Centre, Agriculture Canada, Ottawa, Ontario K1A OC6, and B.K. Thompson, Engineering and Statistical Research Institute, Agriculture Canada.

Chromarods result in band spreading and poorer resolution between lipid classes. In addition, the flame ionization detector (FID) response was less for an equal amount of a mixture of molecular species compared to one molecular species of a lipid class. Therefore, a correction factor determined for a single component lipid class is not applicable for a mixed component lipid class which is usually present in biological samples. It was confirmed that $CuSO_4$ impregnation of Chromarods greatly improves the rod to rod variation and produces a uniform FID response which was proportional to the mass of the component rather than the shape. The $CuSO_4$ impregnation of the chromarods, however, retarded the migration of unsaturated lipids, presumably because of metal complexes, with double bonds. The effect of metal complexing was removed by the addition of traces of formic acid into the developing solvent. These improvements increased the precision and accuracy of the Iatroscan technique.

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ANALYSIS OF GLYCERIDES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH POST-COLUMN DERIVATIZATION. Satoshi Takano and Yukihiro Kondoh, Tochigi Research Laboratories, Kao Corp., 2606, Akabane, Ichikai-machi, Tochigi 321-34, Japan.

Many studies on the analysis of glycerides were reported by means of high performance liquid chromatography with a refractive index detector in almost all cases. This detector has low sensitivity and no selectivity. A few attempts to develop a post-column reactor detector were made. However, high sensitivity was not obtained and satisfactory resoltuion for the analysis of natural fats and oils could not be accomplished. The authors developed a new post-column reactor with high sensitivity, high selectivity and molar responsibility. Glycerides eluted from a column are hydrolyzed with potassium hydroxide, and the resulting glycerin is oxidized to formaldehyde with periodic acid. Then, formaldehyde is reacted with acetylacetone in the presence of ammonium acetate to form 3,5-diacetyl-1,4-dihydrolutidine, which is detected at 410 nm. The detector, for example, can detect 0.1 μg of trilaurin and give a linear working curve between 0.2 and 38 μg of trilaurin. By using this post-column reactor, triglycerides such as palm oil are sufficiently separated and quantitatively analyzed with nonaqueous reversed phase chromatography. Monoglycerides in commercial margarines are also determined quantitatively without any pretreatment.

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ANALYSIS OF CYCLOPROPENE FATTY ACIDS IN COTTON BY HPLC. Randall Wood, Biochemistry and Biophysics Department, Texas A&M University, College Station, TX 77843.

We have previously described a high performance liquid chromatography (HPLC) method for the quantitative analysis of the common fatty acids as phenacyl derivatives [*J. Chromatog. 254*:237-246 (1983)]. This method has now been applied to the analysis of cottonseed oil that contain cyclopropene fatty acids: malvalic (8,9-methylene-8-heptadecenoic) and sterculic (9,10-methylene-9-octadecenoic). Several types of columns were examined which led to the selection of a tandem two-column system that allowed the resolution of malvalate, sterculate and dihydrosterculate from other fatty acids, including the separation of elaidate from sterculate. The cyclopropene and cyclopropane fatty acid content of glanded and glandless cotton varieties and seed components have been determined. The data indicate that malvalic, sterculic and dihydrosterculic acids are located almost exclusively, if not completely, in the hypocotyl of the seed embryo while the cotyledon is essentially free of these acids in both glanded and glandless seed.

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IDENTIFICATION AND CHARACTERIZATION OF FATS AND OILS BY A TRIGLYCERIDE MOLECULAR SPECIES ANALYSIS USING HPLC AND A FLAME IONIZATION DETECTOR. Orville S. Privett, Warren L. Erdahl and Frederick C. Phillips, The Hormel Institute, University of Minnesota, 801 16th Avenue N.E., Austin, MN 55912.

Fats and oils from a wide variety of animal and vegetable sources were analyzed by reversed-phase HPLC using a flame ionization detector as previously described (*Lipids 19*:142-150; 880-887, 1984), to demonstrate the use of the method in oil and fat technology and in metabolic or nutritional experiments with fats. The triglyceride species composition of samples of the most common vegetable oils, lard and beef tallow were characterized via an analysis by reversed-phase HPLC using two Zorbax octadecylsilane (ODS) columns (250 × 4.6 mm) in tandem. It was found that identification and characterization of many oils could be made on the basis of a computer analysis of the five most abundant species. The method was demonstrated on blends of common oils, the detection of adulteration and the analysis of the triglyceride species of genetically altered soybean and rapeseed oil varieties. A method was also developed for the detection of oils containing partially hydrogenated fats and applied to the analysis of triglyceride species containing *trans* acids in tissues of rats in nutritional experiments.

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ANALYSIS OF TRIGLYCERIDES BY GC-MS. Makoto Fukatsu and Toshitake Tamura, College of Science and Technology, Nihon University, 1-8, Kanda-Surugadai Chiyoda-ku, Tokyo, Japan.

A model triglyceride mixture was prepared by random interesterification of coconut oil at 80 C for 1 hr using sodium methoxide as catalyst. The mixture was separated into several fractions by TLC on silica impregnated with $AgNO_3$ according to the degree of unsaturation. The separated fractions were analyzed by gas chromatography-mass spectrometry (GC-MS) and it was shown that each triglyceride group was eluted from the gas chromatography (1% OV-1 column) on the basis of total acyl carbon number. The fatty acid composition of each triglyceride group having the same total acyl carbon number was determined by the relative intensity distribution of the specific fragmentation ion containing one of three acyl groups of a triglyceride molecule. Correction of the fatty acid composition was carried out using a correction factor derived from the GC-MS data of the standard diacid triglycerides which were synthesized by interesterification of relevant monoacid triglycerides. The fatty acid composition determined by the GC-MS method agreed well with that of theoretical calculation according to random distribution.

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APPLICATION OF THE FREEZE-DEEP-ETCHING TECHNIQUE FOR AN IMPROVED ULTRASTRUCTURAL CHARAC-

TERIZATION OF INTERFACIAL LAYERS IN O/W EMULSIONS. W. Buchheim, Federal Dairy Research Centre, H. Weigmann Str. 1, D-2300 Kiel, West Germany, and Y. Matsumoto, The Nisshin Oil Mills Ltd., Yokohama, Japan.

Until very recently transmission electron microscopy methods did not allow to obtain larger planar views of interfacial layers in O/W emulsions. The commonly used methods of thin-sectioning or of freeze-fracturing can provide only limited information about the structure of adsorbed interfacial material because either only narrow zones or cleavage planes through individual oil droplets are available for ultrastructural analysis. The freeze-deep-etching method offers fundamentally new possibilities because up to 50% of the surface of individual droplets can be inspected. The method requires mainly a removal of dissolved, non-interfacial material from the aqueous phase (e.g. salts, carbohydrates, proteins) by repeated washing under suitable conditions, such as centrifugation and redispersion in distilled water. Such washed emulsions are rapidly frozen, freeze-fractured and subsequently deep-etched, i.e., the ice phase is sublimated at low temperature (e.g., −100 C) to a suitable depth (ca. 100–300 nm). The deep-etched msurface is replicated by a commonly used platinum-carbon evaporation. Using this method of preparation various types of interfacial (protein) layers in O/W emulsions have been investigated, such as skim milk proteins, whey proteins, sodium caseinate and soy protein isolate under varying processing conditions (e.g. pressure and temperature of homogenization, thermal treatment, pH-value).

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF PHOSPHOLIPIDS WITH FLAME IONIZATION DETECTION. Merlin D. Grieser, A. E. Staley Mfg. Co., 2200 E. Eldorado St., Decatur, IL 62525.

A flame ionization detector (FID) is utilized with high performance liquid chromatography in the direct quantitative analysis of lecithins in oil. The flame ionization detector allows the use of chloroform or methylene chloride as the weak solvent in gradient elution. These solvents have excellent solubilizing and chromatographic properties for lecithins in oil. The method utilizes a Beckman Ultrasphere silica column with a solvent gradient composed of chloroform and premixed methanol and ammonium hydroxide. This chromatographic system produces excellent resolution of the three major phospholipids and triglyceride component as well as the minor constituents. The order of elution is triglycerides, phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylinositol (PI). Ammonium hydroxide improves peak shape and resolution but causes the peaks for the phospholipids to appear as doublets. Quantitative analysis is accomplished by the external standard method. The detector produces a linear response over a 25-fold concentration range. The chromatographic conditions coupled with the FID are useful in overcoming the difficulties experienced with ultraviolet detection of phospholipids.

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ANALYSIS OF LIPID CLASSES OF PLANT AND ANIMAL TISSUES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY VIA A FLAME IONIZATION DETECTOR. Orville S. Privett, Frederick C. Phillipis and Warren L. Erdahl, The Hormel Institute, University of Minnesota, 801 16th Ave. N.E., Austin, MN 55912.

Ramifications in the analysis of the lipid classes from various plant and animal tissues by high performance liquid chromatography (HPLC) with a flame ionization detector (FID) as previously described (*Lipids 17*:992–997, 1982) are reported. The FID permits the use of a wide range of solvent systems and gradients to insure a high degree of resolution of the lipid classes as demonstrated with the lipid extract of immature soybeans which contain a complex mixture of neutral and glycolipids as well as phospholipids. The FID also provides a quantitative analysis of the lipid classes on the basis of a proportionality of peak area of separated components. The efficiency and versatility of the method are demonstrated with a variety of columns varying in diameter from 2 to 250 mm and 4 to 50 cm in length, as demonstrated with a concentrate of soybean phosphatides and liver lipid. The method is applied to the analysis of the lipid classes of the tissues and body fluids of rats fed different fat supplements.

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A TOTAL SYSTEM FOR THE GENERATION OF HIGH ACCURACY ANALYSIS OF FATTY ACID METHYL ESTERS BY GAS CHROMATOGRAPHY. J.D. Craske, C.D. Bannon and A.E. Hilliker, Unilever Australia Limited, P.O. Box 9, Balmain, NSW, 2041, Australia.

The generally accepted approach to the analysis of fatty acid methyl esters (FAME) by gas chromatography is to analyze a standard mixture of known composition and to determine therefrom empirical correction factors for individual FAME. It is proposed that this approach is fundamentally unsound as a means of generating consistently accurate results. Rather, the stand is taken that it has now been proven that theoretically calculated FID response factors are valid, both for the saturated and unsaturated FAME commonly encountered in edible oils, and that these should be used as the only response factors. Thus, the proper approach to the generation of highly accurate results is to optimize both equipment and operator technique so that a correct answer is obtained for a primary standard when the theoretical factors are used, rather than to introduce an empirical correction factor other than the theoretical response factor to take account of faulty practice. Several facets of equipment operation or operator technique have been identified which require to be addressed. These will be detailed and an indication given of the importance of high accuracy analysis for control of manufacturing operations.

Session T Friday morning Processing of Oils I

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EXTRUSION OF COTTONSEED AND SOYBEAN IN PREPARATION FOR SOLVENT EXTRACTION. J.T. Farnsworth, Food Protein R&D Center, Texas A&M University, Fm 183, College Station, TX 77843; L.A. Johnson, Iowa State University, and T. Wondrafrash and J.P. Wagner, Texas A&M University.

Extrusion preparation of flaked cottonseed and soybean meats was explored as a means of preparing oilseed products for direct solvent extraction to enhance the oil recovery, increase extraction rates, reduce energy requirements for desolventization and increase extractor capacity. Selected conditions for extrusion appreciably increased the rate of extraction and increased extractor capacity. All extruded pellets had greater bulk density and drained more completely leaving less solvent hold-up than the flakes. Some preparation conditions produced pellets which extracted very well, while other conditions extracted very poorly. The results of the pilot plant extrusion and extraction were included in the economic model for cottonseed oil milling to evaluate the economics of including extrusion preparation in operating oil mills. Capital costs for extruders as well as extractors with increased capacity were included in the computer model with their maintenance and energy requirements. Extrusion preparation was found to increase the average net revenues for direct solvent extraction plants in all sections of the country.

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POSSIBILITY OF USE OF ENZYMES IN OIL EXTRACTION. Krystyna Sosulski and Ewen Coxworth, Saskatchewan Research Council, 15 Innovation Blvd., Saskatoon, Saskatchewan S7N 2X8, and Frank W. Sosulski, University of Saskatchewan, Saskatoon, Saskatchewan.

Extractability of oil from canola seeds depends greatly on mechanical disintegration of cell walls, commonly achieved by cooking, flaking and/or pressing. The potential of enzymatic hydrolysis to increase the permeability of canola cell walls and thus enhance the extractability of oil was investigated. Enzymes produced by several manufacturers and of different specificity were tested and compared on canola exposed to the various steps in the conventional processing of vegetable oil. Extraction efficiencies and quality of oil were determined.

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MODELING THE SOLVENT EXTRACTION OF OILSEEDS. G. Abraham, R. Hron and S. Koltun, USDA, ARS, Southern Regional Research Center, P.O. Box 19867, New Orleans, LA 70179.

A computer model has been developed which calculates the solvent to flake ratio and chain speed (residence time in extractor) for optimum performance in a moving bed extractor. The percent residual lipids in the extracted flakes is also calculated. Experimentally measured data are presented which allow computation of the above extractor parameters for cottonseed with any of the following solvents: ethyl alcohol, isopropyl alcohol, or hexane. With input parameters such as feed rate, number and spacing of solvent spray nozzles, bed width, and temperature of solvent, the model solves a quasi-linear set of material balance and equilibrium equations to give the extractor settings. The model, written in FORTRAN, is relatively simple and can be run on most microcomputers.

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SUNFLOWERSEED OIL EXTRACTION WITH ETHANOL. Marisa A.B. Regitano-d'Arce and Urgel de Almeida Lima, Esc. Sup. Agric. "Luiz de Queiroz" - U.S.P., Av. Pádua Dias, 11 - Caixa Postal 9, CEP - 13.400, Piracicaba, São Paulo, Brazil.

The authors carried out this work to check the possibility of ethanol use as a solvent for oil seed extraction. Sunflower was used because a special program of development of this plant is being studied in Brazil. As microdistilleries for ethyl alcohol production for fuel purposes are being installed in the country, ethanol as solvent in concentrations less than 99% was used in the research. Laboratory test extractions were carried out in Soxhlet and Butt extractors for different times, solvents and raw material rates to study performance of laboratory equipment. As no uniform results were found, a special extractor with 8 vessels was built in order to permit temperature control and solvent exchange as is done in intermittent industrial installations. With this equipment it was found that 99% ethanol showed the best extraction capacity, comparable to hexane in the experimental conditions at atmospheric pressure. The best rate of solvent and raw material was 2:1, moisture content of seeds being less than 5%. Preparation of seeds included drying and flaking. Extraction was done for an immersion period of 8 hr average, efficiency with pure solvent was 81% for hexane and 83% for anhydrous ethyl alcohol for the Contisol cultivar and 69% and 75% for Anhandy cultivar. Efficiency extraction rate was limited because the seed was used in integral form. If press cake was used efficiency was higher but miscellae separation by cooling was never attained due to low oil concentration. By gas chromatographic analysis hexane and alcohol extracted oils did not show differences in fatty acid composition, acidity, saponification and iodine value. Unsaponifiable matter contents were higher for alcohol extracted oils. Amino acid contents of meals from alcohol extractions revealed no difference regarding the original seed. Chlorogenic acid contents of seeds were not affected by hexane and anhydrous alcohol extractions. Ethanol 96%, 93% and 90% extracted more chlorogenic acid from seeds than hexane and anhydrous alcohol.

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APPLIED TECHNIQUES FOR ASSESSING THE POTENTIAL OF PROCESSING COMMERCIALLY IMPORTANT COMPOUNDS VIA SUPERCRITICAL EXTRACTION. W.G. Engelhart, Milton Roy Company, Flow Control Division, 201 Ivyland Road, Ivyland, PA 18974-0577.

Supercritical extraction technology has not been widely applied to commercial compounds for a number of reasons. Principal among these is the absence of basic solubility and equilibrium data to initiate meaningful process studies. To date, this information has been incomplete or unavailable to process researchers for proprietary reasons. The use of a special high pressure ultraviolet-visible light spectrophotometer enabling researchers to rapidly ascertain compound solubilities and optimize supercritical extraction conditions for scale-up is discussed. Results obtained on such substances as algae, citrus oils, bioengineered fungal mycelium, deaactivated palladium catalyst and diesel fuel-oil mixtures will be discussed.

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A COMPARISON OF QUALITY PARAMETERS FOR ANIMAL FAT FROM DIFFERENT RENDERING PROCESSES. Henrik Holst-Pedersen, Baltorpvej 154, DK2750 Ballerup, Copenhagen, Denmark.

Traditionally, most animal fat has been dry rendered. New rendering methods introduced over the last years, especially the low temperature wet rendering process, have brought an improved quality of animal fat on the market. Different rendering processes are discussed with respect to processing conditions that affect the quality. Quality parameters for fat from different rendering processes are compared.

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THE FRACTIONATION OF FATTY ACIDS AND TRIGLYCERIDES BY SUPERCRITICAL EXTRACTION. T.E. Boland, R.J. Robey and S. Sunder.

Fatty acids and triglycerides are difficult to fractionate because of their low vapor pressures and high molecular weights. Fatty acids are traditionally separated by distillation at very low pressures, typically below 20 mm of mercury. Triglycerides are fractionated by fractional crystallization. Supercritical extraction has been proposed as an alternative to both of these processes. The results of experimental studies on the deodorization of coconut oil, the purification of a branched chain fatty acid, the separation of a monobasic acid from a dibasic acid and the fractionation of fatty acid methyl esters will be discussed. Both batch and continuous countercurrent extraction results will be presented. The solvents used include carbon dioxide, propane and ethane. In addition, preliminary results for the production of a cocoa butter substitute or extender from a triglyceride mixture will be discussed. Preliminary process design and economics have been developed for most of these studies. These economics indicate supercritical extraction can be competitive with conventional processes for applications which can take advantage of supercritical extraction's unique characteristics.

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FRACTIONATION OF SOFT COCOA BUTTER. H. Traitler, A. Dieffenbacher and P. Ducret, Nestlé Research Dept., Nestec Ltd., Ave Nestlé 55, CH-1800 Vevey, Switzerland.

Soft cocoa butter with iodine values of around 39 can be upgraded by careful selective fractionation with various solvents and solvent systems. Optimum fractionation temperatures are between −10 and 0 C and solvent to fat ratios are preferably between 2:1 and 5:1. After fractionation times of between 6 and 24 hr, two fractions could be obtained. One fraction which accounts for approximately 15% of the total cocoa butter is a liquid oil at ambient temperature with an iodine value of around 64 (cocoa butter olein) and a solid fraction (cocoa butter stearin) with an iodine value of around 35. Fractionation principle is based on the selective separation of POO,OOO and SOO triglycerides, which in normal cocoa butters account for approx.

6-7% whereas in soft cocoa butters they can reach levels of ca. 15%. High resolution capillary gas chromatography is the method of choice for the analytical backup of the fractionation efficiency. Possible applications of CB-olein are cosmetic as well as food, e.g., in chocolate spreads whereas the stearin fraction gives normal to slightly harder chocolate qualities. Fatty acid composition of the stearin fraction is within the limits of natural variation of cocoa butters.

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FRACTIONATION OF BLACK-CURRANTSEED OIL. H. Traitler, H.J. Wille, A. Studer, Nestlé Research Dept., Nestec Ltd., Avenue Nestlé 55, CH-1800 Vevey, Switzerland.

Black-currantseed oil is known to be one of the richest natural sources of γ-linolenic acid, with values of up to 19% of this acid. These concentrations are sufficient for most of the applications of the oil as such but some particular utilizations require higher concentrations of γ-linolenic acid from between 70 to 95% purity. Black-currant oil also contains up to 14% of α-linolenic acid. Different fractionation techniques have been evaluated. Distillation as well as fractionated crystallization at various temperatures did not give any reasonable results. Preparative high performarnce liquid chromatography gives only separations on reversed phase columns (RP-18 type) but α- and γ-linolenic acid cannot be separated by this technique. Surprisingly enough, urea fractionation in methanol gives a specific separation of α- and γ-linolenic acid, whereas stearidonic acid (18:4,, n-3), which is present at around 3% in the black-currant seed oil, cannot be separated by urea-fractionation but only by subsequent preparative HPLC on RP-18 columns. Stearidonic acid, as γ-linolenic acid has a double bond in the Δ6 position, which makes these two acids quite unique in this respect. This most probably explains their similar behavior toward urea-occlusion. On a large scale basis of up to ton scale it was possible to enrich black-currantseed oil fatty acids to 75-80% γ-linolenic acid in a preferably 2-stage fractionation process. Further preparative HPLC-separations allowed fractions of 95% γ-linolenic acid.

Session U Friday morning Biological Oxidation of Lipids III

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LIPID PEROXIDES AS AGENTS CAUSING ATHEROGENESIS. Kunio Yagi, Institute of Applied Biochemistry, Yagi Memorial Park, Mitake, Gifu 505-01, Japan.

We have reported that intravenous injection of linoleic acid hydroperoxide into a rabbit provoked injury to the endothel-ial cells of the thoracic aorta, along with adherence of aggregated platelets. From these aggregates a growth factor causing smooth muscle cells (SMC) to proliferate and chemoattractants, which force SMC to migrate from their normal medial site into the intima, are known to be released. It was recently found by using cultured cells that SMC are less susceptible to injury by linoleic acid hydroperoxide than are endothelial cells. Thus, it seems that SMC still survive when the endothelial cells are injured by the hydroperoxide. Nevertheless, the hydroperoxide has a definite effect on the uptake of low density lipoprotein (LDL) by SMC. Binding of LDL to cultured SMC from rabbit aorta was increased when the cells were incubated with LDL in the presence of 3-6 nmol/ml of the hydroperoxide (in terms of malondialdehyde) as determined by the thiobarbituric acid method. Under these conditions, the internalization of LDL was increased compared with that in the absence of the hydroperoxide. Obviously cholesterol carried by LDL into the cells tends to accumulate. This would account for the formation of foam cells from SMC. In the case of cultured phagocytes, accumulation of lipids in the cells was increased when LDL was previously incubated with hydroperoxide, indicating the possible formation of foam cells from phagocytes. From these observations, we propose that an increase in serum lipid peroxide level both initiates and promotes atherogenesis.

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PEROXIDATION OF UNSATURATED FATTY ACIDS IN LIPOSOMAL MEMBRANES. Junzo Sunamoto, Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852, Japan.

The peroxidation of unsaturated lipids at various levels caused by enzymatic and/or nonenzymatic processes leads to serious problems such as damage of cell membranes, inflammation, arteriosclerosis, cataract and so forth. Therefore, the investigation of lipid peroxidation occupies an important position in understanding a class of compounds that play a central role in a variety of biological events and food products. In order to understand the effect of microenvironment on lipid peroxidation, liposomal membranes have been employed in this work. In the peroxidation of unsaturated fatty acids such as linoleoyl and arachidonyl acids in DMPC or DPPC liposomes, autoxidation was largely retarded with a decrease in the membrane fluidity by adding cholesterol or other additives, while lipoxygenase peroxidation was accelerated by decreasing the membrane fluidity. Lipoxygenase oxidation was, however, effectively prohibited by coating the surface of liposomal membranes with polysaccharide derivatives or by doubly encapsulating liposomes into the hydrogels. In the oxidation with Fe(II)-ascorbic acid, both the copper(II) and manganese (II) ions were found to be good antioxidants.

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LIPID PEROXIDATION AND CHEMILUMINESCENCE IN ANIMAL TISSUES. Teruo Miyazawa and Kenshiro Fujimoto, Tohoku University, Food Chemistry Department, Tsutsumidori Amamiyamachi 1-1, Sendai 980, Japan, and Takashi Kaneda, Koriyama Women's University.

Chemiluminescence (CL) is generally known to be associated with oxidation reactions of some organic compounds involving molecular oxygen. A similar CL could be expected to occur under physiological conditions. The objective of this study is to detect and characterize the weak light emissions from in vivo lipid peroxidation induced by the feeding of oxidized oils to animals. With this aim, a synchronous single photon counting apparatus was developed and used, which enables to detect quantitatively as weak a CL from tissue samples as below 10^{-17}W. By the oral administration of oxidized oils and methyl linoleate hydroperoxides to rats, the tissue CL intensities and TBA values were obviously increased in liver, kidney, heart and lung, and the increases were inhibited by the supplements of α-tocopherol and riboflavin butyrate to animals. The luminescence spectrum recorded by a filter spectral analyzing method gave definitive emission peaks at 585 nm and 635 nm for CL of liver homogenates of rats fed oxidized oils. The spectrum was identical to that for singlet molecular oxygen. A very strong CL was also found when lipid hydroperoxides were added to rat liver homogenates in vitro, and was quenched by the presence of free radical scavengers and β-carotene. The free radicals essentially responsible for tissue CL were identified as lipid peroxy radicals which were adducted in vitro and in vivo systems by ESR studies using a spin-trapping technique. These results show that the tissue CL reflects the occurrence of short-lived free radicals and excited species derived form lipid peroxidation which was induced by the feeding of oxidized oils to animals.

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INHIBITION OF PEROXIDATIONS OF LIPOSOMAL- AND BIO-MEMBRANES BY WATER SOLUBLE ANTI-

OXIDANTS. Etsuo Niki, Yorihiro Yamamoto and Yoshio Kamiya, University of Tokyo, Faculty of Engineering, Hongo, Tokyo 113, Japan.

Aerobic organisms have an array of protective mechanisms both for preventing the formation of active oxygen species and lipid peroxidation and for repairing oxidative damage. Vitamin E is accepted as a lipophilic antioxidant in biological membranes. In this work, we have studied the possible role of uric acid, ascorbic acid, cysteine and glutathione as water soluble antioxidants. These compounds scavenged the free radicals in an aqueous phase efficiently and suppressed the oxidations of methyl linoleate micelles, soybean phosphatidylcholine liposomes, and erythrocyte ghost membranes in aqueous dispersions initiated with a water soluble azo compound which generated the initiating free radicals at a constant and known rate. However, these compounds located in an aqueous phase could not scavenge the lipid peroxy radicals in the membranes and, hence, could not suppress the oxidations when the initiating radicals were generated in the lipid region of the membranes. Ascorbic acid showed synergistic inhibition with vitamin E: ascorbic acid in an aqueous phase could regenerate vitamin E probably by giving a hydrogen atom to chromanoxy radical as observed in homogeneous solution. However, other compounds did not show an appreciable synergistic effect with vitamin E. The effect of these compounds on in vivo peroxidations also will be discussed.

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DRUGS, LIPID PEROXIDATION AND GLUTATHIONE STATUS OF MOUSE LIVER. Albrecht Wendel, University of Tübingen, Hoppe-Seyler-Str. 1, 7400-Tübingen, West Germany, and Hartmut Jaeschke and Chritin Kleinwächter.

Lipid peroxidation is believed to represent a primary lesion in acute drug intoxication. The aim of this work was to evaluate the general significance of LPO in vivo in mechanistically different models of hepatotoxicity in male albino NMRI mice. LPO was assessed in vivo by exhalation of ethane and pentane, and in vitro by determination of thiobarbituric acid-reactive material. The extent of liver damage was measured by quantification of transaminases release into the circulation as well as by the degree of hepatic glutathione (GSH) depletion. When paracetamol was administered as a model drug metabolized by the membrane-bound microsomal monooxygenase system, a dose-dependent LPO parallel to the severity of the liver lesion was observed. In the isolated perfused liver, it was shown that LPO preceded cell death. Pretreatment of the animals with either liposomally entrapped GSH or soybean lecithin fully protected the animals against otherwise lethal doses of paracetamol and abolished LPO. These effects were not due to a block in the metabolism of the drug. Allyl alcohol (AA), as the second model hepatotoxin used, is metabolized by the soluble cytosolic enzyme alcohol dehydrogenase to acrolein, the presumable ultimate toxic metabolite. Administration of AA produced an immediate massive dose-dependent LPO in vivo and in vitro, fulminant liver destruction and total depletion of hepatic GSH. Pretreatment of the animals with inhibitors of alcohol dehydrogenase prevented all symptoms. This is the first demonstration of in vivo LPO induced by cytosolic non-oxygen-dependent metabolism of a xenobiotic. Intravenous pretreatment with soluble GSH protected the liver and suppressed LPO. Only sulfhydryls available for intrahepatic GSH biosynthesis such as N-acetyl cysteine or methionine were protective. In contrast, intravenous pretreatment with lecithin abolished the signs of LPO but failed to show hepatoprotection. This divergence seems not compatible with a causal involvement of LPO in liver toxicity of AA. The mechanism by which reactive metabolites generated within the cytosol of liver cells peroxidize membrane lipid is discussed.

Session V Friday afternoon
Edible Uses of Oils and Derivatives

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MICROSTRUCTURE AND INTERACTIONS OF DISPERSED SOY LECITHIN IN MILK. W. Buchheim, A. Wiechen, D. Prokopek and A. Funke, Federal Dairy Research Centre, H. Weigmann Str.1, D-2300 Kiel, West Germany

There are indications that the enzymic and acid coagulation of milk proteins (casein micelles) may be influenced by the presence of lecithin, thereby also affecting composition and texture of cheese. Using a commercial soybean lecithin ('Metarin K,' Lucas Meyer, Hamburg, F.R.G.) which contained 20–23% PC, 21–24% PE and 18–22% PI, the microstructure and the interactions with milk proteins have been studied transmission electron microscopically by application of the freeze-fracture technique. When dispersed into skimmed milk by ultrasonics or high pressure homogenization the lecithin formed globular, liposome-like vesicles with a volume/surfacer average diameter (D_{vs}) of ca. 200 nm. Thermal sterilization of a milk-lecithin mixture resulted in a partial fusion of individual vesicles and, furthermore, in the formation of complexes with protein. During acidification of cheese milks by a starter culture the lecithin vesicles transformed into a complex network-like structure including aggregated protein. Whereas lecithin vesicles of a non-heated milk-lecithin mixture exhibited only a weak interaction with milk proteins and were accumulated mainly in the cheese whey, those which had been heated were located mainly on the surface or within the protein matrix of the cheese curd. Possible effects on composition and texture of cheese will be discussed.

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A COCOA BUTTER EQUIVALENT FROM *SHOREA ROBUSTA* FAT. Kazuo Itagaki and Shoji Maruzeni, Asahi Denka Kogyo K.K., 4, 8-Chome, Higashi-Ogu, Arakawa-Ku, Tokyo, Japan.

Sal fat extracted from *Shorea robusta* seeds is rich in 2-oleoyl-1,3-disaturated alkanoyl triglycerides, and its mid-fraction proved to be one of the valuable materials formanufacturing cocoa butter equivalents (CBEs), through the investigation of characteristics of the CBEs and the practical evaluation of chocolate made from them. Sal mid-fraction and palm mid-fraction were prepared by solvent fractionation on a laboratory scale, and physical behavior of these mixtures was examined by solid fat content (SFC) curves and compared with that of cocoa butter. The SFC curve of the mixture of 40% sal mid-fraction and 60% palm mid-fraction was identical with that of cocoa butter. The products were subjected to various chocolate-making processes. Chocolates were subjected to practical evaluation tests such as solidification, hardness and anti-blooming tests, and proved to be improved in all respects by incorporation of the CBEs. Shea butter (fat from *Butyrospermum parkii*) traditionally used in manufacturing CBEs will also be discussed in comparison with sal fat.

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EFFECT OF FOOD EMULSIFIERS IN FLUID SHORTENING ON THE ONE STAGE MIXING OF CAKE MAKING. Nobuya Matsui, Niijima Gakuen Women's Junior College, 53 Showa-machi, Takasaki-city, Japan.

Fluid shortening is used for the manufacturing of butter sponge cake in Japan. Fluid shortening is available for one-stage mixing in the continuous mass-production line. We can find many types of fluid shortening on the market. However, this report is concerned with only one type of liquid oil and some types of food emulsifiers. It was reported that food emulsifiers are effective in multi-component systems. Fatty acid esters of propylene glycol, glycerine, sorbitan and sugar are allowed as food emulsifiers in Japan. The effect of these emulsifiers on foaming and cake making

in one-stage mixing will be shown especially in relation to the stearic acid esters of propylene glycol, sugar and polyglycerine.

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INTERACTION BETWEEN AMYLOPECTIN AND MONOGLYCERIDES IN MODEL SYSTEMS IN RELATION TO BREADMAKING CONDITIONS. Lilian R. Batres and Pamela J. White, Iowa State University, 107 Mackay Hall, Ames, IA 50011.

The ability of glyceryl monomyristate (GMM), glyceryl monopalmitate (GMP) and glyceryl monostearate (GMS) to form insoluble complexes with amylopectin was studied in model systems. By using different solvent extractions, it was demonstrated that amylopectin complexed to the greatest extent with GMP, followed by GMM and GMS, respectively. The degree of complex formation was statistically different ($p < 0.01$) among monoglycerides. Iodimetric titrations of the complexes showed that the presence of GMP and GMM in the model systems significantly ($p < 0.01$) decreased the iodine affinity of the amylopectin when compared to the control. The presence of GMS in the model systems did not significantly decrease the iodine affinity of the amylopectin, however a slight effect was seen. The decrease in iodine affinity caused by the three monoglycerides was statistically different ($p < 0.01$) when compared among treatments. A negative linear relationship ($r = -0.999$, $p < 0.01$) was found between the amount of complex formed and the iodine affinity of the complexes.

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THE EFFECT OF A MICROWAVE TEMPERING SYSTEM. Hiroaki Hoshino, Miyoshi Oil & Fat Co. Ltd., 1-48, 7-Chome, Karumo-Dori, Nagatu-Ku, Kobe, Japan, and Hirohisa Sasaki, Koichiro Marusugi, Moriya Sano and Takeo Saotome, Miyoshi Oil & Fat Co. Ltd, Kobe Factory.

Ordinary tempering processes have been designed to keep the temperature of materials below the melting point during a long period. In this report, we tried to use microwave energy to complete this process in a short time, and to get products with high whipping value. A microwave generator was introduced in the manufacturing process. The apparatus we used was able to control the temperature of materials within ±1 C of the desired temperature. Our discussion is concerned with the effect of tempering period and temperature, and the result was that the latter has great influence on the tempering process. The microwave tempering method and the ordinary method were equivalent to one another in getting high whipping value. It was suitable to measure the solid fat content curve for the determination of the suitable temperature of microwave tempering. Quality of the products was evaluated mainly by a whipping test. Electron microscopy, DSC and X-ray diffraction pattern were also used for the evaluation.

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RHEOLOGY OF WHIPPED TOPPINGS IN RELATION TO LIPID-PROTEIN INTERACTIONS. Niels Krog, Grindsted Products A/S, Edwin Rahrs Vej 38, DK-8220 Brabrand, Denmark.

Whippability and foam stiffness of toppings are studied as a function of fat composition. Hydrogenated coconut oil (HCNO) is usually used as topping fats. As substitutes partially hydrogenated soybean oil (HSBO, m.p. 35 C) and hydrogenated fish oil (m.p. 35 C) were tested in toppings formulation with propylene glycol monostearate as surfactant. The solid fat content of HSBO and fish oil were identical to that of HCNO at 25 C. Lipid-protein interactions in the dry topping powders were measured by low resolution pulse-NMR technique and correlated to whippability and viscoelastic properties of the whipped toppings. Only HCNO gave good foam stiffness. Dynamic complex modulus, $G = 5140$ Pascal at oscillation frequency 0.1.Hz. The foam of whipped HCNO toppings is stabilized by crystalline platelets of fat forming a network around the air cells. The strength of this network is proportional to the amount of crystals formed which depends on the degree of supercooling of the fat phase. Toppings produced with the other fats gave low foam stiffness ($G = 800$ Pascal). The poor functionality of HSBO or fish oil was followed by a low lipid-protein interaction in contrast to toppings with HCNO. There was no or very little supercooling of the fat phase in the HSBO or fish oil toppings; thus, no crystallization took place after reconstitution, and therefore these whipped topping foams had no internal structure to support their texture. The dynamic complex modulus, G. increases proportionally to surfactant concentration, while the loss factor, G''/G', is constant.

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SPECIFIC APPLICATIONS OF PALM KERNEL OIL IN THE FOOD INDUSTRY. K.G. Berger and M.S.A. Kheiri, Palm Oil Research Institute of Malaysia.

The availability of palm kernel oil is increasing annually, whereas supplies of coconut oil, the major lauric oil, are rather static. Palm kernel oil has a higher content of unsaturated C18 acids than coconut oil and therefore a range of products can be made by hydrogenation and/or fractionation. The physical properties of these products make them especially suitable for applications in confectionery, bakery coatings and imitation creams. Palm kernel oil has valuable properties in margarine either in straight blends or after interesterification. A number of specialty fats based on palm kernel oil are commercially available.

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BEEF LIPID DEGRADATION IN STEW WITH AND WITHOUT VEGETABLES. Sharon L. Melton, Myung Joo Han, M.J. Riemann and F.A. Draughon, University of Tennessee, Food Technology and Science, P.O. Box 1071, Knoxville, TN 37901.

The effect of cooking, 4 different treatments (T), 2 processes (P) and storage (S) was determined on malonaldehyde (MA) content of beef stew and concentrations of individual phospholipids (PL) and fatty acids and aldehydes of PL in beef. The treatments were beef cooked alone (T1), with either onion (T2), or carrot (T3) or with both vegetables (T4). The two processes were storage in a barrier bag (P1) at 0 C or a polyethylene container (P2) at 5 C. Storage times for P1 were 0, 2 and 4 weeks and for P2, 0, 2 and 4 days. Two replications were run. A replication ($n = 24$) consisted of homogeneous lot of diced beef of the Biceps femoris and semitendinosus muscles from the right (replication 1) or left (replication 2) side of "A" maturity steer carcasses and homogeneous lots of dried onions and frozen carrots. The order in which each stew was cooked was randomized across T, S and replication for each process. Cooking the beef resulted in a 40% loss of cephalin and a 20% loss of lecithin, both expressed on a beef dry matter basis, and a 20 to 25% loss in the fatty aldehydes with 16 and 18 carbons expressed on a total lipid basis. The major degradation products of the phospholipids were lysocephalin and lysolecithin. MA content increased linearly across storage in each process, but the rate of increase was drastically reduced by storage in the barrier bag (P1) compared to conventional storage (P2). Addition of carrots to the stew (T3 and T4) caused significantly greater concentrations in cooked beef of cephalin and lecithin and the PL fatty acids, $20{:}3_{\omega}6$ and $20{:}4_{\omega}6$, than in beef cooked alone (T1) or with onions (T2). Storage did not affect ($P < 0.05$) individual PL content or PL fatty acid and aldehyde concentrations in either process.

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EFFECTS OF PIGMENTS ON THE OXIDATION STABILITY OF OLIVE OIL. Nickolaos Fakourelis, Eun-Ok Choe Lee and David B. Min, Ohio State University, Department of Food Science and Nutrition, 122VH - 2121 Fyffe Rd., Columbus, OH 43210.

Virgin olive oil was purified by silicic acid and charcoal column chromatography. The effects of chlorophylls and carotenes on the oxidation stability of virgin and purified oils were studied by the combination of oxygen disappearance, volatile compounds formation and peroxide value determination. Virgin oil was

oxidized faster than the purified oil under light, and the reverse was true under dark storage. Results also showed that chlorophylls in purified oil acted as photosensitizer for singlet oxygen formation to accelerate the oil oxidation under light and acted as an antioxidant in purified oil under dark. Carotenes worked as an antioxidant under light due to its light-filtering effect, but did not show any effect on the oxidative stability of virgin or purified oil under dark. Experiments clearly suggested that two different oxidation mechanisms were involved in the oil oxidation during storage under light and dark in the presence of chlorophyll. Singlet oxygen was responsible under light, and free radical reaction involving triplet oxygen was responsible under dark for the oil oxidation.

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NUTRITIONAL IMPACTS OF OILS AND FATS CONSUMPTION TRENDS IN DEVELOPED AND DEVELOPING COUNTRIES. S.H. Fatemi, Iranian Research Organization of Science and Technology, 118 Felestin St., Felestin Sq., Tehran, Iran.

Food energy deficiency is the most pressing nutritional problem of the unindustrialized world. Oils and fats are valuable sources of energy; increasing the ratio of energy derived from oils and fats to the total energy in diet, however, can be characterized as a sign of food affluence with its specific consumption pattern. In this study, changing trends of oils and fats consumption versus total energy intakes are investigated at three-year intervals during the period from 1965-1982. This is done on a group of 22 countries with food calorie intakes below their requirements but with relatively high levels of fat (oil) and protein, and 31 industrialized countries. Correlation coefficients for calorie deficient countries indicate a distinct tendency to utilize more fat (oil) in the diet rather than to improve the whole energy deficient diet through proper approaches. The lowest coefficient is found for 1974-1976 interval ($r = 0.15$). World food crisis in the mid-1970s, along with higher prices of petroleum products and food commodities, could account for food energy shortages in some developing countries. A considerable increase in fat (oil) consumption, however, implies that at the time of economical and agricultural crisis, affluent trend may even grow at the expense of depressing the staple diet. Such a trend which also intensifies uneven distribution of foods, has caused many developing countries to experience complications, resulting from both a high fatty diet and an energy deficient one, simultaneously. The ratio mentioned earlier can be employed as an index to evaluate nutritional status and estimate the actual energy intakes by malnourished people in many developing countries. Higher correlation coefficients are observed for industrialized countries. Again, the lowest coefficient belongs to 1974-1976 interval ($r = 0.39$). In these countries, the growth of energy intake basically results from higher fat (oil) consumption, of which invisible animal fats constitute a major part. Thus, to overcome nutritional complications of a high fat (oil) diet, emphasis should generally be focussed on the reduction of animal products consumption.

Session W Friday afternoon Role of Lipids in Cancer II

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UNUSUAL UNSATURATED FATTY ACIDS IN HUMAN HEPATOCELLULAR CARCINOMA. Nobuo Okazaki, Department of Internal Medicine, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo, Japan, and Eiji Araka and Keiko Oda, National Cancer Center Hospital.

Eicosatrienoic acid n-9 is known to increase in essential fatty acid-deficient mammals. This unusual fatty acid has been found to increase in tumor tissue as well as blood of patients with hepatocellular carcinoma irrespective of their nutritional conditions. The present study was undertaken to demonstrate an abnormal metabolic pattern of fatty acid in human hepatocellular carcinoma (HCC). The producing ability of eicosatrienoic acid n-9 was maintained in a human HCC tissue (Li-7) transplanted into nude mice over 50 generations, which were fed on high linoleic acid diet (60% of total fatty acids). Eicosatrienoic acid n-9 of the tumor was distributed in all lipid fractions and was especially high in glycerophospholipid fraction, but not in the host liver. The percentages of linoleic and arachidonic acids in the total fatty acid contents were not different from those in the host liver. Increment of a fatty acid was found in Li-7, which could be due to an increase in eicosadienoic acid n-9. These data indicate that in human HCC (Li-7) synthesis of eicosatrienoic acid n-9 is not suppressed by essential fatty acids.

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CALORIC RESTRICTION AND EXPERIMENTAL CARCINOGENESIS IN RATS. David Kritchevsky, Maxine M. Weber, Carrie L. Buck and David M. Klurfeld, The Wistar Institute, 3601 Spruce St., Philadelphia, PA 19104.

Moreschi (1909) demonstrated that caloric restriction inhibited growth of transplantable sarcomas in mice. Tannenbaum and Lavik and Baumann (1940s) found caloric restriction to lower incidence of chemically induced skin tumors in mice. We (*Cancer Res. 44*:3174, 1984) found that rats maintained on a calorically restricted (by 40%) diet exhibited a significantly lower incidence of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors than ad libitum-fed controls. Two further studies are reported here. In the first, female Sprague-Dawley rats were fed ad libitum (AL) a diet containing 5% corn oil and compared with rats pair-fed to receive 10, 20, 30 or 40% reduction in calories (R) but still ingest 5% fat. Incidence of DMBA-induced tumors (%) was: AL, 60; 10% R, 60; 20% R, 40; 30% R, 35; and 40% R, 5. Average tumor burden (tumors per tumor-bearing rat × tumor weight) was: AL, 10.1; 10% R, 5.4; 20% R, 4.7, and 30% R, 0.9. In the 40% R group one rat had one tumor. Rats were also fed AL diets containing 5, 15 or 20% cornoil. Two pair-fed groups were maintained on diets providing 75% of calories but still giving 15 or 20% corn oil. Incidence (%) of mammary tumors in the AL groups was: 5%, 65; 15%, 85, and 20%, 80. In the calorie restricted rats incidence was: 15%, 60; and 20%, 30. Tumor burden in the five groups was: 5%, 4.2; 15%, 6.6; 20%, 11.8; 15% R, 1.5; and 20% R, 2.3. Rats maintained on regimens low in calories exhibit fewer tumors even when the restricted diet is high in fat.

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EARLY EVENTS: CHANGES IN HOST METABOLISM PRECEDING TUMOR GROWTH. Randall Wood, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843.

After a susceptible host is inoculated with a transplantable tumor, there is a latent period when tumor growth is not detectable and the host animal appears to be completely normal. Buffalo stain rats transplanted with Morris hepatoma 7288CTC exhibit a latent period of 10 to 14 days. Although host animals appear normal in the latent period, changes in the lipid composition and metabolism have been detected. The ratio of oleate to vaccenate in the plasma phospholipids is detectable by the third day and significantly different by the 6th day after transplantion (*Lipids 14*:70-71, 1979). The concentration of plasma free fatty acids and triglycerides decreased significantly at days 3 and 6, respectively, before returning to normal levels at day 12, at which time the concentration of all neutral lipid classes increased sharply (*Lipids 15*:421-427, 1980 and *17*:771-779, 1982). Additional evidence that the lipid metabolism of the host animal is affected early by the tumor was obtained with studies using methyl 2-hexadecynoic acid, an acetylenic acid (*Lipids 15*:141-150, 1980). The data suggest that one or more enzymes responsible for the metabolism of the acetylenic acid was absent from the host animals, but not the controls. These data and others indicate the lipid metabolism of the host animal is affected soon after transplantation and may be involved in the preparation of the host for the tumor growth.

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ABNORMALITIES IN LIPID METABOLISM IN CANCER: AN IN VITRO MODEL FOR LIPID MOBILIZATION. Philomena F. McAndrew, UCLA/Wadsworth VA Medical Center, Wilshire and Sawtelle Blvds., Los Angeles, CA 90073, and Shinichi Kitada, James F. Mead and Esther Hays, University of California at Los Angeles.

Weight loss leads to significant morbidity in the cancer afflicted population. Several studies have shown that the magnitude of specific lipid metabolic abnormalities and fat depletion exceeds that of protein loss in animals and humans with cancer. Anorexia and decreased energy intake alone are insufficient to explain these changes. Recently, a factor (Lipid Mobilizing Factor—LMF) has been recognized in the sera and tumor extracts of AKR lymphoma bearing mice which causes lipid mobilization in an in vivo bioassay (mice with radiolabeled adipose tissue). Sera of cancer patients (9/12) with a variety of advanced tumors were also found to have this activity. Serum free conditioned medium from a subclone of AKR lymphoma contains a significant LMF activity according to this assay and is the source of material for the purification of this factor. We have developed an in vitro assay with cultured pre-adipocytes to study this lipid mobilization that can be used to assay quantitatively for this peptide during the purification as well as to study its mechanism of action. These studies into the mechanism of cancer-induced lipid stores depletion may provide an insight into specific means of blocking these metabolic abnormalities and possibly inhibit tumor growth.

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CHARACTERIZATION OF LIPID MOBILIZING FACTOR (LMF) FROM MURINE MALIGNANT T LYMPHOMA CELL LINES. Shinichi Kitada, Brenda Guthrie, Philomena McAndrew, Minerva G. Elepano, Esther F. Hays, James F. Mead and Joseph R. Reeve Jr., University of California, Los Angeles, Laboratory of Biomedical and Environmental Sciences, 900 Veteran Ave., Los Angeles, CA 90024.

In previous studies using AKR mice with implanted adipose tissue labeled with [1-^{14}C]-linoleic acid, we have shown that in the fed mouse having a thymic lymphoma, fat is mobilized rapidly and appears in part in the membrane phospholipids of the tumor (*Lipids 15*:168-174, 1980; *Prog. Lipid Res. 20*823-86, 1981). LMF was originally detected in serum of lymphoma-bearing mice by the dramatic increase in the expired CO_2 when injected into normal fed mice with radioactive adipose grafts. Activity was also detected in serum-free conditioned medium from murine malignant lymphoma cell line SL 12.4 by in vivo assay. In vitro lipolytic activity has been demonstrated by analysis of glycerol liberated from adipocytes after incubation with conditioned medium and used for isolation. LMF is a relatively heat stable, small protein (molecular weight about 5,000). LMF may play a part in adipose tissue depletion in lymphoma-bearing AKR mice enabling the growing tumor to derive fatty acids necessary for membrane formation.

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LIPIDS AND CANCER: A COMMENTARY. Roslyn B. Alfin-Slater, University of California, Los Angeles, School of Public Health, Center for Health Sciences, Los Angeles, CA 90024.

Of the three most prevalent types of cancer in the U.S., i.e., lung, mammary and colon, epidemiological studies have suggested an association between a diet high in fat and the latter two conditions. The possibility exists that high fat diets may stimulate the biosynthesis of prolactin which may induce or enhance mammary tumors, and may result in the overproduction of bile secretions which then may form carcinogens by bacterial action in the colon. Whether fats are initiators or moderators in the etiology of cancer, whether fats are involved only because of their high caloric contributions to the diet, whether both saturated and unsaturated fats are equally involved, whether they act as carcinogens or cocarcinogens, and/or whether they affect the immune system thus making the body more susceptible to external factors, still require further investigation. It is interesting to note that experiments with animals given a carcinogen, a high-fat, calorie-restricted diet, resulted in fewer tumors than in rats fed ad libitum suggesting that calories rather than fat play a role in carcinogenesis.

Session X Friday afternoon Symposium on Oilseed Proteins—Fermentation

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MICROBIAL ENVIRONMENTS IN SOLID SUBSTRATE FERMENTATIONS. Richard E. Mudgett, Food Science and Nutrition Department, University of Massachusetts, Amherst, MA 01003.

The microbial environment may significantly affect growth and product formation rates and yields in koji fermentations by filamentous fungi or bacteria that can grow in mycelial forms. Although the liquid phase is the locus of biological activity in such fermentations, it is not accessible to direct measurement and control because it exists on solid surfaces in thin films that cannot be monitored by sensors used in submerged cultures. The gas environment is accessible to direct measurements and can be monitored in terms of gas pressures, flow rates, humidity and temperature, variables which may lend themselves to new adaptive process control techniques through the development of new sensors and control algorithms based on biological models related to heat and mass transfer. Oxygen consumption and carbon dioxide evolution in solid-state processes are of interest not only in their role as indicators of biological activity in the liquid phase, but also for their effect on equilibria between the liquid phase and the external gas environment. Research on gas environment effects in open and closed aeration systems is discussed that suggests direct regulation of metabolism by oxygen or carbon dioxide for solid substrate fermentations including the traditional rice koji process, a novel process for leaf protein recovery and the conversion of natural birch lignin. Oxygen and carbon dioxide transfer rates in open aeration of rice koji in packed beds are also shown to be unusually high, compared with transfer rates normally seen in submerged cultures at laboratory scale. Some advantages of closed aeration systems in maintaining constant gas environments are also considered in terms of their potential for process control.

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HETEROGENEOUS BIOREACTORS FOR LIPID DERIVATIVE SYNTHESIS. Yukihisa Tanaka, Jiro Hirano and Tadashi Funada, Nippon Oil and Fats Co. Ltd., Tsukuba Research Lab. Tokodai 5-10, Toyosato-machi, Tsukuba, Ibaraki, 300-26 Japan.

A lipase can hydrolyze substrates and synthesize at the interface of oil and water, because these solvents are not mutually soluble. Generally stirring and an emulsifier were used for increasing the area of an interface and improving efficiency of the enzymatic reactions. The authors have devised a bioreactor which is suitable for heterogeneous lipase reactions and saving energy. Two principles were applied when developing this bioreactor: (i) The substrates (oil, fatty acids, etc.) are insoluble in water. (ii) Their specific gravities are smaller than that of water. This bioreactor was composed of cylinder units, one of which is 30 mm diameter, 70 mm height. At the connection between the units, there are dispersers. When the substrates go through the dispersers, they become fine spheres of about 0.1-1.0 mm diameter. The area of interface increases by using the dispersers, and the reaction rate can be accelerated as the area of interface increases. In this report, it was concluded that a triglyceride was synthesized by the bioreactor. Conditions: Number of dispersers, 7; rate of water in glyceride, 10%; temp., 40 C; enzyme, the lipase derived from *Can. cylindracea* (1000 μml). Results: Time of the reaction, 7.5 hr; rate of the synthesis, 78.1%; rate of TG in the reaction mixture, 32.7%. These results show that this bioreactor can be used more efficiently than batch type reactors.

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BIOCHEMICAL MODIFICATION OF FATS BY MICROORGANISMS. S. Koritala, C.W. Hesseltine and T.L. Mounts, Northern Regional Research Center, USDA/ARS, 1815 N. University St., Peoria, IL 61604, and E.H. Pryde, deceased.

Industrially useful chemicals are currently derived from fats and oils by chemical modification at high temperatures and pressure, often in the presence of metallic catalysts. Enzymes or microbial whole cells could carry out some of these reactions with greater rapidity and better specificity under milder conditions. At the Northern Regional Reserach Center, we have instituted a survey of microorganisms capable of growing in the presence of soybean oil. Microorganisms surveyed include bacteria, actinomycetes, fungi and yeasts. Lipase appeared to be extensively distributed, especially in fungi. In some instances, over 90% of the soybean oil was hydrolyzed to free fatty acids (FFA). The composition of the fractions recovered from both the medium and cells indicated that some microorganisms preferentially utilize polyunsaturated fatty acids.

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PRODUCTION OF γ-LINOLENIC ACID BY FUNGI. Osamu Suzuki and Toshihiro Yokochi, National Chemical Laboratory for Industry, 1-1, Higashi, Yatabemachi, Tsukuba-gun, Ibaraki, Japan 305.

γ-Linolenic acid (all-*cis*-6,9,12-octadecatrienoic acid) is one of the precursors of prostaglandins and their physiological activities. Recently it has been publicized as a component of healthy foods and a raw material of medicines. The most common source of this fatty acid is Evening Primrose (*Oenotbera biennis*) seed oil. A very efficient microbiological method is proposed for the preparation of γ-linolenic acid or a lipid containing γ-linolenic acid. The fatty acid was found to occur in considerable amounts in fungal lipids in a species of the *Mortierella* genus fungi. The cells were grown very rapidly in an unconventionally high concentration of the carbon source, carbohydrates, of 200 g/l, to give a yield of the fungal cells of 80 g/l, on a dry basis, containing 48% by weight of the lipid corresponding to a yield of lipid of 38 g/l. The optimal conditions of culture are D.O. 2 ppm, pH 4.0 and 30 C. The fungal lipids were extracted with $CHCl_3$/MeOH (2:1, v/v) and analyzed for composition of fatty acids and for total, neutral and polar lipid fractions by gas liquid chromatography. The main fatty acids of *M. vinacea* lipids are 27.0% palmitic, 4.8% stearic, 45.4% oleic, 13.0% linoleic, 7.8% γ-linolenic fatty acids. A concentrate containing 97% of methyl γ-linolenate was prepared from the subsequent urea complex separations of non-adduct fractions of fungal lipids. Characterization of the isolated γ-linolenic acid ester by PMR and ^{13}C-NMR gives a consistent identity of its chemical shifts after assignment of all ^{13}C-NMR signals.

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PRODUCTION OF EPA BY FRESHWATER UNICELLULAR ALGAE. Hiroaki Iwamoto and Shinichio Sato, Faculty of Agriculture, Meiji University, 1-1-1, Higashimita, Tamaku, Kawasaki-shi, Japan.

A highly unsaturated fatty acid, EPA, is important as a precursor of prostaglandin. Marine Chlorella is an alga which has been investigated as a possible source of EPA in addition to fish oil. EPA-rich algae are found mostly in marine species, but *Monodus subterraneus* is the only freshwater alga which contains extremely EPA-rich lipid. This alga shows a high lipid content even in nitrogen sufficient culture and also shows a high EPA content in its total fatty acids under the same condition. Effects of temperature and incident light intensity on growth, lipid content and EPA content are investigated. Lower temperature and lower light intensity favored increased EPA content. EPA is contained mostly in the polar lipids, so high grade lipid accumulation udner nitrogen deficiency is not always advantageous for EPA production. The EPA content changed remarkably with NaCl concentration and heterotrophic or mixotrophic culture condtions. Production of EPA by culturing *Monodus* and other algae is discussed.

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SOYBEAN SOAPSTOCK FERMENTATION. C.W. Hesseltine, Northern Regional Research Center, USDA/ARS, 1815 N. University St., Peoria, IL 61604.

In the refining of soybean oil about 6% of the volume of crude oil becomes soybean soapstock (also referred to as "foots"). About 318,300,000 lb of this strongly alkaline material (pH 11.5) are produced annually from all fats and oils. Soapstock can be acidulated with sulfuric acid, dried, and used in animal feed, or the fatty acids may be recovered. Little or nothing is known about which microorganisms will grow in this material, either with the pH unadjusted or when neutralized. Soapstock was tested for growth of microorganisms at pH unadjusted and at 8.0. The fermentation medium, consisting of 2% soapstock in distilled water, was inoculated with appropriate microorganisms and shaken for 5 days at 28 C. Subsequently, the inoculum was plated on yeast-malt extract agar. Approximately 90 microorganisms, including bacteria, actinomycetes, yeasts and fungi, were grown on this soapstock medium. Surprisingly, 15 strains of microorganisms grew with the pH unadjusted, and an additional 30 strains grew at pH 8. Thirty-three strains failed to grow under either condition and 12 gave questionable growth. Soapstock was examined for amino acids, triglycerides, trace elements, carbohydrates and total nitrogen. Of the microorganisms tested, yeasts provided the most strains that grew on soapstock without pH adjustment.

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EFFECT OF NATURAL LACTIC ACID FERMENTATION AND SELECTED BACTERIA ON NUTRITIONAL VALUE OF CEREAL AND LEGUME MEALS. Marion Fields, Department of Food Science and Nutrition, 21 Agriculture Building, University of Missouri-Columbia, Columbia, MO 65211.

Analyses of cereal and legume meals after a natural lactic acid fermentation showed that there were declined in pH and increases in titratable acidity. Yields of fermented cornmeal depended upon the solids to water ratio in the fermentation. The more the water the less the yield after drying the solids. Benefits derived from the fermentation were increased mineral availability, decreased stachyose and raffinose and decreased trypsin inhibitor. Increased levels of vitamin B_{12}, riboflavin, and folacin were produced in fermented cornmeal. Increased protein quality (% relative nutritive value, %RNV) as measured by *Tetrahymena pyriformis*, was also produced.The following bacteria in known mixtures also developed significant increases in %RNV in cornmeal: *Pseudomonas maltophilia, Bacillus subtilis, Bacillus cereus; P. maltophilia, B. subtilis; B. subtilis* and *B. cereus.* On the other hand, chicken embryo studies (water and oil extracts of fermented and nonfermented meals of sorghum were placed in the air sac of fertile eggs) showed that the fermentation increased the toxicity of mycotoxins in the sorghum meal. Both fermented and nonfermented meal contained ochratoxin A and zearalenone which occurred naturally in the growing and handling of the sorghum prior to our experimentation.

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RECENT ADVANCEMENTS IN CUCUMBER FERMENTATION. Henry P. Fleming, USDA-ARS, Box 7624, NorthCarolina State University, Raleigh, NC 27695.

Cucumbers constitute the largest volume of vegetables preserved by brine fermentation in the United States. Primary fermentation is by lactic acid bacteria, which convert sugars in the vegetables to lactic acid and small amounts of acetic acid and other end products. Sodium chloride directs the course of the fermentation and prevents enzymatic softening of the cucumbers,

and traditionally has been added in excess as insurance against various spoilage problems. Technological advancements are being introduced into the cucumber fermentation industry that are intended to favor fermentation by selected or genetically modified bacteria with superior traits; permit the use of novel fermentations involving mixed cultures of lactic acid bacteria and yeasts; permit use of lower concentrations of sodium chloride, and eliminate gaseous, textural and other spoilage problems. The advancements include purging of carbon dioxide from brines of fermenting cucumbers with nitrogen to prevent gaseous deterioration of the cucumbers, addition of calcium salts to reduce requirements for sodium chloride, buffering of the brine to assure rapid and complete conversion of fermentable sugars to stable end products, and development of an anaerobic tanking system for improved fermentation control.

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SOLID STATE FERMENTATION TO PRODUCE EDIBLE MUSHROOMS AND ANIMAL FODDER. Krystyna Sosulski and Ewen Coxworth, Saskatchewan Research Council, 15 Innovation Blvd., Saskatoon, Saskatchewan, S7N 2X8.

Production of mushroom of *Pleurotus* spp., known commonly as oyster mushrooms, can be viewed as the solid state fermentation of lignocellulose by extracellular and hydrolytic enzymes released to the substrate by mycelium. The substrates, as agricultural and forestry wastes, are being hydrolyzed to support the growth of fruiting bodies, mushrooms. Two varieties of *P.* mushrooms, *P. sajor-caju* and *P. ostreatus*, were grown on straw supplemented at the 2% level with nitrogen of plant origin. The yield of fresh weight of mushrooms was equal to 130% and 84% of substrate DM, for *P. sajor-caju* and *P. ostreatus*, respectively. The protein content of mushrooms as an average of 5 flushes harvested, was 34% of DM. The composition, in vitro organic matter digestible and utilization of spent substrates are also reported.

Session Y Friday afternoon Surfactants and Detergents IV—Sequestrants

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THE FUNCTIONALITIES OF POLYMERS IN DETERGENT FORMULATIONS. George T. McGrew, Alco Chemical Corp., Alco Industries Co., 909 Mueller Dr., Chattanooga, TN 37406.

Polymers function as sequestrants, dispersants and threshold inhibitor agents in detergent formulations. Data is presented on calcium binding constants (sequestration), thinning curves (dispersancy) and threshold activity for a variety of polymers and copolymers and related to functionality in formulations. Also presented are comparisons of polymers and copolymers to other builders and builder assists relating to the effects on detergency, ash and antiredeposition in low and zero phosphate formulations.

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EFFECT OF WATER HARDNESS ION CONTROL ON SURFACTANT FORMULAS. Patrick C. Hu and Melvin E. Tuvell, Ethyl Corp., P.O. Box 14799, Baton Rouge, LA 70898.

Foaming characteristics (foam generation and foam stability) of alkylbenzene sulfonate solution in the presence of various water hardness ions and water hardness controlling agents were examined using a modified maximum bubble pressure method. The modified maximum bubble pressure method was used to measure the dynamic surface tension as a function of the bubble forming rate (or stress rate) and to generate monodisperse foam columns of fixed foam cell size for subsequent study. It was found that a surfactant concentration substantially higher than the critical micelle concentration was required to achieve optimum foam conditions ad indicated by the generation of the finest foam cells. On the other hand, stable foam can be generated at a much lower surfactant concentration. The presence of water hardness ions and water hardness ion controlling agents (sequestrants and zeolite A) affects the foam generation markedly but contributed very little to the stability of the foam once it is generated.

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ZEOLITE AND PHOSPHATE: ADDRESSING FUNCTIONAL DIFFERENCES TO ACHIEVE PERFORMANCE PARITY. Richard T. Coffey and Steven L. Rock.

Sodium tripolyphosphate has long been recognized as an excellent detergent builder. It exhibits such desirable characteristics as calcium and magnesium sequestration, reserve alkalinity, dispersant activity, and calcite crystal growth inhibition. Challenges facing the detergent formulator have been to provide these characteristics at a cost lower than that of phosphate or without the use of phosphate in areas in which it is banned. This paper will highlight the fundamentally different chemical natures of zeolite and STPP as well as the resulting functional differences in their detergent builder performances. Furthermore, this paper will introduce formulation strategies wherein the desired functional characteristics that can be provided by STPP are incorporated into zeolite-built detergents. These desired characteristics are contributed through the judicious selection of appropriate commercial detergent ingredients such as silicates, surfactants and polyacrylates. The resulting zeolite-built detergents achieve performance parity with STPP as measured by detergency testing. A formulator, therefore, can develop premium quality zeolite-built detergents with raw material costs lower than that of STPP. Additionally, these formulations can often be produced without the use of phosphate.

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AN IMPROVED METHOD FOR EVALUATING DETERGENT BUILDERS FOR WATER HARDNESS CONTROL. J.A. McDonnell and G. Liu, Economics Laboratory, Inc.

Commercial detergent additives to control water hardness may act through sequestration, crystal growth inhibition, precipitation or ion exchange. These builders lower the free hardness (Ca^{++}, Mg^{++}) concentration by different mechanisms. A factorially designed experiment has been developed to evaluate builders functioning by the sequestration or crystal growth inhibition of calcium carbonate or magnesium hydroxide. The builders performance is determined by its ability to prevent precipitation while in the presence of carbonate and hardness ions. The tests are based on incubation followed by filtration and determination of calcium and magnesium in the filtrate by AA or ICP. Variables in the designed experiments include builder concentration, temperature, pH and time. Results for several commercial builders will be presented.

Session Z Friday afternoon Surfactants and Detergents V—Analytical

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THE RAW MATERIAL, FINISHED PRODUCT, AND DUST PAD ANALYSIS OF DETERGENT PROTEASES USING A SMALL SYNTHETIC SUBSTRATE. T.M. Rothgeb, P.H. Garrison, L.A. Smith and M.G. Venegas, The Procter & Gamble Co., 5299 Spring Grove Ave., Cincinnati, OH 45217.

An improved method for the analysis of commercial detergent proteases is presented. Protease activity is assayed using a small synthetic polypeptide: N-succinyl-alanyl-alanyl-prolyl-phenyl-alanine-p-nitroanalide. Advan tages of the method are improved speed, sensitivity, precision, and accuracy versus the auto-

analyzer Dimethyl Casein method (with trinitrobenzene sulfonate detection). Of particular importance is the use of this method for the analysis of airborne enzyme dust levels at a detergent manufacturing plant. A 25-fold increase in sensitivity versus DMC is obtained with no detergent matrix or primary amine interferences. The method is fully automated and can be used to measure enzyme raw material stocks, formulated finished products, and dust pads. Comparative data for commercially available proteases (Alcalase, Maxatase, Savinase, Esperase and Maxacal) will be given.

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DETERMINATION OF HLB VALUE BY HPLC. Jiao Shouan and Tian Shuzhi, Research Institute of Petroleum Exploration and Development, Oilfield Chemistry Laboratory, P.O. Box 914, Beijing, People's Republic of China.

Using high performance liquid chromatography two type of nonionic surfactants, polyoxyethylene nonylphenyl ether and the block copolymer of propylene oxide with ethylene oxide, have been studied. Their retention values (t_r) in reverse phase chromatography as a function of the ethylene oxide and propylene oxide, and their HLB value, were given. We found that there is a linear relationship in log t_r-HLB and log t_r-log EO for polyoxyethylene nonylphenyl ether type, and in log t_r-log EO/PO for the block copolymer of propylene oxide with ethylene oxide. The results show that it is possible to determine HLB value of nonionic surfactants by high performance liquid chromatography.

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ANALYSIS OF ETHANOLAMINES IN AIR BY ION CHROMATOGRAPHY. John F. Goelz, The Drackett Co., 5020 Spring Grove Ave., Cincinnati, OH 45232.

Ethanolamines are volatile weak bases used in the chemical and pharmaceutical industries as intermediates in the production of emulsifiers, detergents, solubilizers, drugs, cosmetics and textile finishing agents. Furthermore, ethanolamines are used in the gas processing industries to scrub carbon dioxide and hydrogen sulfide from light hydrocarbons. Numerous analytical methods are currently available for ethanolamines present in solution. Several published methods claim detection limits in the ppm range; however, all require a derivatization or a pre-concentration step. For air sampling, the low vapor pressure of the ethanolamines dictates the use of a method with low detection limits or large sampling volumes. We report a method using chemically suppressed ion chromatography with detection limits of 1 ppm. This allows direct air sampling into a solution, without the usual requirement of pre-adsorption onto filters. The method requires less than 15 min for analysis and will simultaneously determine mono-, di- and triethanolamines.

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RAPID QUANTITATIVE HPLC ANALYSIS OF POLYETHOXYLATED NONIONICS. Haruo Yoshimura, Toyoki Sugiyama and Toshio Nagai, Lion Corp., Analytical Research Center, No. 13-12, 7-chome, Hirai Edogawa-ku, Tokyo, Japan.

Polyoxyethylene alkyl ether is widely used in household products together with anionics. There have been a number of investigations on HPLC analysis for polyethoxylates; however, these methods are generally limited to determining their distribution of alkyl radical and/or of adducted numbers of ethylene oxide. Application of these HPLC methods to quantitative analysis for polyethoxylates brought some difficulties for practical use because of their mutual separation according to the distribution mentioned above. Our objective in this work was to develop a quantitative analytical method for alkyl polyethoxylates which would be applicable to quality control in a factory. Theoretical consideration about LC elution based on plate theory suggested that polyethoxylates might be eluted as one chromatographic peak regardless of the distribution by switching flow direction of eluent to reverse (back flush) while the polyethoxylates were not yet eluted out of a column. According to this possibility, the quantitative analytical methods were developed for unreacted alkyl polyethoxylate in alkyl ethoxy sulfate and polyethoxylated nonionics in mixtures with anionics. In the conditions employing an ODS type column (Unisil QC-18) and aqueous methanolic eluent, anionics were less retained than alkyl polyethoxylates. Therefore, the flow direction should be switched to reverse after anionics pass through the column. In the case of the heavy duty detergent we studied, an additional column (LiChrosorb RP-2) was employed to remove the interference owing to co-existing more strongly retained species.

Session AA Friday afternoon Analysis of Lipids IV

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HPLC OF ETHOXYLATED FATTY ACID DERIVATIVES. L. Yodual and G. Szajer, Akzo Chemie America, 8401 W. 47th St., McCook, IL 60525.

This paper presents chromatographic separation of compounds obtained from the addition of ethylene oxide to fatty acids and fatty acid derivatives. Included are ethoxylated fatty acids, ethoxylated fatty amines and ethoxylated quaternary ammonium compounds, all of tallow or coconut oil origin. The separation of individual ethylene oxide adducts is accomplishedusing high pressure liquid chromatography. Ethoxylated species and glycol by-products are separated on a cyano modified silica column using a variety of solvents. Column selection and solvent effects are discussed, and ethylene oxide adducts are identified.

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CHROMATOGRAPHIC ANALYSIS OF MONOACYLGLYCEROLS. Yutaka Itabashi and Toru Takagi, Department of Chemistry, Faculty of Fisheries, Hokkaido University, Minato-cho, Hakodate, Japan.

Enantiomer resolution of monoacylglycerol (MG) racemates was examined by high perfomance liquid chromatography (HPLC) on chiral stationary phases. Complete resolution into enantiomers of saturated and unsaturated MG racemates with acyl carbon numbers 8–22, as 3,5-dinitrophenyl urethane derivatives, was obtained on (S)-2-(4-chlorophenyl) isovaleric acid and its amide derivatives chemically bonded to γ-aminopropyl silanized silica. HPLC analysis was done at ambient temperature using a stainless steel column (25 cm × 4 mm I.D.) packed with the 5-μ particles, which had 9,000 theoretical plates for the urethane derivative of 3-monopalmitoyl-*sn*-glycerol. A mixture of hexane-ethylene dichloride-ethanol (40:12:3, v/v/v) was used as a mobile phase at a flow rate of 1 ml/min. The urethane derivatives dissolved in chloroform were injected into installation. Peaks were monitored by a UV detector set at 254 nm. The chromatograms obtained were characterized by sharp and symmetrical peaks resolved within adequate retention times, faster elutions of 1-monoacyl-*sn*-glycerols than the corresponding 3-monoacyl-*sn*-glycerols, and no resolution based on acyl carbon numbers. The separation factors and peak resolution were 1.12–1.14 and 2.35–2.44, respectively, for all MG racemates used in this study.

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SYNTHESIS AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF 1-ALKYL-2-ACYL-*sn*-

GLYCEROLS. Takashi Iwama, Nippon Oil and Fats Co. Ltd., c/o Eastern Regional Research Center, 600 E. Mermaid Ln., Philadelphia, PA 19118, and Thomas A. Foglia, Eastern Regional Research Center, Philadelphia, PA.

A series of 1-alkyl-2-acyl-*sn*-glycerols was synthesized to provide precursors for the preparation of platelet activating factor (PAF) homologues. The general intermediate in the synthesis of this class of lipids with natural configuration is 1,2-isopropylidene-*sn*-glycerol. The latter compound was obtained in high enantiomeric purity by the oxidative cleavage of either 1,6-ditrityl-D-mannitol or 1,2,5,6-diisopropylidene-D-mannitol. Key intermediates in the synthesis of the title compounds are 1-alkyl-2-acyl-3-tritylglycerols. However, the final step of the sequence, removal of the trityl protective group, was complicated by the concomitant migration of the 2-acyl moiety to yield 1-alkyl-3-acyl-glycerol isomers. The separation of the 1,2- and 1,3-glycerol isomers was accomplished by high performance liquid chromatography (HPLC). The effects of experimental conditions and various reagents on the extent of this 2-acyl to 3-acyl migration in the detritylation step were evaluated by the developed
HPLC methods. Additionally, the utility of the analytical HPLC methods for the large scale separation and purification of these 1,2 and 1,3 isomers was demonstrated.

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QUANTITATIVE DETERMINATION OF MEDIUM CHAIN TRIGLYCERIDES IN INFANT FORMULA BY REVERSE PHASE HPLC. Theresa W. Lee, Ross Laboratories, 625 Cleveland Ave., Columbus, OH 43216.

Two methods were developed for the separation of medium chain triglycerides (MCT) using reverse phase HPLC. Both methods employed a C18 microbond HPLC column as the stationary phase and an isocratic solvent system. The first method described consists of acetonitrile/acetone as the mobile phase with a differential refractometer as the detector. In the second method, acetonitrile/water was used as the mobile phase and a UV detector at 210 nm. Trinonanoin was used as the internal standard for quantitative determination. This method is suitable for milk, whey and soy protein based matrices. With minor modification, it is applicable to MCT levels ranging from 10 to 50% of total fat.

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HPLC ANALYSIS OF SOYBEAN PHOSPHOLIPIDS. Kikuo Shimbo, Makoto Shimazaki and Naoe Taguchi, Sugiyama Chemical and Industrial Laboratory, 11 Kagetori-cho, Totsuka-ku Yokohama, 245 Japan.

Recently, a large number of papers was published by authors who had applied HPLC to the separation of phospholipids. Nevertheless, only a few of them achieved "baseline resolution" of phospholipids. We developed a new HPLC technique using a column packed with aminopropyl group bonded silica. PC, PE, PI, PG, PS, PA and LPC were clearly separated by isocratic elution. Each peak eluted from the analytical column was pure enough to analyze molecular species. Then an HPLC method for molecular analysis with microgram-size phospholipids was investigated using soybean lecithin. Before analysis, fractionated phospholipids (PC, PE, PI) were converted to diglycerides with phospholipase C (from *B. cereus*) and then derived to dinitrobenzoate. Among some derivatives of phospholipids, diglyceride dinitrobenzoate was suitable because its preparation procedure was mild enough not to decompose acyl moiety of phospholipids, and because it had strong UV absorption. HPLC was performed with a column packed with octadecyl group bonded silica. Major molecular species of soybean PC was shown to be dilinoleoyl type, and 15 peaks were detected. The composition of molecular species of soybean PE and PI will be discussed.

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APPLICATIONS OF SILVER RESIN CHROMATOGRAPHY: THE FRACTIONATION OF FISH OILS AND OTHER POLYUNSATURATED FATS. R.O. Adlof, NRRC, ARS-USDA, 1815 N. University, Peoria, IL 61604.

Silver resin chromatography has been used to separate a variety of unsaturated compounds, according to the number and configuration of the double bonds. For example, applications of silver resin chromatography include separation of a wide variety of fatty esters, acids and triglycerides, containing *cis*, *trans* and acetylenic bonds from plant, animal and fish oils. Other compounds which have been separated are unsaturated hydrocarbons, a variety of insect pheromones, resin methyl esters and sterols. The main advantages of silver resin columns over other columns containing conventional silver ion-impregnated packing materials are the high-capacity factors, long column lives and high flow rates at low pressures. The performance of silver resin columns is related to the surface area of the sulfonic acid ion exchange resin used. Superior separation normally is obtained with macroreticular resins such as XN1010 (Rohn and Haas). The most recent applications of silver resin chromatography have used mixed solvent systems or gradient elution to separate polyunsaturated fats. Fatty acids and esters isolated on a preparative scale include geometric and positional isomers of 18:2, 18:3, 20:2 and 20:3 from plant lipids as well as 20:4, 20:5 and 22:6 isolated from fish oils. Elution of these compounds requires solvents (i.e. CH_3CN in acetone) that destabilize the silver ion-double bond complex and permit separation based on total number of double bonds.

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DETERMINATION OF VITAMIN E ISOMERS IN VARIOUS SEED OILS USING SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC). Allan D. Scott Jr.

The role of vitamin E as an antioxidant in biological systems is well documented. There is an increasing need for quick methods which allow one to isolate and quantitate the various isomers of vitamin E. This proposed method for vitamin E determination using supercritical CO_2 with a polar modifier demonstrates the effectiveness of supercritical fluids chromatography as a routine analytical tool. The method is specific and sensitive, utilizes low cost solvents, and offers rapid analysis with minimal sample preparation. The isomers of vitamin E can be baseline resolved in less than seven minutes. Tocopherol concentrations of various seed oils have been determined and compared to conventional HPLC results. In summary, supercritical fluid chromatography offers many well documented advantages. However, its acceptance as a routine analytical tool has been hindered by the lack of practical applications. Applications of this nature offer exceptional resolution of difficult to resolve isomers in a short period of time, making SFC an attractive alternative to conventional HPLC.

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DISTRIBUTION OF HEXADECENOIC, OCTADECENOIC AND OCTADECADIENOIC ACID ISOMERS IN HUMAN TISSUE LIPIDS. R.O. Adlof and E.A. Emken, NRRC, ARS-USDA, 1815 N. University St., Peoria, IL 61604.

The *trans* 16:1, 18:1 and 18:2 fatty acid compositions of various human organ lipids and individual lipid classes were measured to determine if *trans* isomers accumulate in specific tissues. *Trans* isomers are defined as fatty acids containing one or more *trans* double bonds. A 100-meter SP2560 fused silica capillary (gas chromatography) column was used to separate by GC many of the various configurational and/or positional fatty acid methyl ester isomers present in adipose, kidney, brain, heart and liver tissues (obtained from autopsies). Distribution of *trans* 16:1 ranged from 0.1% in the kidney, brain and heart to 0.3% in adipose tissue while the percent of *trans* 18:1 ranged from 0.2 in the brain to 4.0 in adipose tissue. The total *trans* 18:2 isomers content ranged from

0.0% in the brain to 0.6% in adipose tissue. Data for the individual *trans,trans-,cis,trans-* and *trans,cis*-18:2 isomers also were obtained. Ratios of *trans* 18:2 vs. *trans* 18:1 incorporation were found to range from near 0 in the brain to 0.35-0.45 in the liver. Comparison of these ratios to ratios obtained for adipose tissue and hydrogenated oil indicate the *trans* 18:2 isomers are preferentially excluded from several tissues and lipid classes. Ratios for *cis* 16:12 (Δ9 vs. Δ7) and *cis* 18:1 (Δ11 vs. Δ9) were very consistent, which suggests that these ratios might be useful for the diagnosis of metabolic disorders.

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THERMAL AND COMPOSITIONAL PROPERTIES OF COCOA BUTTER DURING STATIC CRYSTALLIZATION. Paul S. Dimick, Pennsylvania State University, 116 Borland Laboratory, Department of Food Science, University Park, PA 16802, and Douglas M. Manning, M & M Mars, Inc., Elizabethtown, PA.

Studies were conducted using differential scanning calorimetry (DSC) and high performance liquid chromatography (HPLC) to determine the thermal properties and glyceride composition of cocoa butter crystals formed under static conditions. In addition to these studies, visual characterization of the individual crystallites were obtained with polarized light microscopy (PLM). Crystals were formed under controlled static or motionless conditions at formation temperatures of 26.0, 28.0, 30.0, 32.0 and 33.0 C. Preparatory techniques were developed using laminated polyethylene with plastic hoops in order to grow the crystals for isolation and visual identification by PLM prior to DSC assay. Crystallization of cocoa butter was also conducted from liquid oil directly in the DSC pans prior to thermal assay. At each crystal formation temperature (26-33 C), various crystallite types grow, each with varying triglyceride composition (PLiP, POO, PLiS, POP, SOO, SLiS, POS, SOS, SOA). As an example, the 'feather' and 'individual' crystals formed at 26.0 C exhibited significant increases in SOS and significant decreases in POP when compared to the original butter. It was determined that the amount of SOS significantly increases in the crystallite as the incubation temperature increases from 26-32 C. These static crystallization studies yielded data that aid in our understanding of the mechanism of bloom formation, a factor detrimental to product quality.

Session BB Friday afternoon Processing of Oils II

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PROCESSING AND HANDLING FACTORS THAT LEAD TO CORN OIL DEGRADATION AND RESULTING CHEMICAL CHANGES. Roger D. Sinram and F.T. Orthoefer, A.E. Staley, 2200 E. Eldorado, Decatur, IL 62525.

This study examined reasons for degradation of unhydrogenated deodorized corn oil, due to variations in processing and handling. Corn oil samples were placed under poor storage conditions, including exposure to heat, air, light, moisture and heavy metals. The effects of the above conditions on several quality parameters, including color, peroxide value, free fatty acids, flavor and volatiles, were monitored over a period of time. The extent of degradation was dependent upon both storage conditions and initial processing steps, including method of crude oil extraction and bleaching ingredients used. Several correlations were completed, including color reversion versus flavor stability and peroxide value versus free fatty acid. The color reversion was explained by changes in tocopheroi, chlorophyll and carotenoid levels. Flavor degradation was related to increasing total volatiles.

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DEGUMMING AND NEUTRALIZING METHODS FOR VEGETABLE AND ANIMAL OILS AND FAT. C. Zehnder and K. Carlson, Alfa-Laval, Tumba, Sweden.

Even though various methods of degumming and neutralizing of oils and fats are well known, there is often confusion about how to apply these methods. This paper presents the methods proposed by Alfa-Laval with emphasis on producing high quality products at minimum operating cost. Presentation will include degumming methods, water degumming, cold degumming, acid conditioning, acid degumming, super degumming, neutralizing methods, long mix and short mix, and miscella neutralization.

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INTERESTERIFICATION OF FAT DURING PHYSICAL REFINING. Tsugio Izumi, Nobuo Sagi and Hiroyuki Mori, Fuji Oil Co. Ltd., 1-Sumiyoshi-cho Izumisano City, Osaka, Japan.

Physical refining of fats and chemical reaction during the process are discussed. Physical refining is used to refine palm oil in Malaysia due to simplified process, cost saving and solution of waste water compared with chemical refining. There are currently many papers concerning the comparison between physical refining and chemical refining of edible fats and oils, some of which have pointed out the relation of residual phospholipids, chlorophyll to qualities of physically refined fats and oils and mentioned that generally high quality fats and oils have been produced from chemical refining but suitable pretreatment has been required for physical refining. M.G.A. Willems reported palm oil has isomerized during steam refining and produces asymmetrical triglycerides (PPO) which might have been caused by interesterification, which is a negative effect on quality. The authors have studied chemical reactions during physical refining in detail using a model system and have found that there have been interesterifications among fatty acids, diglycerides and triglycerides in the system during physical refining when both fatty acids and diglycerides exist in the system above the 5% level and asymmetrical triglycerides have been produced. On the contrary, there has not been observed an increase of asymmetrical triglycerides when either fatty acids or diglycerides have been present at less than 5% in the system.

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NEW PRODUCTS VIA ADSORPTIVE SEPARATIONS. Stanley A. Gembicki and Kenneth U. Johnson, UOP Inc., Algonquin and Mt. Prospect Rds., Box 5017, Des Plaines, IL 60017-5017.

Significant product development effort must be applied to the discovery of new products and the chemistry and feedstocks necessary for their production. However, due to the reality of low conversion, impurities and byproducts, new product economic viability is often determined by the separation processes required for concentration and purification of raw materials and crude products. This paper illustrates the use of continuous, adsorptive separation technology to economically accomplish difficult separations and thus contribute to new product opportunities in the fats and oils industry. Experimental results from several pilot plant operations are reviewed.

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PALLADIUM(II)-CHLORIDE AS A HOMOGENEOUS CATALYST FOR SELECTIVE HYDROGENATION. Jan A. Heldal, Norwegian Herring Oil and Meal Industry Research Institute, N-5033 Fyllingsdalen, Bergen, Norway.

Palladium(II)-chloride in the presence of different solvents was used as a catalyst for selective hydrogenation of methyl esters of linoleic acid, soybean oil and capelin oil at atmospheric pressure and 50 C. The properties of these catalytic systems were compared to similar hydrogenations with the heterogeneous Pd/C catalyst.

The solvents, in addition to unsaturated fatty esters with two or more double bonds, valence-stabilized Pd(II) through a possible complex formation. The presence of solvents such as propanol and acetylacetone strongly improved the activity and selectivity compared to $PdCl_2$ without solvent and Pd/C. The selectivity during hydrogenation of soybean oil methyl esters in these catalytic systems ($PdCl_2$ + acetylacetonate) was high (S_{Ln} = 6,8 and S_{Lo} = 64) compared to other PD-catalysts, and the specific isomerization (% *trans*/WIV) increased from 0.5 to an equilibrium value of 0.8. The specific isomerization during hydrogenation of capelin oil methyl esters was 0.4–0.5. The accumulation of the methylene-interrupted *trans,trans*-isomer of linoleate was significant (max 5%) during hydrogenation of linoleate and soybean oil with all the examined Pd-catalysts.

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ENVIRONMENTAL PERMITTING AND FACILITIES FOR A NEW FATTY CHEMICALS PLANT. Michael J. Boyer and Malcolm E. Burman, Applied Engineering and Science, 5404 Peachtree Rd., Chamblee, GA, and Roger E. Burke, Resinall Inc.

Resinall Inc. is a major producer of rosin and hydrocarbon resins, fatty esters and related fatty chemicals. In early 1984 Resinall reached a decision to construct a new grass roots resin plant in Hattiesburg, Mississippi. The site had formerly been occupied by a previous resin processor and had been a forest products/pulp chemicals site since the mid-1800s. The new facilities did not incorporate any of the limited active facilities. Applied Engineering and Science was retained in June 1985 to handle the design and construction management of all phases of the project. As part of this, AES also handled environmental permitting. This paper describes the program implemented in defining environmental needs, obtaining permits and special problems which developed. The environmental facilities design was closely integrated into the process facilities. This will be described in depth.

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IMPROVED SULFONATION PLANT POLLUTION CONTROL VIA SECOND GENERATION ELECTROSTATIC PRECIPITATOR. Burton Brooks, Chemithon Corp., 5430 W. Marginal Way, S.W., Seattle, WA 98106.

Chemithon developed a new electrostatic precipitator (ESP) utilizing patented high intensity corona discharge electrodes. This ESP increases sulfonation plant effluent gas particle collection efficiencies from 97 to 99+%. Resultant stack opacities are reduced to 0–5%. Electrode voltages are increased to 50–55 KVDC, while the specific collection area is reduced by a factor of 2.3. Operating data from commercial sulfonation plants is contrasted to pilot data and to data obtained with conventional wire and tube designs.

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CONSIDERATIONS ON THE DESIGN OF A WASTEWATER FREE EXTRACTION PLANT AND REFINERY. Klaus Weber.

Increasing concern on the environmental issues makes the disposal of wastewater one of the major problems when defining the location of a new plant. Also more stringent water pollution regulations are creating additional problems and costs for actually operating plants. By incorporating in the design of an extraction plant the recycling of the effluent water eventually a wastewater free plant can be conceived. A similar re-use of the wastewater can be considered for refineries reducing at least the COD-load by 90% and transforming the effluent from the soapstock splitting into a disposable solid waste. Additionally, considerable energy recovery can be achieved by using such a system. The energy and mass balance of such systems incorporated into extraction plants and refineries are described, as is the basic design concept for new facilities and the required alterations in existing plants to achieve the above aim.

Session CC Friday afternoon Biological Oxidation of Lipids IV

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PARACRINE AND CLASTOGENIC ACTION OF OXIDATIVE METABOLITES OF ARACHIDONIC ACID IN TUMOR PROMOTION. Peter A. Cerutti, Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research, 1066 Epalinges/-Lausanne, Switzerland.

Inflammation is necessary but not sufficient for promotion in mouse skin. An early event following the application of phorbol-myristate-acetate (PMA) and many other promoters is the infiltration of polymorphonuclear cells (PMN) and later monocytes/macrophages in response to chemotactic signals. These phagocytic cells react to the exposure with PMA and other membrane-active compounds with an oxidative burst and the release of a complex mixture of phospholipids, free arachidonic acid (AA) and AA-metabolites. Because this mixture of compounds contains highly active agonists and is clastogenic (i.e., induces chromosomal damage) it may exert a promotional effect on initiated epithelial cells. We have biochemically characterized the clastogenic factor (CF) released from human monocytes in response to PMA. It consists of H_2O_2, thromboxane B_2/prostaglandin $F_{2\alpha}$, prostaglandin E_2, HHT, hydroxyl-derivatives of AA, free AA and small amounts of mono- and diacylglycerol and phospholipids. The hydroxyl-derivatives of AA contained in CF are formed from the corresponding hydroperoxy-AA precursors, e.g., PGG_2 and 5,11- and 15-hydroperoxy-AA (HPETEs). When produced in the tissue these hydroperoxy- derivatives are sufficiently stable to reach neighboring target cells. Therefore, we have studied the capacity of HPETEs to induce DNA strand breakage in intact mouse embryo fibroblasts C3H 10T1/2. Using the alkaline elution assay we found that HPETEs efficiently induced DNA single strand breaks in a Ca^{2+}, Mg^{2+}-dependent reaction. A mechanism of action of HPETEs is suggested by the observation that HPETEs caused the release of mitochondrial Ca^{2+}. Our results suggest that the oxidative metabolites of AA which are released by PMA stimulated inflammatory cells exert a promotional effect on neighboring target cells because they disturb Ca-homeostasis and induce DNA damaged.

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LIPID PEROXIDATION IN CELL PROLIFERATION AND DIFFERENTIATION. David G. Cornwell, Hanfang Zhang, Elizabeth C. Downs and Ronald L. Whisler, Ohio State University, 260 Meiling Hall, 370 W. Ninth Ave., Columbus, OH 43210-1238.

Karl Mason, certainly the most perceptive investigator in the vitamin E field, noted as early as 1933 that this lipid antioxidant was essential for tissues in which cell proliferation and differentiation were unusually rapid. Recent studies from our laboratory, while confirming his insight, have identified many and sometimes paradoxical effects of lipid peroxides on growth and differentiation. Different lipid peroxides are synthesized by several biochemical reactions and these reactions are not common to all cells. Some lipid peroxides act directly in suppressing cell growth and other lipid peroxides, through the control of prostanoid synthesis, act indirectly in enhancing cell growth. Prostaglandins enhance growth in some cells and some lipid peroxides through their stimulatory effect on phospholipase and cyclooxygenase activities may enhance proliferation in these cells. Prostacyclin suppresses growth in other cells and other lipid peroxides, through their inhibitory effect on prostacyclin synthetase, may enhance proliferation in these cells. Since prostaglandins diminish the production of both interleukin-1 and Ia-like antigens by monocytes, lipid peroxides suppress monocyte accessory cell function and immune responsiveness by stimulating prostaglandin synthesis. Disorders as diverse as atherosclerosis and cancer may

be caused in part by the paradoxical effects of lipid peroxides and agents that destroy their balance. Intervention strategies based on vitamin E and antioxidant drugs are potentially capable of either enhancing or suppressing the disease.

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THE NEURONAL CEROID-LIPOFUSCINOSES: NEW BIOCHEMICAL FINDINGS. A.N. Siakotos and L. Bray, Indiana University School of Medicine, Department of Pathology, 635 Barnhill Dr., Indianapolis, IN 46223; A. Sevanian, University of Southern California, Los Angeles, CA, and N. Koppang, Norwegian Veterinary Institute, Oslo, Norway.

The pathogenesis of the neuronal ceroid-lipofuscinoses (NCL) has remained an enigma since 1826 when Stengel first described four affected siblings with this disease in Rŏraas, Norway. These neurogenetic disorders, with both automsomal recessive and dominant modes of inheritance, are characterized by visual impairment, seizures, mental deterioration, and the abnormal deposition of a pathognomonic autofluorescent lipopigment in all cells, but more specifically, pigment deposition in neuronal tissue. These autofluorescent lipopigments are the "end products" of accelerated lipid peroxidation unique to these diseases. A number of investigators have separated different clinical types based on common features in the clinical pathologic course of affected patients. With the advent of more specific tests, such as the presence of high levels of urinary dolichols or the positive identification of the NCL specific autofluorescent lipopigment in tissue biopsies, the diagnosis of such patients can be more reliable. Although a number of hypotheses have been proposed for the pathogenetic basis for these disorders, to date no reliable biochemical discoveries exist that are of value in detecting presumptively affected patients or screening individuals as carriers of these genetic traits. Our studies on lipid peroxide metabolizing enzymes have revealed a number of specific deficiencies in some membrane-bound enzymes required for oxidant metabolism. Of the membrane-bound enzymes studied, both a heme-dependent peroxidase and a glutathione peroxidase appear to be most useful. The distribution of these enzymes in peripheral blood cells, e.g., platelets, monocytes, lymphocytes and neutrophils, provides a reliable non-invasive diagnostic indicator for various types of NCL in human patients and the canine model of the disease. In the canine model the findings with peripheral blood cells are corroborated with deficiencies of heme-dependent peroxidase in affected organs, e.g., the brain and the eye. In addition, these unique biochemical changes provide a reliable means of screening individuals as carriers of these specific genetic traits.

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FORMATION OF OXIDIZED CHOLESTEROL PRODUCTS IN LIPOSOMES AND IN VIVO PEROXIDATIONS. Guey-Shuang Su, Robert A. Stein and James F. Mead, University of California, Los Angeles, Laboratory of Biomedical and Environmental Sciences, 900 Veteran Ave., Los Angeles, CA 90024.

Recent recognition of the broad range of biological activities of oxidized cholesterol products has stimulated attention to the formation of these products in artificial bilayers and in tissues. Cholesterol, incorporated into dipalmitoylphosphatidylcholine liposomes, oxidized slowly to give 7β-hydroxy, 7-keto and 5,6-dihydroxycholesterol as major secondary products. These products presumably are derived from the initially formed 7β-hydroperoxide, as has been recognized in other models of cholesterol autoxidation. The 25-hydroxycholesterol previously established as one of the major products in the bulk phase autoxidation was absent in the cholesterol liposomes. The non-polar side chain is apparently situated in the hydrocarbon interior of bilayers and thus is less susceptible to the oxidative reactions occurring at the hydrophilic surface. Cholesterol oxidation products, however, are rarely seen in tissues without a preceding decrease in the level of tissue antioxidants or protective enzymes. Wistar rats were fed a vitamin E-deficient diet until they became severely vitamin E deficient. Initiation of in vivo tissue peroxidation in these rats by free radical generating hepatotoxins, such as CCl_4, produced 7-keto, 7β-hydroxy, 5,6-epoxycholesterol and 3,5-cholestadiene-7-one in the liver. Without the administration of CCl_4, the severely vitamin E deficient rat livers also produce similar cholesterol oxidation products but in much smaller quantities. The product distribution obtained from these tissues appears to be similar to that from cholesterol liposomes, except that a larger proportion of 5,6-dihydroxycholesterol was present in the liposome preparation. In animals supplemented with vitamin E, the small amounts of cholesterol derivatives detectable are 5,6-epoxycholesterol and its hydration product, 5,6-dihydroxycholesterol. The in vivo formation of these cholesterol products in tissue will be discussed in relationship to the altered level of enzymes, in particular, cholesterol 7-hydroxylase, in vitamin E deficiency.

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MECHANISMS OF LIPID PEROXIDATION INDUCED BY QUINONE AND QUINOID ANTICANCER DRUGS. Hiroko Nakano-Totsune and Minoru Nakano, College of Medical Care and Technology, Gunma University, Showa-machi, Maebashi, Gunma, Japan.

Quinoid and quinone anticancer drugs produce dose-dependent hepatic and cardiac disorders which may be attributable to peroxidative cleavage of cell membranes. The mechanism has been studied using reconstituted microsomal systems containing certain drugs. Ferric ion-ADP-adriamycin (ADM) complex, which may non-enzymatically convert to perferryl ion complex by an intramolecular electron transfer in air and in Tris-HCl buffer, acts as a powerful initiator for lipid peroxidation. Lipid peroxidation induced by this complex is strikingly inhibited by ceruloprasmin. During the reduction of Fe^{3+}-ADP-ADP complex by NADPH-cytochrome P-450 reductase (enzyme) in a Tris-HCl buffer, the system does not produce $^{\bullet}OH$, but possesses much more strong lipid peroxidation. On the other hand, in phosphate buffer in which Fe^{3+}-ADP-ADP could be dissociated to Fe^{3+}-ADP and ADM, $^{\bullet}OH$ is produced during the enzymatic reduction of $^{3+}$-ADP-ADP, but it is not involved in phospholipid peroxidation. Therefore, lipid peroxidation induced by ADM is initiated by a proposed perferryl ion complex, which may be generated by interaction of Fe^{3+}-ADP-ADM complex with O_2. In contrast to ADM, a quinone anticancer drug, mitomycin c, does not produce coordination complex with Fe^{3+}-ADP, even in Tris-HCl buffer. Both O_2^- and H_2O_2 are generated during the aerobic enzyme-catalyzed reduction of mitomycin c as well as ADM. $^{\bullet}OH$ is formed in the reaction by the reduction of H_2O_2. This is catalyzed by Fe^{2+}-ADP complex in a phosphate buffer or to a lesser extent when in a Tris-HCl buffer. The reduction of Fe^{3+}-ADP to Fe^{2+}-ADP is mainly achieved by O_2^-. The resulting Fe^{2+}-ADP in the presence of O_2forms a perferryl ion complex, a powerful initiator of lipid peroxidation. However, the formation of such an iron-O_2-complex is strongly inhibited by phosphate ions, which do not interfere with the generation of $^{\bullet}OH$. These findings suggest that, since lipid peroxidation occurs in a Tris-HCl buffer (but not in a phosphate buffer), $^{\bullet}OH$ is unlikely to be involved in the observed lipid peroxidation process. Vitamin K_3 behaves like mytomycin c on lipid peroxidation, but possesses very weak activity.

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CIRCULATING MICRORESERVOIRS: A LOW DENSITY LIPOPROTEIN ANALOG DRUG DELIVERY SYSTEM. Barry

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One of the major disadvantages of liposomes as drug delivery systems is their instability in vitro and in vivo, and their tendency to be sequestered by the reticuloendothelial system when injected intravenously. To overcome these barriers, a new approach has been developed to produce a phospholipid based drug delivery system for therapeutic use. These new drug carriers are composed of phospholipids and cholesterol esters and have a striking similarity in terms of biophysical properties and physiological fate to low density lipoproteins. The cholesterol ester component, because of its molecular packing in a liquid crystalline state, imparts unique stability to this drug delivery system which has been termed as circulating microreservoirs or CMR in order to distinguish this approach from liposomes. There are two distinct classes of CMR, vesicular and nonvesicular. The vesicular CMR are approximately 250 A in diameter and contain a very limited aqueous internal space. Nonvesicular CMR, which range from 250 to 1000 A, do not contain any internal aqueous at all. Which type of CMR is formed and the size is determined from the ratio of phospholipid to cholesterol ester. Whole body autoradiographic studies in rats using radiolabeled CMR show that intravenously administered CMR are not selectively sequestered by the reticuloendothelial system. Plasma kinetics and $^{14}CO_2$ metabolism studies are consistent with intravenously administered CMR leaving the plasma compartment and entering into the lymphatic system and being metabolized by peripheral tissue. Experimental data relative to the ability of CMR to increase the therapeutic index of anti-tumor compounds, and to alter the physiological disposition of contraceptive agents and anti-parasitic agents, will be presented.

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CHOLESTEROL INHIBITS CELL LYSIS INDUCED BY LYSOPHOSPHATIDYLCHOLINE. Barbara Malewicz, S. Parthasarathy and Wolfgang J. Baumann, University of Minnesota, The Hormel Institute, 801 16th Ave. N.E., Austin, MN 55912.

Novikoff hepatoma cells adapted to serum-free conditions were grown in shaker culture. Cell cholesterol levels were modulated by culturing the cells for 24 hr in cholesterol-supplemented media. Cell cholesterol increased in a linear fashion in response to increased cholesterol levels in the media, ranging from 7 μg cholesterol/mg protein for cells grown in unsupplemented medium to 45 μg/mg for cells grown in medium supplemented with 100 nmol cholesterol per ml of medium. Up to 100 nmol/ml, cell growth remained unaffected as judged by cell count and [^{3}H]thymidine incorporation into TCA precipitates. The increase in cell cholesterol ester content was insignificant. More than 90% of the cell cholesterol was not removable by washing the cells with 0.4% bovine serum albumin, indicating that the sterol was incorporated into the cell membrane. The effect of lysophosphatidylcholine (lysoPC) on the cells was measured by following $^{51}Cr^{+3}$ release and thymidine uptake. We found that cells grown in unsupplemented medium were most sensitive to lysoPC and that lysoPC at levels as low as 10–12 nmol/ml induced 50% $^{51}Cr^{+3}$ release from these cells within 60 min. However, cells grown in cholesterol-supplemented media were protected from lysoPC attack in proportion to the amount of cholesterol present in the cells.

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HYDROXYLATION OF FATTY ACIDS AND ALCOHOLS BY MICROSOMAL CYTOCHROME P-450 SYSTEM FROM GERBIL LIVER. Yoshiro Miura and Harumi Hisaki, Department of Biochemistry, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173, Japan, and Sen-ichi Oda, Research Institute of Environmental Medicine, Nagoya University.

The Mongolian gerbil, *Meriones unguiculatus*, has been used for many studies on lipid biochemistry in recent years. We report the substrate specificity and other properties of the cytochrome P-450 fatty acid hydroxylase system in gerbil liver microsomes. The contents of cytochromes P-450 and b_5 were 1.6 ± 0.16 and 0.79 ± 0.10 nmol/mg of microsomal protein, respectively. NADPH-cytochrome *c* reductase activity was 0.18 ± 0.02 μmol/mg/min. Lauric acid was the most active substrate among saturated fatty acids (C^8-C_{18}): relative hydroxylation activity; C_{12} (100, actual conversion: 5.51 nmol/mg/min), C_{14} (82), C_{13} (80), C_{16} (53), C_{10} (45), C_{18} (20) and C_8 (17). It was noted that the specific activity of laurate hydroxylase in gerbil liver was much higher than that in other species. The activity of dodecane was very low (0.33 nmol/mg/min). The apparent Km was 13 and 35 μM for Ω- and (Ω-1)-hydroxylation of laurate; 91 and 87 μM for Ω- and (Ω-1)-hydroxylation of 1-dodecanol. The hydroxylation activity of laurate was maximal at about pH 7.0, but the Ω/Ω-1-hydroxylation ratio increased with increasing pH. The effect of detergents on laurate hydroxylation was examined. Sodium deoxycholate, Tween 20, Emulgen 913 and Triton X-100 showed an inhibitory effect on Ω-hydroxylation, but almost no inhibitory effect on (Ω-1)-hydroxylation. On the other hand, the opposite was found to be true for the use of sodium cholate. A gerbil liver microsomal hydroxylase system was less susceptible to inhibition by Emulgen 913 than the rat liver microsomal hydroxylase system.

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HEPATIC LIPID ABNORMALITIES IN A MOUSE MODEL FOR REYE'S SYNDROME. M.G. Murphy, L. Archambault-Schertzer, J. VanKessel and J.F.S. Crocker, Department of Pharmacology, Sir Charles Tupper Medical School, Dalhousie University, Halifax, Nova Scotia Canada B3H 4H7.

We have examined the development of liver lipid abnormalities in a mouse model for Reye's Syndrome (RS) which duplicates many features of the frequently fatal childhood disease. Neonatal mice exposed dermally to nontoxic doses of an industrial surfactant had significantly elevated levels of hepatic cholesterol, with otherwise normal liver lipid content. Subsequent inoculation of the mice with sublethal doses of mouse-adapted human Influenza B virus had several significant effects on liver lipids, including a transient increase in phospholipid content, reduction in neutral glyceride levels, and abnormalities in fatty acid profiles. The latter included elevated short chain fatty acids and increased ratios of phospholipid arachidonic to docosahexaenoic acids. Histologically, there was little evidence of hepatic fat accumulation at the time when the mortality rate in the combined-treatment mice began to increase (96 hr post infection); however, hepatic mitochondria had severe structural abnormalities similar to those seen in RS patients. The data from these studies suggest that surfactant-induced changes in liver lipids increase susceptibility to viral infection, and that increased viral virulence is associated in some manner with severe disruptions in lipid metabolism.

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GRANULOMA FORMATION IN LUNGS AND SPLEEN IN MICE BY THE NEW MYCOLIC ACID-CONTAINING GLYCOLIPIDS IN *NOCARDIA* AND RELATED GROUPS. Ikuya Yano, Department of Bacteriology, Niigata University School of Medicine, Asahimachi-dori 1, Niigata 951, Japan; Ikuko Tomiyasu, Tezukayama College; Kenji Kaneda, Niigata University School of Medicine, and Yukie Sumi and Keiko Kato, Sawai Pharmaceutical Co.

The most characteristic component of the cell walls of the bacteria belonging to Actinomycetes, such as *Mycobacteria* and *Nocardia*, has been recognized to be "mycolic acid," a very high molecular weight, 2-alkyl 3-hydroxy fatty acid. The mycolic acids play important roles for the maintenance of hydrophobic properties and acid-fastness in the cell walls, and their composition varies significantly by genus or species. Furthermore, the cell wall components containing mycolic acids, such as cell wall skeleton or cord factor, possess unique biological activities in infectious diseases, such as immunoadjuvant activities, macrophage activ-

ating activities and antitumor activities. Oil associated BCG cell wall dispersed in Tween-saline causes a significant increase in lung weight when injected intravenously into mice. Histologically, it was revealed the increase in lung weight resulted from granuloma formation. Furthermore, it was reported that a cord factor may play an important role in the pulmonary granuloma formation by BCG CWS vaccination, probably by the cooperation of protein antigen and adjuvant active glycolipids. However, owing to the highly hydrophobic properties, mycobacterial cord factors possess strong toxicity for mice and therefore, appear to be unsuitable for the antitumor agents or adjuvant active components for human use. We have examined the occurrence, structure analysis and granuloma forming activity of mycolic acid-containing glycolipids in *Nocardia* and related taxa, which are closely related to *Mycobacteria* taxonomically, but are usually not human-pathogenic. We report here a variety of glycolipids possessing various mycolic acids and trehalose or glucose from these bacteria can produce massive granulomatous changes in the lungs and spleen of mice.

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EFFECTS OF ESTROGEN IMPLANTATION ON ENDOGENOUS HYPERTRIGLYCERIDEMIA IN CHICKS. Jeong Ro Park, University of Illinois, Burnsides Research Laboratory, Department of Food Science, Urbana, IL 61801, and B.H.S. Cho, Burnsides Research Laboratory, University of Illinois, and Harlan E. Moore Heart Research Foundation, Champaign, IL.

The implantation of estradiol in chicks resulted in a marked elevation of plasma lipids, and the increase was greatest in triglyceride followed by phospholipid and cholesterol. Just one day after implantation of estrogen, triglyceride level increased almost threefold, and this pattern of elevation progressed linearly up to day 14. During the period of two weeks, plasma triglyceride level increased to almost 45 times that of control (6838 vs 141 mg/dl). Although the elevation of cholesterol was not as dramatic as that of triglyceride, the level of cholesterol increased steadily during the 14-day period, attaining nearly a sixfold increase in comparison to the level of control (873 vs 147), and the level of plasma phospholipid elevated markedly from 209 to 2,861 during the same period. The untreated chicks had only a small amount of VLDL. However, VLDL-cholesterol level increased dramatically after estrogen implantation. LDL-cholesterol level also elevated substantially, but HDL-cholesterol level decreased markedly with estrogen treatment. Dietary fat level (5% vs 15%) had no significant effect on either plasma lipid or lipoprotein cholesterol levels. Triglyceride secretion rate in control and estrogen treated chickens were measured in vivo following the administration of Triton WR-1339. During 60 min post-Triton period, linear increases in plasma triglyceride concentrations were obtained with high correlation coefficient in both control and estrogen treated chickens. In control chickens, the plasma triglyceride levels increased from the initial level of 40.6 to 257.0 mg/dl during 60 min of post-Triton injection, resulting in a net triglyceride secretion rate of 112.6 mg/dl/hr. Chickens implanted with estrogen for 1 and 3 days showed a significantly higher triglyceride secretion rate than the control birds, 162.3 and 266.9 mg/dl/hr, respectively. Triglyceride removal rates measured following intralipid (10% fat emulsion) administration revealed no significant differences between control and estrogen implanted chickens. Present findings on triglyceride secretion and its removal rate provide further evidence that the hypertriglyceridemia associated with estrogen treatment in chicks is due mainly to the hepatic overproduction of triglyceride rather than to a diminished clearance of triglyceride from plasma.

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FATTY ACID SYNTHESIS AND CONSERVATION BY RAT HEPATOCYTES CULTURED IN FAT-FREE, SERUM-FREE MEDIUM. Darshan S. Kelley and Gary J. Nelson, U.S. Department of Agriculture, Western Human Nutrition Research Center, ARS, P.O. Box 29997, Presidio of San Francisco, CA 94129.

Fatty acid composition and synthesis was studied in cultured rat hepatocytes. Cells were isolated from the livers of rats that were fed a stock diet (F) or fasted for 24 hr (S) or fasted for 48 hr then refed a fat-free high carbohydrate diet for 48 hr (RF). They were maintained up to 120 hr as monolayer cultures in a glucose-rich serum-free medium (Waymouth's MB 752/1 containing dexamethasone, insulin and triiodothyronine). The rates of fatty acid synthesis (determined from incorporation of C^{14}-acetate into cellular lipids) and the activities of acetyl-CoA-carboxylase and fatty acid synthetase were measured. Synthetic rates decreased rapidly in cells from RF animals and increased in cells from S and F animals with time in culture. The synthetic rate was inversely correlated to the intracellular fatty acid content ($r = -0.9$) but was not related to the activity of these lipogenic enzymes. In the F and S animals palmitic, stearic, oleic, linoleic and arachidonic acids were the major fatty acids of liver; in RF animals palmitic, palmitoleic, stearic, oleic and *cis*-vaccenic acids were the predominant fatty acids. The saturated and monounsaturated fatty acid content of the cells increased markedly with time in culture in all three groups of hepatocytes. This caused an apparent decrease in the percent of polyunsaturated fatty acids (PUFA). However, if the amount of PUFA in the cells was analyzed as μg fatty acid per mg cellular protein, the content of the cells remained constant for the duration of the experiment. Cells in culture reflected the metabolic characteristics related to their prior metabolic status in the intact animal. Thus, it may be that isolated hepatocytes can provide a powerful model system to study the effects of dietary fat on lipid metabolism in the intact animal.

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MASS CHROMATOGRAPHIC ANALYSIS OF C_{70-90} α-MYCOLIC ACIDS FROM MYCOBACTERIA. Kenji Kaneda and Ikuya Yano, Niigata University School of Medicine, Asahimachi-dori 1, Niigata 951, Japan, and Seiko Mizuno, Soai Woman's College, Osaka, Japan.

The most characteristic cell wall component in acid-fast bacteria, such as *Mycobacterium* and *Nocardia*, is well recognized to be "mycolic acid," an extremely high molecular weight, 3-hydroxy fatty acid with a long alkyl chain at the 2-position. The structure of mycolic acids has been known to vary greatly by genus and species, and also by cultural conditions. Therefore, in the past several decades, studies on the structure analysis of mycolic acids have been carried out widely and extensively in several laboratories. However, until recently, the precise determination of molecular species composition of mycolic acids using gas chromatography has shown limited success, because of the instability of mycolic acids at high temperature. Recently, we have succeeded in the direct GC/MS analysis of trimethylsilyl (TMS) ether derivatives of α-mycolic acid methyl esters ranging from C_{70} to $C_{¿}86$ and revealed to be especially useful for the structure identification of individual molecular species or the determination of mycolic acid composition of each *Mycobacterium* species. Thirteen rapidly growing and 12 slowly growing species were harvested, hydrolyzed and then transmethylated. These fatty acid methyl esters were separated into several subclasses of mycolic acids on TLC. α-Mycolic acid methyl esters on recovery were converted to TMS ether derivatives and analyzed by gas chromatography. Using GC and GC/MS, the molecular species of α-mycolic acids were separated as the TMS ether derivatives of the methyl esters, according to their total carbon numbers. From the $[M]^+$, $[M-15]^+$ and $[M-90]^+$ ions, the total carbon and double bond numbers of mycolic acids were detemined, while the straight and branched chain structures were identified by the mass fragment ions $[A]^+$ due to C_2-C_3 cleavage $[R\text{-}CH\text{-}O\text{-}Si(CH_3)_3]^+$, AND $[B]^+$, C_3-C_4 cleavage $[(CH_3)_3\text{-}Si\text{-}O\text{-}CH\text{-}CH(R')\text{-}COOCH_3]^+$, respectively. The concentration of odd and even carbon numbered mycolic acids was clearly determined by mass chromatography monitoring $[M-15]^+$ ions and was revealed to differ remarkably by the species of

mycobacteria. Based on the molecular species composition, the average carbon numbers of the α-mycolic acids were calculated. Each species of the bacteria was demonstrated to possess a characteristic profile of α-mycolic acid composition. Therefore, GC/MS analysis of α-mycolic acid gives the most useful information for the chemotaxonomy of *Mycobacterium*.

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INFLUENCE OF DIETARY *TRANS* FATTY ACIDS ON THE LIPID METABOLISM OF THE BRAIN. Jan Pettersen, Norwegian Herring Oil and Meal Research Institute, Tjaereviken, 5033 Fyllingsdalen, Norway.

In two comprehensive experiments on the nutritional effects of *trans*fatty acids, we studied the fatty acid metabolism of the heart and brain of the offspring of sows (e.g. 3-wk-old suckling piglets) fed partially hydrogenated oils. The results showed that low but significant amounts of *trans* fatty acids were incorporated into the brain lipids (e.g. phosphatidylethanolamines) when the dietary content of essential fatty acids (EFA; e.g. linoleic acid) was restricted, but not when a liberal dietary supply of EFA was provided. A high content of *trans* fatty acids in the diet did not influence the fatty acid profile in the brain when the content of EFA was low but did lead to certain changes when the EFA content was high. Regardless of the level of EFA in the sows' diet, the PE of the heart in the suckling piglets contained significant amounts of *trans* fatty acids. Further, the presence of high levels of *trans* fatty acid in the sows' diet increased the level of linoleic acid and decreased the level of arachidonic acid in the heart PE. This effect was especially pronounced when the dietary level of EFA was restricted. These results indicate that *trans* fatty acids reduce the activity of Δ-6 desaturase in the heart but not in the brain. The experimental results will be presented and discussed.

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EFFECTS OF TYPE OF DIETARY FAT ON LIPID AND GLUCOSE METABOLISM IN PIGS. Laura A. Woollett and D.C. Beitz, Iowa State University, 313 Kildee Hall, Ames, IA 50011.

We have shown that type of dietary fat will affect triglyceride and cholesterol deposition in tissue of pigs. These effects are postulated to occur because of changes in turnover rates of triglyceride and cholesterol from specific plasma lipoproteins in response to changes in concentrations of glucoregulatory hormones in plasma. Thus, a study was designed to evaluate effects of type of dietary fat on in vivo kinetics of triglyceride, cholesterol and glucose metabolism in diabetic and nondiabetic pigs. Twenty 8-wk-old pigs were used; 10 pigs were made diabetic with alloxan. Five pigs from each group were fed either soy oil or beef tallow as the primary fat, providing 40%of dietary calories. After 6 wk of feeding fat treatment, glucose tolerance tests were performed on each pig to measure effects of fat on in vivo clearance of blood glucose. Type of fat did not alter rates of glucose clearance in nondiabetic pigs. Diabetic pigs fed tallow-based diets, however, removed glucose at a slower rate than did diabetic pigs fed soy oil-based diets. The dietary fat-induced changes in glucose clearance probably were the result of changes in concentrations of glucoregulatory hormones in plasma. After completion of glucose tolerance tests, pigs were injected with very low density lipoproteins (VLDL) that were labeled with [^{3}H]-triglyceride (TG) and [^{14}C]-cholesterol (CH). Serial blood samples were collected for the next 24 hr. Amounts of TG and CH were determined in VLDL, low density lipoproteins (LDL) and high density lipoproteins (HDL) of each sample. Approximately 80% of radioactive TG and CH of VLDL were cleared from circulation within 15 min in nondiabetic pigs, whereas 30 min were needed for 80% clearance in diabetic pigs. Clearance of TG and CH in VLDL was not affected by type of dietary fat. After an initial equilibration of radioactive TG and CH between VLDL and LDL, amount of labeled lipids in LDL had decreased to a relatively constant amount by 15 min. By 1 and 2 hr, respectively, after injection of labeled lipids, an increasing amount of TG and CH began to appear in LDL, suggesting a conversion of VLDL to LDL as a result of lipoprotein lipase activity. Rate of conversion of VLDL to LDL seemed greater in diabetic pigs and in pigs fed the tallow diets. Rate of transfer of TG and CH from VLDL to HDL was not influenced by diabetes. Pigs fed tallow diets, however, incorporated more TG and CH of VLDL into HDL than did pigs fed soy oil diets. In summary, regulatory mechanisms that result in the hyperlipidemia commonly found in diabetics could result from slower VLDL turnover rates and (or) greater amounts of VLDL lipids being transferred to LDL. Feeding a saturated fat increased transfer of TG and CH of VLDL to LDL and HDL.

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VESICLES FROM SUCROSE FATTY ACID ESTERS. Yutaka Ishigami, National Chemical Laboratory for Industry, Higashi 1-1, Yatabe, Tsukuba, Ibaraki, Japan, and Hajime Machida, Mitsubishi Chemical Industries Ltd.

Studies on the properties of the vesicles composed of sucrose fatty acid esters were carried out for the purpose of the development of new vesicle-forming materials aiming at drug carriers. Sucrose fatty acid esters examined were mainly composed of the diesters containing monoesters and triesters as the constituents. These compositions were analyzed by chromatographic analyses. Fatty acid residues of the esters were from caprylic to stearic acid, respectively. Some of them were the diester enriched samples which were prepared by repeating chromatographic fractionation. Vesicles were prepared by vortexing the dried lipid films consisting of sucrose fatty acid esters on adding water or buffer solutions over the phase transition temperatures. It was confirmed that sucrose fatty acid esters formed vesicles, from the observation using an electron microscope (Hitachi HU-12A) after staining negatively with aqueous uranyl acetate solutions. The range of the particle size of the vesicles consisting of sucrose dilaurate were apparently 70∿700 nm in the longer diameter of individual vesicles from the photographic observation, while the weight-average particle size of it was 424 nm by means of the photo correlation method (Coulter N4 sub-micron particle analyzer). The amounts of 6-carboxyfluorescein trapped in the vesicles of sucrose fatty acid esters were determined fluorimetrically. For example, the vesicle of sucrose distearate had the central water phase of 1.9 l water/mol ester, and showed a very slow release of 6-carboxyfluorescein from the central water phase after the preparation of the vesicle. The effects of some kinds of additives on the barrier functions of these vesicle membranes also were investigated.

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METABOLISM OF *CIS*-12, *TRANS*-15-OCTADECADIENOIC ACID COMPARED TO COMMON DIETARY FATTY ACIDS IN MAN. E.A. Emken, W.K. Rohwedder, R.O. Adlof and H. Rakoff, Northern Regional Research Center, ARS-USDA, 1815 N. University St., Peoria, IL 61604, and R.M. Gulley, St. Francis Medical Center, Peoria, IL.

Mixtures oftriglycerides containing deuterium-labeled 16:0, 18:0, 9c-18:1, 9c,12c-18:2 and 12c,15t-18:2 were fed to two young adult male subjects. Plasma lipid classes were isolated from blood samples collected over a 48-hr period. Absorption, turnover and incorporation into plasma lipid classes of the fed deuterium-labeled fats were followed by gas chromatography-mass spectroscopy analysis of the lipid class methyl ester derivatives. Unexpected results were (1) endogenous fat contributed about 35% of the total fat incorporated into chylomicron triglycerides, (2) reaction rates

for elongation and desaturation of the deuterated fats were negligible and (3) the polyunsaturated isomer (12c,15t-18:2) was metabolically similar to the saturated and 9c-18:1 fatty acids rather than to 9c,12c-18:2. As expected, absorption of the saturated fats (16:0 and 18:0) was 30-40% less than for the unsaturated fatty acids (9c-18:1, 9c,12c-18:2, 12c,15t-18:2). Data for the 1- and 2-acyl positions of phosphatidylcholine and for cholesteryl ester fractions reflected the well-known high specificity of phospholipid acyltransferase and lecithin:cholesteryl acyltransferase for 9c,12c-18:2. The various results confirm that the use of ratios automatically compensated for the effect of unequal isotopic dilution by endogenous fatty acids. Thus, comparison of the ratios of the various deuterated fatty acids in the fed mixture to the ratios found in the plasma lipids reflect the sum of the overall rate constants.

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EFFECTS OF GESTATIONAL EFA SUPPLEMENTATION ON OFFSPRING BEHAVIOR DURING STRESS. David E. Mills and Ron P. Ward, Department of Health Studies, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Behavioral and cardiovascular hyper-reactivity to stress increases risk of heart disease. EFAs reduce cardiovascular reactivity to isolation stress, but their effects on behavior are unknown. The present study examined the effects of prenatal and early postnatal EFA supplementation on behavioral responses to isolation stress. Adult female rats (n = 6/group) received osmotic pumps releasing olive oil (OL), or OL plus linoleic (LA), gamma linolenic (GLA), arachidonic (AA) or alpha linolenic (ALN) acid. A sixth group received dummy (DUM) pumps. Animals were then bred. On day 1 postpartum, three females/group had pumps removed, while the rest underwent sham surgery. Offspring behavior during stress was tested in an open field apparatus on days 25 and 50. Samples were taken on days 1, 26 and 50 postpartum for brain EFA analysis. EFAs altered behavior on both days, although pre- vs pre- plus postnatal treatment had no effect. OL lowered rearing, sniffing and locomotor activity vs DUM, while the n3 and n6 EFAs acted in an antagonistic manner to OL. EFAs also differentially affected learning ability in the adult offspring. EFA effects were pronounced in the females, but only marginal in males. Profiles of brain EFAs will be discussed. These data suggest diet during pregnancy may be a potent determinant of postnatal behavioral reactivity to stress. This effect appears to last until maturity and be more pronounced in females than in males.

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ANTICARIOGENICITY OF LOW LEVELS OF DIETARY LAURICIDIN. J.J. Kabara and C.M. Wernette, Department of Biomechanics, Michigan State University, E. Lansing, MI 48824; and P. Lynch and R.A. Schemmel, Department of Food Science and Human Nutrition.

Studies previously conducted have indicated that Lauricidin (glycerol monolaurate) at levels of 2% lowered the incidence of smooth surface caries (54-65%). The effect of lower concentrations of Lauricidin was studied in 48 female Osborn Mendel rats divided into six treatment groups. Animals were placed into groups receiving 0.0, 0.5, 1.0 or 2.0% Lauricidin in their diet. The first four groups of animals were then inoculated with *Streptococcus mutans* while two groups were non-inoculated and received either 2% Lauricidin or 2% Crisco. After six wk on the dietary regiments, animals were killed and the teeth were evaluated for microorganism populations (total count and *Streptococcus mutans*) as well as carie scores. The food intake and weight gains between the various groups were not statistically different. While the total bacterial count for control rats was much higher than the three treated groups, the difference in *Streptococcus mutan* levels were not consistently lower for the treatment groups. The largest difference (< 80% reduction) in carie scores were in the buccal-lingual area of the lower jaw; the upper jaws were essentially free of smooth area caries in both control and treated groups. Other carie scores for sulcal and proximal areas were also lower in the treated groups. The role of Lauricidin in dental health will be discussed.

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THE EFFECT OF DIETARY FAT TYPE ON CHOLESTEROL AND LIPOPROTEIN METABOLISM IN RABBITS FED SEMIPURIFIED DIETS. John E. Bauer, University of Florida, J-144 JHMHC, Department of Physiological Sciences, Gainesville, FL 32610.

Soy protein isolate/dextrose semipurified diet was formulated to which either 13% hydrogenated coconut oil plus 1% corn oil (HCO) or 14% safflower oil (SAF) was added. Two groups of New Zealand white rabbits (n = 6/group) were pair-fed the HCO and SAF diets for a period of 15 wk. A third group of animals were fed the basal diet containing 2.5% corn oil (SD). At the end of the experiment, serum was collected and lipoproteins were isolated by a single-spin density ultracentrifugal method. Lipoprotein fractions of the following density ranges were obtained: VLDL + IDL_1, $d < 1.012$ g/ml; IDL_2, $1.012 < d < 1.028$ g/ml; LDL, $1.028 < d < 1.067$ g/ml; HDL, $1.067 < d < 1.140$ g/ml. Serum VLDL ($d < 1.006$ g/ml) were additionally isolated by preparative ultracentrifugation, washed once at this same density and concentrated to a final triglyceride concentration of at least 3.5 mg/ml for in vitro lipolysis studies. Increases in all serum lipid subclasses were observed in the HCL diet fed rabbits. Increases in lipoprotein mass of all fractions were also seen in animals fed this diet. Weight ratios of core to surface (C/S) lipoprotein components, however, were not significantly different in any of these fractions. Compositionally, with regard to the major lipid and total protein components, the VLDL + IDL_1 and HDL fractions were similar among all dietary groups. The IDL_2 and LDL of rabbits fed the HCO diet were cholesteryl ester enriched when compared with that of the other dietary groups. When this VLDL was used as an in vitro substrate for postheparin plasma mediated lipolysis using both dansyl phosphatidylethanolamine and ^{3}H-triolein probe labeled VLDL, a twofold, statistically significant greater lipolytic rate was observed. Reasons for this difference may be due to the type or amount of apo VLDL peptides present in VLDL of rabbits fed the hydrogenated fat diet. The cholesteryl ester enrichment of both intermediate and low density lipoproteins seen with saturated fat feeding may be, in part, the result of this increased rate of VLDL triglyceride lipolysis since these particles were also relatively triglyceride poor compared with the polyunsaturated fat containing diet.

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EFFECTS OF FATTY ACID SUPPLEMENTATION ON FLUID AND ELECTROLYTE REGULATION DURING STRESS IN THE RAT. David E. Mills and Ron P. Ward, Department of Health Studies, University of Waterloo, Waterloo, Ontario N2L 3G1 Canada.

Certain n6 and n3 EFAs attenuate cardiovascular responses to chronic isolation stress in rats, but their mechanism of action is unknown. The current study examined effects of n3 and n6 EFA administration during chronic isolation stress on body fluid and salt regulation. Group-reared male rats were fasted three days and then placed on a fat-free diet. After 2 wk, animals were divided into seven groups, and osmotic pumps (Alza) releasing olive oil (OL), or 18:2n6, 20:4n6, 18:3n3 or 20:5n3 in OL, were implanted ip. Another group received dummy pumps (DUM). Two weeks later, rats were isolated in metabolic cages for a 4-wk stress period. Food, water, and salt intake, body weight, and urinary water and salt output were measured over 24 hr intervals upon isolated and at weeks 1-4. Food, Na and K intake were decreased by 20:4n6 and in 18:3n6, but increased by 18:3n3. Water intake increased in DUM, OL, 20:4n6 and in 18:3n3 groups. Na and K accumulation paralleled Na and K intake. Water accumulation increased in DUM and 20:4n6 groups and decreased with 20:5n6, 18:3n6 and OL. Body weight gain did not vary in one run, but in a second, OL, 18:3n6 and 18:3n3

decreased weight gain vs DUM, 18:2n6 and 20:4n6. These data suggest that (1) n3 and n6 EFAs exert differential effects on salt and water regulation, (2) of the EFAs studied, 18:3n6 most effectively reduces food intake, and water and electrolyte retention during stress, and (3) food and electrolyte intake in response to n3 and n6 EFA during stress parallels reported blood pressure responses.

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THE EFFECTS OF DIETARY FISH OIL CONTAINING EICOSAPENTAENOATE ON THE PRODUCTS OF THE PLASMA LECITHIN:CHOLESTEROL ACYLTRANSFERASE IN HUMAN SUBJECTS. Bruce J. Holub, Dorothy Bakker and Murray Skeaff, Department of Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

A high intake of marine fish and fish oils containing eicosapentaenoic acid (EPA) has been implicated in a reduced incidence of arterial thrombosis associated with cardiovascular disease in certain population groups. In addition to its potential effect on human platelets, dietary EPA can affect the level of circulating lipids and lipoproteins. The mechanisms of action of dietary EPA in mediating these changes have not been elucidated. In the present work, an encapsulated preparation of fish oil containing EPA (MaxEPA) was given daily to one group of human subjects and a capsule containing olive oil (OO) to a control group. The MaxEPA group, but not the OO group, showed a marked reduction in fasting plasma triglyceride levels with intervention as well as a moderate elevation in plasma HDL-cholesterol levels. In vitro assays using cholesterol as a substrate also showed a dramatic rise in the formation of cholesterol eicosapentaenoate via lecithin:cholesterol acyltransferase activity using the plasma of those given MaxEPA. These results may be of importance in accounting for the effects of dietary EPA on plasma cholesterol and lipoprotein metabolism.

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LEVELS OF FATTY ACIDS IN NORMAL POPULATIONS—A WORLDWIDE STUDY. M.S. Manku, N. Morse-Fisher and D.F. Horrobin, Efamol Research Institute, P.O. Box 818, Kentville, Nova Scotia B4N 4H8, Canada.

Fatty acid levels both in plasma and red cell membranes in populations of normal individuals from various parts of the world will be reported. Blood samples were obtained from the following eight countries: Canada, Britain, Australia, USA, New Zealand, Zimbabwe, Finland and Sweden. In all, plasma and RBD phospholipid fractions were analyzed from 520 individuals. The fatty acids analyzed wer as follows: 16:0, 18:0, 18:1ω9, 18:2ω6, 18:2ω6, 18:3ω3, 20:3ω6, 20:4ω6, 20:5ω3, 22:4ω6, 22:5ω6, 202:5ω3 and 22:6ω3. Complete comparative fatty acid data will be presented.

Session FF Saturday morning Symposium on Oilseed Proteins—Chemical Modification

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CHEMICAL AND PHYSICAL LIPOPHILIZATION OF PROTEINS. Makoto Kito, Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan.

The importance of improving the functional properties of food proteins has been stressed in recent years. In the native protein, the hydrophobic core is tucked away from the aqueous environment for which the hydrophilic outer shell has an affinity. However, if sufficient hydrophobic patches could be created on the surface without significantly altering the solubility, they could constitute binding sites for lipid and thereby enhance the functional property of the protein. With a view to enhance the amphipathic nature of food proteins, chemical and physical modification was carried out. (1) We lipophilized them by chemically attaching naturally occurring fatty acids to the hydrophilic soybean 11S protein and α_{s1}-casein molecules. The covalent attachment of fatty acyl residues to these proteins caused an increase in their emulsification capacity. (2) Soy proteins were associated with soy lecithin by sonication. The emulsification activity of soy protein isolate, 7S and 11S proteins complexed with lecithin was increased after they were treated with 50% ethanol. By this procedure, the conformation of these proteins was changed resulting in aggregation. Emulsification activity of the soy protein-lecithin complex was apparently increased by boiling. This treatment, however, only caused 11S protein to aggregate resulting in better emulsification activity, regardless of whether it was complexed or not. The increase in emulsification activity caused by ethanol treatment was not affected by NaCl, whereas the increase by boiling was reduced in the presence of NaCl. Thus, it seems likely that ethanol and heat treatments change the conformation of soy proteins complexed with lecithin in different ways.

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CHEMICAL INACTIVATION OF SOYBEAN TRYPSIN INHIBITORS. David J. Sessa and Philip E. Ghantous, USDA, ARS, Northern Regional Research Center, 1815 N. University St., Peoria, IL 61604.

Sodium metabisulfite and glutaraldehyde were used alone and in combination to inactivate Kunitz trypsin inhibitor (TI) in model systems and soybean protein isolate TIs. Treatment of Kunitz TI, 0.79 TI/mg, in 0.1 M sodium phosphate buffer, pH 7.0, for 1 hr at 25 C with 0.2–1.0% glutaraldehyde resulted in 60–75% reduction in activity. Treatment of soybean protein isolates, 0.08 mg TI/mg, with up to 3% glutaraldehyde under the same conditions reduced TI activity only 40%, however. Acidification to pH 5 caused increased loss of soybean TI activity and insolubilized proteins, but a temperature of 75 C had little effect on TI activity. Sodium metabisulfite (0.3 mM to 1.0 mM) inactivated 90% Kunitz TI within 1 hr at 75 C and 85--94% soybean protein isolate TIs. The combination of 0.6 mM metabisulfite followed by 0.5% glutaraldehyde at 75 C inactivates 99% Kunitz TI and combination with up to 3% glutaraldehyde inactivates 83% of soybean protein isolate TIs versus 92% inactivation with salt alone. Reason for reversal of soybean protein TI activity upon salt-aldehyde treatment is unknown. Metabisulfite treatment can cleave disulfide bonds, and glutaraldehyde in excess of that which interacts with metabisulfite yields bisulfite adducts and also can react with protein functional groups. Thus, our results show that combined reaction of metabisulfite and glutaraldehyde inactivates Kunitz Ti better than either one alone, while bisulfite alone best inactivates soybean protein isolate TI.

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APPLICATION OF PROTEOLYTIC ENZYMES TO STRUCTURAL ANALYSIS OF α-$_{s1}$-CASEIN ADSORBED ONTO AN OIL SURFACE OF AN EMULSION AND IMPROVEMENT OF THE EMULSIFYING PROPERTIES OF THE PROTEIN. Shuichi Kaminogawa, Makoto Shimizu and Kunio Yamauchi, University of Tokyo, Department of Agricultural Chemistry, Bunkyo-ku, Tokyo, Japan.

α_{s1}-Casein, a major protein of bovine milk, has a unique amphiphilic structure which may provide excellent emulsifying properties. The present study was undertaken to analyze the structure of α_{s1}-casein adsorbed onto an oil surface of a stable emulsion, and to improve the emulsifying properties of α_{s1}-casein by proteolysis. The emulsion was prepared with α_{s1}-casein and soybean or coconut oil. By using trypsin and chymotrypsin, the enzymic cleavage of α_{s1}-casein, adsorbed on an oil surface of the

emulsion, was compared with those dissolved in an aqueous solution. Cleavage of 13 peptide bonds in the adsorbed α_{s1}-casein in the emulsion was difficult, as compared to those in solution. These peptide bonds were probably among definite regions inaccessible for the proteases. Based on the results, a preliminary model is proposed for the structure of α_{s1}-casein at an oil/water interface. Emulsifying activity (EA) of α_{s1}-casein increased by the pepsin digestion. The peptide fraction (PF), composed of α_{s1}CN(f1-23) and a small amount of other peptides, was separated from a peptic hydrolyzate of α_{s1}-casein. PF showed a similar EA to that of α_{s1}-casein at neutral pH and maintained a high EA in the acidic region where α_{s1}-casein lost its EA. The removal of the small amount of the peptides from PF resulted in the marked decrease of the EA. However, the addition of the removed peptides to α_{s1}CN(f1-23) restored the EA. Some synergistic effect between α_{s1}CN(f1-23) and the other peptides in emulsification was suggested.

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IMPROVING THE NUTRITIONAL PROPERTY OF SOY PROTEIN BY ENZYMATIC MODIFICATION. Soichi Arai, Department of Agricultural Chemistry, University of Tokyo, Bunkyo-ku, Toyko 113, Japan.

In recent years our views on the absorption of protein digestion products have undergone a radical change. It is now believed that intralumen hydrolysis of fed proteins to free amino acids is only partial. A great deal of effort is being made to evaluate superior nutritional properties of partial hydrolysates of proteins and to produce an oligopeptide rather than free amino acid nitrogen source for use in alimentary diets. We have developed a sophisticated technique for producing oligopeptide mixtures from soy protein isolate (SPI) by modification with proteases. SPI was thus modified to oligopeptide mixtures having covalent methionine levels of 1% and 3%. Each of them had an average molecular weight of 600–900 daltons. They were compared with corresponding amino acid mixtures as well as with SPI for protein efficiency ratio (PER) and other nutritional parameters. Normal and malnourished rats were used for the comparison tests. When malnourished rats were subjected to a feeding test at a methionine level of 1% in nitrogen source, the oligopeptide mixture, OPM_1, gave a significantly higher PER value than any of the SPI and the amino acid mixtures. At a methionine level of 3%, both normal and malnourished rats utilized the oligopeptide mixture, OPM_3, with higher efficiency than the amino acid mixture. These results suggest that the oligopeptide mixtures were utilized similarly to or more efficiently than the SPI and the amino acid mixtures. An economic feasibility of modifying soy protein into such a nutritionally superior oligopeptide mixture is also discussed.

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EFFECT OF LIMITED PROTEOLYSIS ON THE FUNCTIONAL PROPERTIES OF OVALBUMIN. Etsushiro Doi and Naofumi Kitabatake, Research Institute for Food Science, Kyoto University, Uji, Kyoto 611 Japan.

Egg white coagulates upon heating. This heat coagulated gel is used for many food products. The present experiments have been carried out to modify the properties of gels of ovalbumin, the main constituent of egg albumin, by limited proteolysis. We found very limited proteolysis of ovalbumin by pepsin at pH 4. Pepsin split off a small peptide (about 3,000 in molecular weight) from the ovalbumin molecule. The cleavage site was determined to be between His 25 and Ala 23 of the ovalbumin amino acid sequence by analysis of the N- and C-terminals of the product. The residual protein (about 42,000 in molecular weight) was designated as P-ovalbumin. This cleavage site is quite different from those for subtilisin, which attacks the C-terminal portion of ovalbumin and causes the formation of plakalbumin. Some of the functional properties of P-ovalbumin were examined, and an interesting property of its heat-induced gel was examined. The heat-induced gel of P-ovalbumin was transparent. The original ovalbumin gave a white turbid gel under the same condition. The transparent gel made of ovalbumin should prove useful for preparation of new types of food products.

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LIMITED PROTEOLYSIS AND COVALENT ATTACHMENT OF PHOSPHATE AND AMINO ACIDS TO PROTEINS TO IMPROVE THEIR FUNCTIONAL AND NUTRITIONAL PROPERTIES. John R. Whitaker, University of California, Department of Food Science and Technology, 1480 Chemistry Annex, Davis, CA 95616; M. Sitohy, Zagazig University, Zagazig, Egypt, and J.M. Chobert, National Institute of Agronomical Research, Nantes, France.

Seed proteins generally have lower water solubility and lower nutritional quality than do animal proteins. Chemical modification has been proposed and tested by several researchers as one means of improving solubility and nutritional quality. Dispersal in alkaline solution is widely used to solubilize more of the soy proteins; however, several types of chemical modification can occur in the alkaline solution, catalyzed by the hydroxide ion. In the present work, we have explored the use of highly specific proteases to selectively hydrolyze a few peptide bonds to produce controlled-size peptides with improved solubility characteristics, especially in the range of the isoelectric point of the protein. Zein, the major protein of corn, is insoluble in water and has a very low PER (protein efficiency ratio) value. Phosphorylation of zein with $POCl_3$ improved its water solubility via covalent incorporation of up to 21 moles of phosphate groups per mol of zein. $POCl_3$ simultaneously catalyzed the covalent attachment of free amino acids to zein when added singly or as mixtures of amino acids. The effect of phosphorylation and simultaneous covalent attachment of lysine, threonine and tryptophan to zein on solubility, emulsifying and whipping properties and nutritional quality will be described.

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FUNCTIONALITY OF ACYLATED FOOD PROTEINS. Khee Choon Rhee, Texas A&M University, Food Protein Research and Development Center, F.M. Box 183, College Station, TX 77843.

Proteins are modified intentionally for structure-function relationship studies or for development of new and improved products from less useful resources. Acylation of proteins has been of interest in the potential application for nutritional and functional improvements of food proteins. Acylation is the chemical reaction which attaches acyl groups to unprotonated amino groups of protein to form amide derivatives. Application of acylation techniques is able to control deteriorative reactions during processing or transportation, to improve physical or functional properties, to eliminate undesirable substances such as pigments, to improve nutritional properties and to increase the acceptability of unconventional protein resources. Also, it may be helpful in the physical separation of protein from crude animal, plant or microbial materials, in the inactivation of some antibiological substances such as allergens or enzyme inhibitors, and in the development of new raw materials from natural resources for chemical or pharmaceutical industries, as well as for human foods and animal feeds. In spite of such advantages, acylation not only is poorly investigated or understood, but also, attempts to apply it to food protein systems are nonexistent. The objective of this manuscript is therefore to review the current status of food protein acylation studies.

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EXTRACTABILITY OF FOOD CONSTITUENTS IN THE PRESENCE OF ACYLATING AGENTS. Lilian U. Thompson, Department of Nutritional Sciences, University of Toronto, 150 College St., Toronto, Ontario, Canada M5S 1A8.

Acylation introduces a neutral or anionic group in the nucleophyllic groups of amino acid residues of the proteins. This changes the interaction of proteins with other proteins as well as other constituents of the food and consequently their extractabilities and precipitation in aqueous solution. The extractabilities differ with degree of acylation and ionic strength. For example, when rapeseed flour was treated with high levels of acetic or succinic anhydride, the protein was highly extracted from the flour with very little simultaneous extraction of phytic acid. With low level of acylation, the phytic acid was extracted more than double that of the unmodified control while the protein extractability was increased considerably. At low ionic strength, while the protein extractability remained the same, the extractability of phytic acid increased with levels of acylation. The extractability of major minerals followed that of phytic acid. Similar results were observed with legume flour. The study suggests that extraction in the presence of acylating agents may be a good method of separating certain undesirable constituents from the proteins.

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FUNCTIONAL PROPERTIES OF CHEMICALLY MODIFIED EGG WHITE PROTEINS. Hershell R. Ball Jr., North Carolina State University, 339 Schaub Hall/Box 7624, Raleigh, NC 27695-7624.

Functional properties of egg white proteins can be altered through selected chemical reactions. Acylations with acid anhydrides have received the greatest amount of attention. Oleic acid and sodium dodecyl sulfate (SDS) have also been used to effect function of egg white proteins. The charge characteristics of acylated proteins are altered through modification of the N-terminal and epsilon-amino groups. The acid anhydride used and the extent of modification have a major effect on the ionic properties of the protein. The altered ionic properties have been shown to effect the optical properties of protein sols, heat stability, foaming, performance in angel cakes, initiation of gelation, ultimate strength, and freeze-thaw stability of heat-set gels. Although exact explanations of the mechanisms for the interactions of oleic acid and SDS are not available, increases in charge occur and result in gels with physical properties very similar to gels made from succinylated egg protein. The relationships of properties of modified egg white proteins in solution to functional performance will be discussed.

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FUNCTIONAL PROPERTIES OF OAT PROTEINS MODIFIED BY ACYLATION, TRYPSIN HYDROLYSIS OR LINOLEATE TREATMENT. Ching-Yung Ma and D.F. Wood, Food Research Institute, Agriculture Canada, Central Experimental Farm, Ottawa, Ontario, Canada K1A 0C6.

Proteins extracted from defatted oat (*Avena sativa* L.) were acylated with acetic or succinic anhydride at levels of 0.05 and 0.20 g/g protein. The oat proteins were also modified by partial hydrolysis with trypsin or potassium linoleate treatment. Total essential amino acid content was slightly decreased by acetylation but unaffected by succinylation. Gel filtration chromatography showed some dissociation of oat polypeptides by succinylation and linoleate treatment, and considerable protein breakdown by trypsin hydrolysis. Solubility, emulsifying properties and fat binding capacity were all markedly improved by acylation and linoleate treatment. Enzyme hydrolysis also improved solubility and emulsifying properties, but the fat binding capacity was lowered. Water hydration capacity and foam stability were adversely affected by acylation but were increased by linoleate and trypsin treatments. The effect of acylation on oat proteins was also studied in a model wiener system. Succinylation improved the cook yield in beef wiener, and cohesiveness and firmness in pork wiener.

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MODIFICATIONS OF ARACHIN—STRUCTURAL IMPLICATIONS. D. Rajagopal Rao, A.G. Appu Rao and V. Prakash, Central Food Technological Research Institute, Mysore 570 013, India, and R. Shyama Sundar, All India Institute of Medical Sciences, New Delhi, India.

Arachin is the major high molecular weight protein component of groundnut. In order to improve the functional properties of groundnut proteins, they have been subjected to various types of physical treatments and chemical modifications. We have attempted to investigate the structural implications of such modification (principally acylation) of arachin by various methods such as viscosity, sedimentation velocity, polyacrylamide gel electrophoresis, gel filtration, susceptibility to proteolysis, absorption and fluorescence spectra, optical rotation and circular dichroism. The effects of acylation on the structure of arachin are gradual up to 50% succinylation and marked changes occur beyond this level. There is a threefold increase in reduced viscosity. Beyond 60% succinylation, there is fluorescence quenching, dissociation of subunits (predominantly 4S) and increased susceptibility to proteolysis. The susceptibility to proteolysis of acetylated arachin is different from succinylated arachin. The dissociation of the protein due to acylation has a bearing on the acidic and basic subunits of arachin molecule. Analysis of the various biophysical data of the modified arachin indicate that the oligomeric structure is predominantly held by noncovalent interactions.

Session GG Saturday morning Surfactants and Detergents IV—Surfactant Performance and Evaluation

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PROPERTIES OF A SERIES OF SIMPLE ALKYL CAPPED ALCOHOL ETHOXYLATES. Thomas R. Oakes and Roger W. Keppers, Economics Laboratory Inc., The Osborn Building, St. Paul, MN 55102.

The need for truly biodegradable low foam surfactants with both alkaline and acid stability still exists. The ready ultimate biodegradation of the linear alcohol ethoxylates and the fact that simple alkyl capping reduces foam suggested these materials as potential candidates to satisfy this need. We have prepared a series of alkyl capped materials and have examined their foam properties and hard surface cleaning properties in the presence and absence of various defoamers. As expected, foam tendency generally increases with the degree of ethoxylation and decreases with increasing chain length of the capping groups. Defoam properties, in the presence of added defoamers, seems to reach an optimum level that may reflect the proper HLB matching of surfactant and defoamer. Cleaning properties, aquatic toxicity and biodegradability will be discussed.

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INSTRUMENTAL METHOD FOR EVALUATING STATIC CONTROL IN LAUNDRY. Merlin G. Tiede.

The most difficult performance attribute to measure when evaluating a fabric softener has been static control. Several instrumental methods have been used in the past, all with limited success. The most frequently used method has been a subjective evaluation by an experienced operator, but this also has obvious shortcomings. The topic method is based on a Faraday Cage. Test swatches are removed from the dryer and placed in an insulated stainless steel tank. As each swatch is removed a corresponding charge is induced on the tank, which is measured by a high impedence volt meter. The method differentiates well between types or levels of softeners. The effects of different softeners on

various fabric types can also be shown. Reproducibility is considerably better than with other methods. Time involved to record and evaluate the data has been minimized with automated data acquisition and tabulation by means of a computer interface.

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FORMULATING CHARACTERISTICS OF HIGH AND LOW 2-PHENYL SODIUM LINEAR ALKYLBENZENE SULFONATES IN LIQUID DETERGENTS. Joseph C. Drozd, Stepan Company, 22 W. Frontage Rd., Northfield, IL 60093.

Detergent range linear alkylbenzene (LAB) is currently manufactured by two different processes, employing either aluminum chloride or hydrogen fluoride as the alkylation catalyst. The alkylates from each process are not exactly the same. Furthermore, the properties of the linear alkylbenzene sulfonate (LAS) surfactants made by sulfonation of the LABs are also different. Since LAS finds use in most types of detergent products, it is important to know how the properties of each type of LAS differ. This paper compares the formulating characteristics (such as viscosity, solubility and foaming) of high and low 2-phenyl LAS in some typical household cleaning product formulations. It is concluded that the two types of LAS should not be used interchangeably without first checking carefully all the physical properties required in a product.

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DETERGENCY AND FOAMING PROPERTIES OF ALKYL POLYGLYCOSIDE SURFACTANTS. G.M. Verboom, A.D. Urfer, A.H. Malik and N.F. Borys.

The alkyl polyglycosides are a class of surfactants that offer unique properties of direct benefit to the detergent manufacturers. Detergency and foaming properties of alkyl polyglycosides will be presented to highlight their application and contribution to household cleaning products.

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Cancelled.

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CONSIDERATIONS ON THE EFFECTS OF LIPASES AT WASHING CONDITIONS. Erik Gormsen and Niels Elvig, Novo Industri A/S, Novo Allê, DK-2880 Bagsvaerd, Denmark.

Olive oil has been decomposed by lipase to different degrees of hydrolysis. The non-enzymatic removal of partially decomposed olive oil from fabrics is investigated in washing trials. The influence of surfactants, builders, washing time, and pH-value on the removal of the individual components of the hydrolysates is examined. Free fatty acids are removed more easily than triglycerides and diglycerides. The removal of free fatty acids is favored by high pH-value. Evidence of the beneficial effect of an alkaline lipase applied in low temperature washing is given.

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A NOVEL SOAP MAKING PROCESS. Richard J. Bertozzi, E. Gary Myers and Julian R. Story, Armour-Dial Inc., 15101 N. Scottsdale Rd., Scottsdale, AZ 85260.

A process for the preparation of soap products from raw materials normally employed in the manufacture of such products including fatty acids, triglycerides and caustic or alkali by subjecting such raw materials to intensive counter-current mixing whereby saponification takes place in a relatively short time to yield a product, preferably in granular or powder form, which requires no further drying for most uses. The resulting product can, if desired, be then subjected to plodding, extrusion and stamping to form soap in bar form. The starting material can also be a mixture of such raw materials where neutralization has proceeded to some degree, preferably the neat soap stage.

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Cancelled.

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SUNLIGHT PHOTODEGRADATION OF SURFACTANT POLLUTANTS CATALYZED BY SEMICONDUCTING PARTICLE MATERIALS. Hisao Hidaka, Meisei University, Department of Chemistry, 337, Hodokubo, Hino-shi, Tokyo 191 Japan; Nick Serpone, Department of Chemistry, Concordia University, Canada; Ezio Pelizzetti, Universita di Torino, Italy, and Michael Grätzel, Ecole Polytechnique Federale de Lausanne, Switzerland.

A number of surfactants that have been used widely in various fields have caused several problems to the natural aquatic environment. Their biodegradation through bacteria is very efficient. Photoinduced catalytic decomposition with semiconductor powders by solar exposure provides an important route to the environmental degradation of common surfactants (e.g. sodium dodecylbenzene sulfonate, DBS) by a heterogeneous photoassisted oxidation using TiO_2 suspensions. Solar exposure (60 mW/cm^2) or illumination (simulated sun-test lamp $\lambda > 330$ nm, 100 mW/cm^2) of aqueous DBS solution in the presence of aerobic conditions causes a rapid decomposition of the phenyl moiety in DBS (≥90%) in less than 1 hr. The aromatic group in DBS is decomposed rapidly and efficiently, while the long aliphatic chain is more slowly converted to oxidation products. Since a primary degradation, a secondary environmentally acceptable degradation and a final mineralization are considered, oxygen consumption CO_2 evolution, surface tension changes, and the pH dependence of photodecomposition will also be discussed. The cation radical formation is confirmed by a laser flash photolyses technique. The authors postulate the photodegradation of surfactants.

Session HH Saturday morning Analysis of Lipids V

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COMPARING GRAIN PROTEIN DETERMINATIONS USING DIFFERENT KJELDAHL PROCEDURES. Marlowe L. Iverson, R-G AFB, Bldg. 221, Grandview, MO 64030.

The basic Kjeldahl nitrogen method for protein determinations has been in use for over 100 years but procedures have been modified many times for various purposes that involve cost, environmental, safety, and other considerations. Various technical and trade associations publish their own versions for various materials as official methods of their organizations. These procedures and frequent revisions are usually general enough to provide accurate results but they are not always specific enough to prevent discrepancies. A review of published official methods and some in use for marketing purposes in several countries revealed allowed procedural options taht could lead to systematic errors. With the same catalyst, there were sufficient variations in parameters such as sample to acid and salt to acid ratios, initial digestion conditions, and digestion times, to lead to problems in producing accurate data. Experimental data show how variations in these parameters affect the initial and final digestion temperature and the nitrogen yield. The simple tests and test materials that have been recommended in the past need to be used in a routine manner. The quality control data will verify that acid-base standardization and digestion conditions are proper and will allow data to be compared regardless of which procedure or catalyst is used.

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GLC, TLC AND NMR ANALYSES OF TRIGLYCERIDES PRESENT IN EVERY NATURAL JOJOBA OIL. Thomas K. Miwa, The Jojoba Society of America, 2086 E. La Jolla Dr., Tempe, AZ 85282.

Five decades ago, jojoba oil was erroneously reported as devoid of triglycerides because investigators were not able to isolate glycerine during the saponification of the oil. In spite of publications that referred to the presence of triglycerides in small quantities in every natural jojoba oil, the incorrect notion that jojoba oil is uniquely lacking in triglycerides has prevailed. This report proves in detail the presence of triglycerides in every one of more than 500 natural jojoba oil samples tested by gas liquid chromatography (GLC), and corroborative analytical evidences of their presence are provided by thin layer chromatography (TLC) and by nuclear magnetic resonance (NMR). Minimum detection levels of triglycerides must be established, especially with TLC, where sufficient quantity of jojoba oil sample must be applied to the TLC plate to warrant any verdict of absence of presence of triglycerides. The normal level of triglycerides in jojoba oil is 0.5 to 2%, but may be as high as 15% even in the fully mature seed. Triglycerides in jojoba oil may be removed as residual material from fractional distillation of the liquid wax esters. Triglycerides are necessary in jojoba oil biosynthesis during the early maturation stages, when a hydrophilic environment similar to normal oil seeds must be maintained to convert polar precursors into liquid wax products that are retained in the seed as stored lipids to be catabolized during germination.

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HYDROCARBON CAROTENOID PROFILES OF PALM OIL PROCESSED FRACTIONS. Barrie Tan, C.M. Grady and A.M. Gawienowski, Department of Chemistry and Biochemistry, University of Massachusetts, Amherst, MA 01003.

Analysis by gradient-elution normal phase open column chromatography, thin layer chromatography, and ultraviolet-visible spectroscopy (confirmation by two to three peaks), including calculation of peak ratios, revealed 7 previously unreported hydrocarbon carotenoids in palm oil fractions. They were phytoene, phytofluene, ζ-carotene, α-zeacarotene, β-zeacarotene, neurosporene and δ-carotene. In addition, the presence of α-, β-, γ-carotenes and lycopene was confirmed. The carotenoid profiles of crude palm oil, crude palm olein, and filtered palm olein were similar; carotenoids in these fractions totaled 700–800 ppm. Carotenoids were not found in refined, filtered, and deodorized palm olein, while palm kernel oil contained 0.3 ppm of α-zeacarotene. The use of the antioxidant butylated hydroxytoluene protected carotenoids from destruction; the yield dropped 30% when it was not used. HPLC separation of these carotenoids will also be presented. Potential antioxidative and anticarcinogenic effects of some palm oil carotenoids will be discussed.

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DETERMINATION OF TOTAL SULFUR IN CANOLA OIL. V. Abraham and J.M. deMan, University of Guelph, Department of Food Science, Guelph, Ontario, Canada N1G 2W1.

A rapid and sensitive method for the determination of total sulfur in canola oil is described. All forms of sulfur in the oil are quantitatively converted to sulfate in an oxygen bomb. The sulfate is separated from other ions and measured using an ion chromatograph equipped with a conductivity detector. Standards containing different forms of sulfur were prepared and analyzed with this method. Recovery achieved on 11 standards covering the concentration range from 9.3 to 143.5 mg/kg S ranged from 95.7% to 102.2%. The coefficient of variability of total sulfur in canola oil ranged from 1.0% to 2.9%. Sulfate can also be determined as barium sulfate by nephelometry but only at levels exceeding 100 mg/kg S in the oil. For such oils a correlation coefficient of 0.997 between the two methods was found. The oxygen bomb method employs relatively simple equipment and requires less than 40 minutes for a complete analysis. The method can be used reliably for the determination of as little as 0.5 mg/kg of sulfur in canola oil.

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STUDY ON THE IDENTIFICATION OF RICE BRAN OIL. Yasuhiko Takeshita, Kokushikan University Department of Engineering, 4-28-1, Setagaya, Setagaya-ku, Tokyo 154, Japan; Fumio Iwata, Toryo Industry Co. Ltd, and Haruo Yoshida and Kazuo Hinata, Kokushikan University Chemistry Laboratory.

One of the identifying methods for rice bran oil (RBO) is studied. The specification of RBO in J.A.S. was standardized according to fatty acid composition and large content of USM. The JOCS method of identifying RBO adopted UV absorption of the ferulic acid component which was the largest of the natural cereal lipids. Corn germ oil has 1/10 the ferulic acid ester of crude RBO which contains 1–2%. However, if alkali refining of RBO is severe as in recent industrial processing, most phenolic compounds are neutralized and removed. In such cases, ferulic acid content nears that of corn germ oil. As other methods for identifying RBO, e.g. double acid values by FFA and phenolic matter, Emmerie Engel's reaction (yellow coloring by alcoholic potash owing to phenolic OH) and high mp of USM by wax alcohol are popular as industrial or manipulative procedures, but independent analysis is not perfect. On the other hand, the GLC pattern of USM is common to each breed of rice internationally, in spite of distinct differences of other cereal lipids. The general characteristic USM GLC pattern consists of hydrocarbon or wax alcohol, sterol and triterpenalcohol fractions, and it is suitable for crude or refined RBO, especially from other cereal lipids.

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A RAPID METHOD FOR DETERMINATION OF VOLATILE CARBONYLS AND FURFURALDEHYDE IN EDIBLE OILS. R. Przybylski, Department of Foods and Nutrition, University of Manitoba, Winnipeg R3T 2N2 Manitoba, Canada.

A simple and sensitive method was developed to determine volatile carbonyl compounds and furfuraldehyde in edible vegetable oils and fried foods using a chemical trap reaction and colorimetry. A sample of oil or potato chips was heated and purged with nitrogen gas which carried the volatile carbonyls and furfuraldehyde to a chemical reaction trap containing a 10% hydroxylamine hydrochloride solution. A sample of 25 ml and 10 g was sufficient for analyzing these volatile compounds in oils and potato chips respectively. The amounts of carbonyl compounds and furfural were estimated by spectral measurements of the carbonyl oximes at 212 nm and furfuraldehyde at 274 nm. This method could detect levels as low as 0.5 ppm and 0.1 ppm for carbonyls and furfuraldehyde respectively. The percentage recovery using hexanal and furfuraldehyde by this procedure were over 80% for concentrations over 1.0 ppm and 0.2 ppm respectively. The proposed colorimetric method proved both sensitive and reproducible and does not require pre-extraction and concentration of volatile carbonyl compounds and furfural prior to analyses. This paper also reports the analyses of these compounds in canola and cottonseed oils as well as potato chips fried in these oils by this procedure.

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DETERMINATION OF HYDROXY FATTY ACIDS IN FATS AND OILS. D.P. Schwartz, Eastern Regional Research Center, USDA, 600 E. Mermaid Lane, Philadelphia, PA 19118.

Hydroxy fatty acids (HFA) esterified in the glycerides of fats and oils can be isolated and determined using a relatively simple procedure. The oil or fat (0.5 g) dissolved in 5 ml of hexane in a 9-ml vial is transmethylated by vortexing with 0.5 ml of 2N methanolic

KOH for 2 min. One ml of 2N HCl is added, the vial is shaken, centrifuged 2 min and an aliquot of the hexane layer is evaporated under an N_2 stream. The HFA and other alcohols in the residue are esterified with pyruvic acid chloride 2,6-dinitrophenylhydrazone using 1,4-diazabicyclo[2,2,2]octane as catalyst. The highly colored derivatives are then fractionated on a 5-g bed of neutral alumina into a sterol-fatty alcohol fraction and an HFA fraction using hexane/benzene (1.5:1) and benzene, respectively. Both fractions are subsequently quantitated spectrophotometrically at 400 nm. Near quantitative recovery was obtained for a number of methyl hydroxy stearates that were added to pure synthetic triglycerides. The HFA content of a number of fats and oils will be given and a simple method described for regenerating the parent HFA methyl ester from the derivative.

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COMPARISON OF DETERMINATION OF SOLID FAT CONTENT BY NMR AND DILATOMETRY (SFI). R.E. Timms and E.M. Goh, Kempas Edible Oil Sdn Bhd, P.O. Box 75, Pasir Gudang, 81707 Johor, Malaysia.

The theoretical principles of the determination of solid fat content (SFC) by nuclear magnetic resonance (NMR) spectrometry and by dilatometry are discussed. The effect of tempering is emphasized, especially the effect of tempering at 80 F in the AOCS method for SFI in contrast to the European dilatometric procedures. Results are reported for SFI (AOCS method Cd 10-57), SFC using the Praxis SFC-900 NMR spectrometer and SFC using the Bruker PC120 NMR spectrometer. SFI results are substantially different from SFC by NMR results, even when the same tempering procedure is used. The two NMR spectrometers gave similar results. Unlike the Praxis, the Bruker NMR spectrometer is capable of measuring both solid and liquid fat signals simultaneously so that a direct and immediate display of SFC can be given. As a result, parallel rather than series measurements are facilitated. Results are given comparing parallel and series measurements. In general, little difference was found between the two procedures except for very steep melting fats such as cocoa butter substitutes. The parallel procedure has several advantages and the procedure as defined by IUPAC (Method 2.323) has been adopted in our laboratories for routine quality control. SFC measurements at 4–8 temperatures can be made routinely within two hours.

Session II Saturday morning Hydrogenation

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THE SELECTIVE HYDROGENATION OF SOYBEAN OIL IN A CONTINUOUS, FIXED-BED REACTOR. Gail M. Qualetti, Signal Research Center, 50 E. Algonquin Rd., Des Plaines, IL 60017.

Commercial processing of vegetable oils is carried out continuously, except for the hydrogenation, which is carried out in a batch reactor. Continous, fixed-bed hydrogenation processes have been described, but suffer from minimal selectivity. A continuous, fixed-bed hydrogenation reactor system for the selective reduction of these edible polyunsaturated oils, particularly soybean oil, has been developed. Soybean oil was hydrogenated continuously in the presence of a fixed-bed of various alumina supported nickel catalysts. The effect of temperature, pressure and oil flow rate on conversion and product quality was determined. Selectivity was achieved by limiting the concentration of hydrogen at the catalyst surface. The economic feasibility of this continuous, fixed-bed reactor system was demonstrated by preparing a hydrogenated basestock (IV = 78) of the desired physical properties over a multimonth pilot plant test. Throughput was greater than that obtained in the commercial batch reactor system.

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SELECTIVITY RATIO DETERMINATION IN HYDROGENATED FISH OILS. Ray L. Coleman, United Catalysts Inc., P.O. Box 32370, Louisville, KY 40232.

Fish oil was hydrogenated using a variety of catalysts to produce a series of partially hydrogenated oils. These oil samples were analyzed for dropping melting point and solid fat index to determine the relative selectivity of the hydrogenation. Transesterification of the oils followed by GLC analyses was then conducted to give the oil compositions. A method for interpreting the oil composition in a manner analogous to that for soy oil is proposed which yields selectivity ratios similar to those for soy oil. The chromatographic method, the mathematical model proposed, and the interpretation of the results are discussed which yield information on the selectivity of hydrogenating fish oil.

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THE USE OF COPPER CHROMITES IN THE HYDROGENATION OF FATTY ACIDS AND FATTY ACID ESTERS. M. Schneider and K. Kochlocfl, Süd-Chemie AG, Postfach 202240, 8000 Munich 2, West Germany.

Copper chromites represent versatile heterogeneous catalysts, used at present for various reactions such as hydrogenation, dehydrogenation, aminolysis and reductive amination, methanol steam reforming, water gas shift reaction, oxidation, etc. One of the important applications of copper chromites is the hydrogenation of carbonyl compounds, e.g., carboxylic acids and their esters. In the present work we studied the effect of the following parameters on the physical properties of various copper chromites applied in the hydrogenation of fatty acids and fatty acid esters: (a) preparation method including precipitation and thermal treatment of intermediates; (b) promoters like Ba, Mn, Al, Zn, etc., and (c) Cu/Cr-atomic ratio. The trial catalysts were characterized using BET-surface area, pore volume and pore size distribution, particle size determination (sedimentation analysis), DTA, XRD, XRF, SEM and other surface methods before and after their use. The activity and selectivity were investigated in the hydrogenation of saturated and unsaturated fatty acids and fatty acid esters, using autoclaves. The activities were expressed, either by conversions of fatty acids to fatty alcohols or by kinetic constant of the first order rate equation. The mentioned reactions were studied in the temperature range of 200–300 C and pressure between 100–300 bar, using various catalyst concentrations. It has been found that the thermal decomposition of basic ammonium chromates can provide catalysts with various surface areas (20–150 m^2/g) depending on the temperature and atmosphere (N_2)existing during the treatment. The BET-surface area correlates well with their activity in fatty acid esters hydrogenation (high surface, high activity). On the other hand, copper chromites possessig lower surface areas (>30 m^2/g), are much more resistant to free fatty acid and can be applied in its hydrogenation. Promoters like Ba, Mn increase the activity and especially the catalyst's life (higher reuse value). Zn-, Al- and other metals affect the selectivity of copper chromites in the hydrogenation of unsaturated fatty acid esters to unsaturated alcohols. With increasing the Cu/Cr-atomic ratio, the activity of copper chromites increases. However, the thermoresistance decreases. The first stop in the hydrogenation of fatty acids in the presence of aliphatic alcohols is the formation of corresponding esters, accelerated by acid sites (Cr^{6+}) of copper chromites. The active phase for the hydrogenation is metallic copper, deposited on $CuCr_2O_4$ or Cr_2O_3. Finally, copper chromites, poisoned by sulfur, were investigated using various methods.

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HYDROGENATION OF CLAY TREATED SOYBEAN OIL: EFFECT OF PHOSPHORUS. Douglas V. Okonek, Thomas J. Sullivan and Orest Nebesh, Harshaw/Filtrol Partnership, 23800 Mercantile Rd., Beachwood, OH 44122.

Degummed soybean oil has been treated with activated bleaching clays to improve color and remove phosphorous compounds. Hydrogenation tests were performed on oils of different phosphorous levels using a nickel catalyst at a standard set of reaction conditions. The effects of phosphorous level on hydrogenation catalyst performance have been determined. Results indicate the strong poisoning effect of increasing levels of phosphorous on nickel during hydrogenation leading to progressively slower rates of iodine value drop. Oil compositional analysis by gas chromatography was performed to evaluate the effect of phosphorous on preferential selectivity. Trans isomer contents by infrared spectroscopy were also determined. Higher phosphorous levels cause increased saturated formation and decreased trans isomer levels when comparisons are made at equal iodine values. Solid fat content determinations on hydrogenated oil demonstrate the practical effects of feedstock phosphorous levels on melting properties.

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INDUSTRIAL HYDROGENATION OF RICE BRAN OIL, A SUBSTITUTE FOR TALLOW IN SOAP MAKING. R.R. Press and K.S. Holla, The Tata Oil Mills Co. Ltd., Bombay 400 023, India.

Hard fats rich in palmitic and stearic acids are in greatest demand for soap making. Due to limited availability of animal tallows (conventional hard fat for soaps) huge tonnages were imported by India until 1983. The total ban on import of animal tallows recently imposed by the Indian government has led the Indian soap industry to look for alternate indigenous raw materials. Rice bran oil has assumed great importance in this context, with hydrogenated rice bran oil effectively replacing animal tallows as a hard fat for soap making. Much of the rice bran oil produced in India is of inedible grade. Due to the high free fatty acids (as high as 70%) and other impurities like phosphorus and sulfur compounds, hydrogenation of this oil has always been a difficult proposition. Even though process conditions have been optimized, studies on the industrial hydrogenation of rice bran oil have not been published. It is felt that such a study will help in better understanding of the process and will be of advantage for soap industries. In the present investigation, rice bran oil is hydrogenated to a tallow-like consistency on a plant scale at different pressures. The compositional changes occurring during the process and the selectivity aspects are studied using modern analytical tools like GLC and infrared spectroscopy. The distilled fatty acids derived from this hard fat can be a good substitute for tallow in toilet soaps.

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HYDROGENATION WITH A NOVEL MIXER. Lawrence M. Litz and John J. Santalone, Linde Division, Union Carbide Corp., Old Saw Mill River Rd., Tarrytown, NY 10591.

Laboratory and pilot scale hydrogenation tests of fatty acids and soybean oil have been made using the Union Carbide Advanced Gas Reactor (AGR). The AGR gave higher reaction rates at lower mixing power requirements and lower catalyst concentrations than is required in commercial converters utilizing conventional mixers. Other positive features of this new design are the elimination of a hydrogen sparger below the liquid surface and the internal recirculation of the gas in the headspace.

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EFFECT OF BLEACHING ON HYDROGENATION OF FISH OIL: RATE OF HYDROGENATION AND SELECTIVITY. Werner Zschau, Yoshiyuki Tomiyama, Sachio Koizumi, Tsunenobu Chinda and Ray Coleman, Süd-Chemie AG, Munich, West Germany.

The paper reports the influence of the rate of hydrogenation and the selectivity in dependence on the purification of Japanese fish oil by bleaching clay. Results are shown and discussed for different bleaching clays and hydrogenation catalysts. The fish oil used for the tests had an iodine value of ca. 180–185. The tests were performed in a 2-l autoclave.

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HETEROGENEOUS CATALYTIC HYDROGENATION OF CANOLA OIL USING PALLADIUM. L.L. Diosady, N. Hsu, W.F. Graydon and L.J. Rubin, Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A4.

The hydrogenation of canola oil was studied using palladium black as a potential catalyst for producing partially hydrogenated fats with low trans isomer content. Pressure (150–170 psig) appeared to have the largest effect on trans isomer formation. At 750 psig, 90 C and 560 ppm metal concentration, a maximum of 18.7% trans isomer was observed. Linolenate and linoleate selectivities were respectively 1.2 and 2.7. The maximum trans isomer observed ranged from 18.7% to 42.8% (150 psig). Temperature (30–90 C) and catalyst concentration (80–560 ppm) affected the reaction rate with little effect on trans isomer formation and selectivities. At 250 psig, and 50 C, supported palladium (5% Pd/C) appeared to be twice as active as palladium black. At 560 ppm Pd, 5% Pd/C produced 30.2% trans (IV 67.5), versus 19.0% trans for palladium black (IV 68.9). Respective linoleate selectivities were 15 and 6.6, while linolenate selectivities were approximately unity. Analysis of the filtered oil samples by neutron activation showed approximately 1 ppm Pd residue confirming a heterogeneous catalytic reaction. High catalytic activity and reusability make palladium black an attractive, hydrogenation catalyst with a potential for industrial application.

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NEW DEVELOPMENTS IN THE FILTRATION OF CATALYST FROM HYDROGENATED EDIBLE/VEGETABLE OILS AND FATS. F.G. Veldkamp, L.F.C.-Lochem B.V., Havenstraat 5.

L.F.C. Lochem is a producer of pressure filtration equipment for the edible/vegetable oil industry and since 1981 we have become involved in a number of applications for the filtration of Ni-catalyst from hydrogenated oil. We found that many new types of filter equipment were offered in this service but the actual filter media remained unchanged. It was this filter media that caused trouble in the filtration of catalyst with an accent on the discharge of spent filtercake. In relative terms nickel catalyst is the most expensive raw material purchased by a fat hydrogenator. For commercial reasons it is therefore very often re-used. This re-use causes many problems when it has to be handled in drums, and the new L.F.C. Lochem system will allow a totally enclosed re-use system that can be 100% automated. The L.F.C. Lochem test program showed that the filter media is of great importance to optimize the filtration performance, cake drop, filter aid consumption and housekeeping.

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BUSS LOOP TECHNOLOGY FOR HYDROGENATION OF FISH AND SOYBEAN OILS. D. Urosevic, Buss, Ltd., Switzerland.

In this paper will be presented the pilot plant data versus the most modern Buss production plants for hydrogenation of Japanese fish oils as well as American soybean oil. The Buss loop reactor technology/production plants and operating mode will be presented and discussed. Product variety qualities and consumption figures from batch production plants will be shown as a function of raw material specifications.

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Oxidation of Food Oils I

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CHAIN REACTIONS IN AUTOXIDATION OF FATTY ACIDS. Michael G. Simic, National Bureau of Standards, Bldg. 245, Room C216, Gaithersburg, MD 20899.

Chain reaactions of unsaturated fatty acid peroxy radicals HLOO were investigated in aqueous solutions through measurement of oxygen consumption by a Clark electrode. The concentration of fatty acids, H_2L, was kept below 0.1 mM to prevent micelization so that single, isolated, peroxy radical reactions could be investigated. Fatty acid radicals HL and H_2L-OH were initiated by OH, which in turn was generated by radiolysis. Kinetics and absorption spectra of allylic radicals were measured by pulse radiolysis. Oxygen consumption was measured as $G(-O_2)$ under steady-state conditions at low dose-rates, 5nM/s. Under these conditions for linoleic acid G(initial L) = 6, while $G(-O_2) = 21.5$ at 21 C. The chain length increases with lower dose rates and follows the $G(-O_2) = \text{const.}\ D_r^{-1/2}$ relationship. The chain length increases also with temperature, e.g. $G(-O_2) = 35$ at 37 C for linoleic acid. Some of the HLOO radicals were found to eliminate superoxide radical, HLOO. $L + O_2^- + H^+$. Implication of these measurements to oxidative damage to membranes will be discussed.

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OXIDATION OF MILK AND DAIRY LIPIDS. Tom Richardson, University of California, Davis, Department of Food Science and Technology, Davis, CA 95616.

Oxidation of lipids involves complex chemical reactions between lipids and oxygen mediated by a variety of catalysts. Active oxygen species such as singlet oxygen, superoxide anion and hydroxyl radical can be generated by various chemical and biochemical reactions to initiate lipid oxidation which can then be sustained as a chain reaction by ground state oxygen. Intermediate peroxides decompose to yield off-flavors and potentially toxic oxidation products. A wide variety of food constituents can participate in the oxidation processes to hinder or accelerate them. The fat gluble membrane (FGM) surrounding the triacylglycerol droplets in milk is a focal point for lipid oxidation. Metal ions associated with various ligands in the FGM generally act as prooxidants favoring oxidation of unsaturated fatty acid residues in membrane phospholipids. Tocopherols and carotenoids in the FGM oppose prooxidant effects of metal ions. Enzymes in FGM and aqueous phases of milk may combine to exert prooxidant effects. Thermal denaturation of enzymes might also expose prooxidants such as the ferriheme of lactoperoxidase. Constituents in the aqueous phase may markedly affect the course of lipid oxidation. Ascorbic acid and thiols can be antioxidant or prooxidant depending upon the conditions. Photo-excited riboflavin is a very potent, non-discriminating oxidizing agent. The caseins tend to exert an antioxidant effect.

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CHOLESTEROL OXIDATION. G. Maerker, Eastern Regional Research Center, USDA, ARS, 600 E. Mermaid Lane, Philadelphia, PA 19118.

Cholesterol autoxidation has been the center of renewed interest in recent years because of mounting evidence implicating several of the oxidation products in adverse health effects. Initial oxidative attack on cholesterol occurs predominantly at the 7-position. The isomeric 7-hydroperoxides and 7-hydroxides along with the 7-keto derivatives are prominent oxidation products, as are, to a lesser extent, the isomeric 5,6-epoxides and the 3,5,6-triol derived from the epoxides by hydration. Side chain oxidation products, such as the 25-hydroxy derivative, are usually not found when cholesterol is oxidized in aqueous dispersions but are important in the autoxidation of crystalline cholesterol. Oxidation products are present in most cholesterol-containing substrates in very low concentration. Their detection and determination requires a prior concentration step that enriches the oxides and frees them from the bulk of the unoxidized cholesterol and from other neutral and polar lipids. Thin layer chromatography is a good general method for the detection of cholesterol oxides. When the chromatographic plates are sprayed with aqueous sulfuric acid and heated gently many of the oxides exhibit characteristic colors that give a clue regarding their identity. High performance liquid chromatography has been used successfully to separate individual oxides. Capillary gas chromatography and GC/MS offer a sensitive means to resolve and identify individual oxides.

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STEROL OXIDATION PRODUCTS IN THERMALLY OXIDIZED FATS AND OILS. M.A. Amer, Macdonald College of McGill University, Department of Food Science and Agricultural Chemistry, Box 187, 21111 Lakeshore Rd., Ste. Anne de Bellevue, Quebec H9X 1C0 Canada, and F.M. Gharavy and D.B. Kupranycz, Macdonald College of McGill University.

The identification and quantitation of the sterol oxidation products which are formed during thermal oxidation of fats and oils was studied. Butterfat (summer and winter), solid and liquid butterfat fractions (29 C, 19 C) and selected vegetable oils (Canola, soyabean, sunflower seed and corn) were subjected to thermal oxidation at 185 C for 8 and 16 hr in the presence of air. The unsaponifiable fractions were isolated from the heated oils and analyzed by capillary column gas chromatography. The results showed that the cholesterol content was considerably reduced in all of the heated butterfat samples. The cholesterol oxidation products included 3,5-cholestadien-7-one, 4-cholesten-3-one, cholesterol-5α,6α-epoxide, cholesterol-5β,6β-epoxide, 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol. The total quantity of cholesterol oxidation products which appeared after thermal oxidation was less than the total reduction in cholesterol level. This difference was accounted for by the appearance of additional breakdown products which eluted before the cholesterol peak with the hydrocarbons and other unsaponifiable matter. The identification of the phytosterol oxidation products in the thermally oxidized vegetable oils is in progress and will also be presented.

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ANALYSIS OF VOLATILE THERMAL DECOMPOSITION PRODUCTS OF DIMERS AND OLIGOMERS FROM OXIDIZED METHYL LINOLENATE BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY. E.N. Frankel, NRRC, ARS-USDA, 1815 N. University St., Peoria, IL 61604, and W.E. Neff and E. Selke, Northern Regional Research Center, ARS-USDA.

High-molecular weight compounds were found to be significant secondary products of the autoxidation of polyunsaturated fats. The role of dimers and oligomers as flavor precursors was investigated by analyzing their volatile thermal decomposition products by capillary gas chromatography-mass spectrometry. Dimers and oligomers were isolated by gel permeation chromatography from autoxidized methyl linolenate, and from the monohydroperoxides, the hydroperoxy epidioxides and dihydroperoxides of linolenate. Structural studies showed that these oxidative dimers were cross-linked principally through peroxy bridges. The major volatile decomposition products identified from these dimers were similar to those formed from the corresponding monomers. However, the dimers produced more propanal and methyl 9-oxononanoate than the corresponding monomers, but less methyl octanoate and much less or no 2,4-heptadienal and 2,4,7-decatrienal isomers. Significant differences in minor volatile products were also observed between dimers and monomers from oxidized linolenate. These differences in volatile decomposition products are expected to have a significant flavor impact on oxidized fats containing linolenate.

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MECHANISM OF THE FORMATION OF FREE FATTY ACID DURING THE AUTOXIDATION OF OILS. Kenshiro Fujimoto, Faculty of Agriculture, Tohoku University, 1-1 Amamiyamachi-Tsutsumidori, Sendai 980, Japan; O. Furukoshi, Tohoku University, and T. Kaneda, Koriyama Women's College.

The formation of free fatty acids during the autoxidation of vegetable oils were studied. Purified corn oil was oxidized at 50 C by bubbling with air passed through a silica gel column. Long chain fatty acids ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$) and azelaaldehydic acid were found to be the major components throughout the oxidation period of 41 days. Short chain fatty acids derived from the secondary decomposition products of hydroperoxides also increased with the progress of autoxidation, but were minor components, compared with the long chain fatty acids. Although the water activity (Aw) of the atmosphere had a considerable effect on the formation of free fatty acids during autoxidation, a significant amount of free, long chain fatty acids was also produced with the progress of autoxidation, even with Aw = 0. The additive BHA in oils remarkably depressed the formation of free fatty acids. When the oil was stored in an atmosphere of $^{18}O_2$, ^{18}O was incorporated into carboxyl groups of free fatty acids. These results suggst that the hydrolysis of glycerides is an important pathway of free fatty acid formation in autoxidized oils.

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FORMATION OF SHORT CHAIN VOLATILE ORGANIC ACIDS IN THE AUTOMATED AOM METHOD. J.M. deMan, University of Guelph, Department of Food Science, Guelph, Ontario, Canada N1G 2W1, and L. deMan and Fan Tie, University of Guelph.

The end point in the automated AOM stability test for fats is related to the rapid production of volatile acids at the end of the induction period and usually measured by conductivity of an aqueous solution of the exit gases. Loury has postulated the reaction as the transitory presence of a diperoxide which decomposes into two aldehydes and formic acid. The volatile acids produced by several oils were composed mainly of formic acid and significant amounts of acetic acid. In addition acids with 3 or more carbon atoms were detected. It was found that the temperature of the water in the receiving jars was important in relation to retention of the formic acid. At temperature above 20 C significant losses may occur. The effect of temperature of oxidation on reaction rate and formic acid production has been investigated. Exposure of the oils to fluorescent light significantly reduced the AOM stability. Use of a yellow filter with a wave length cut-off of 415 nm provided partial protection against the effect of light.

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AUTOXIDATION OF ETHYL EICOSAPENTAENOATE AND DOCOSAHEXAENOATE. Soon-Yeong Cho, Tohoku University, Food Chemistry Department, Tsutsumidori Amamiyamachi 1-1, Sendai 980, Japan; K. Kiyashita, Hokkaido University; T. Miyazawa and K. Fujimoto, Tohoku University, and T. Kaneda, Koriyama Women's College.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), particularly abundant in fish oils, are currently being studied because of increasing evidence of their therapeutic value. However, they are known to oxidize readily and the resultant oxidation products are toxic to animals. Therefore, much attention is required in handling these polyenoic acids. The oxidation mechanisms of linoleic (LO) and linolenic (LN) acid have been studied extensively in the last decade, however, the details of polyenoic acid oxidation have not been resolved. This presentation deals with the comparison of oxidation ratio and patterns of oxidation products among ethyl esters of EPA, DHA, LO and LN. Sample esters were oxidized in a sealed glass cylinder with constant agitation at 4 C under light irradiation. Oxidation was followed by oxygen absorption, peroxide value, thin layer chromatography, and high pressure gel permeation chromatography. Both EPA and DHA were rapidly oxidized without a distinct induction period, and the relative oxxygen uptake (LO = 1) during a two-day period was as follows: LN 99; EPA 743; DHA 948. Hydroperoxides of polyenoic esters with more than 3 double bonds were much less table than those of LO. In LN, the major secondary products were bifunctionally oxygenated monomers, while polar materials consisting mainly of dimers were the major components of EPA and DHA. These results indicate that oxidation proceeds much faster in EPA and DHA, and the peroxide value is not necessarily a good indication of oxidation in these polyenoic acids because of the instability of their hydroperoxides.

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OXIDATION OF 1-(ALK-1-ENYL)-2,3-DIACYLGLYCEROLS: MODELS FOR PLASMALOGEN OXIDATION. Thomas A. Foglia, Edwin Nungesser and William Marmer, USDA, ARS, NAA, ERRC, 600 East Mermaid Lane, Philadelphia, PA 19118.

Alk-1-enyl ether glycerides, model compounds for plasmalogen lipids, were synthesized for use as substrates in oxidation studies. The neutral plasmalogen glycerides prepared included 1-(hexadec-1-enyl)-2,3-distearoylglycerol and 1-(hexadec-1-enyl)-2-linoleoyl-3-stearoylglycerol. Several procedures for containing and sampling auto-oxidation reactions were tried. A sealed serum vial and constant temperature bath worked best. Extent of vinyl ether and acyl lipid oxidation was monitored by following the disappearance of alkenyl and acyl residues from the glycerides. Oxidation reactions were quenched with H_2SO_4/methanol, which converted unreacted alkenyl residues to dimethylacetals and acyl residues to methyl esters. The unoxidized residues were quantitated by capillary gas chromatography. In the model glyceryl lipids, the alkenyl ether function was found to oxidize more slowly than the polyunsaturated linoleoyl acyl moiety. However, when the linolenyl group was incorporated into the 2-position of the alkenyl glyceride, as in natural plasmalogens, the rate of disappearance of the alkenyl group was comparable to the rate of loss of the linoleoyl group. Moreover, in the presence of methyl linoleate the rate of vinyl ether disappearance from 1-(hexadec-1-enyl)-2,3-distearoylglycerol also was comparable to that of linoleate. These results suggest that oxidation of plasmalogen glycerides should not be ignored as a factor that contributes to the oxidative instability of animal tissue.

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α-TOCOPHEROL OXIDATION MEDIATED BY SUPEROXIDE ANION (O_2^-). A. Saari Csallany and Yeong L. Ha, University of Minnesota, Department of Food Science and Nutrition, 1334 Eckles Ave., St. Paul, MN 55108.

The reactions of O_2^- and O_2^- derived oxygen species with α-tocopherol were investigated. O_2^- was generated by the electrochemical reduction of molecular oxygen in anhydrous acetonitrile, containing tetrabutylammonium bromide as an electrolyte. The basal reaction mixture contained 3.3×10^{-4}M α-tocopherol and 2.0×10^{-6}M O_2^-. The decomposition of O_2^- was achieved by the addition of 10% water to the reaction mixture to produce mostly hydroxyl free radicals. The effect of H_2O_2 (2.0×10^{-3}M) on the decomposition of O_2^- in the presence of water was also investigated. Disappearance of α-tocopherol and the appearance of the oxidation products in the above reactions were measured by a newly developed high performance liquid chromatographic method. Oxidation of α-tocopherol by O_2^-, in the absence of water, produced an unstable intermediate which decomposed into two stabile tocopherol oxidation products. An attempt was made to characterize these compounds by UV, MS, IR and NMR spectroscopy. Oxidation of α-tocopherol with water and H_2O_2 dismutated O_2^-, resulting in the following oxidation products: α-tocopherol quinone, α-tocopheryl quinone oxide, dimer and dihydroxy dimer. Results indicate different types of oxidation of α-tocopherol in the presence of O_2^- or hydroxy free radicals which are produced by O_2^- dismutation.

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Oxidation of Food Oils II

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PROTEIN DAMAGE RESULTING FROM EXPOSURE TO OXIDIZING LIPIDS. John W. Finley and Gareth Templeman, Nabisco Brands Inc., East Hanover Tech Center, DeForest Ave., East Hanover, NJ 07935.

Processing, storage, distribution and final preparation of foods can result in conditions which are conducive to lipid oxidation as well as other process related chemical reactions. Oxidizing lipids can cause secondary reactions which impact food product quality significantly. Oxidizing lipids cause changes in sulfur amino acids which can negatively influence the nutritional quality of the product. Tyrosine and tryptophan are also directly effected by oxidizing lipids. Secondary losses in lysine, histidine and arginine are observed because the aldehydes products of lipid oxidation form additional reaction products with the side chains of these amino acids. In addition to these changes, flavor and color can be effected because lipid peroxides oxidize normal Maillard reaction products in proteins which contribute color and flavor to food products. These reactions will be reviewed and their nutritional, toxicological and aesthetic significance discussed.

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FLUORESCENT PRODUCTS DERIVED FROM THE REACTION OF ADENINE, METHYL LINOLEATE HYDROPEROXIDES, Fe(II), AND ASCORBIC ACID. Kiyosi Hasegawa, Tohoku University, Food Chemistry Department, Tsutsumidori Amamiyamichi 1-1, Sendai 980, Japan; Kenshiro Fujimoto, Tohoku University; Takashi Kaneda, Koriyama Women's College, and W.E Neff and E.N. Frankel, Northern Regional Research Center.

Lipid oxidation products have been known to fluoresce in reaction with DNA. Recently it was found that the intensity of fluorescence is remarkably enhanced by the addition of reducing agents and metals to the reaction mixtures. Therefore the involvement of some secondary oxidation products derived from the reaction of lipid hydroperoxides with reducing agents and metals was suggested. To elucidate the character of the secondary oxidation products responsible for the fluorescence, we investigated the interaction of methyl linoleate hydroperoxides with reducing agents and metals was suggested. To elucidate the character of the secondary oxidation products responsible for the fluorescence, we investigated the interaction of methyl linoleate hydroperoxides with DNA, nucleosides, or bases contained in DNA in the presence of ascorbic acid and Fe(II). In the pH range 4.0 to 9.0, pH did not effect the intensity or spectrum of the fluorescence. In the reaction of methyl linoleate hydroperoxides with the nucleosides, only adenosine produced significant fluorescence, while the other nucleosides (guanosine, cytidine, and thymidine) produced very little fluorescence under the same conditions. Adenine also produced similar fluorescence. These results suggest that amino group at the 6-position of adenine reacted preferentially with the lipid oxidation products to produce fluorescence. The fluorescent substances formed in the reaction of methyl linoleate hydroperoxides with adenine were soluble in most organic solvents such as chloroform, and consisted of at least four major components on TLC. On reverse-phase HPLC using a fluorescent detector, four peaks all showing an excitation maxima at 325nm, an emission maxima at 410nm together with an ultraviolet absorption maxima at 260-270nm were isolated. ^{1}H NMR and IR spectrum of the main fluorescence constitute suggested the presence of moieties both derived from methyl linoleate hydroperoxides and adenine such as ester(s) and hydrocarbon chain(s). However, no aldehyde group was recognized. The detailed structure will be discussed on the bases of spectroscopic data.

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CHOLESTEROL, CHOLESTEROL OXIDES AND ATHEROSCLEROSIS. Paul B. Addis, Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108.

Researchers of atherosclerosis have reported contrasting findings with regard to the potential roles of cholesterol and its autoxidation products in atherogenesis. However, much evidence suggests that autoxidation products of cholesterol are more active than cholesterol itself in the initiation and promotion of atherosclerosis. This report will review relevant literature concerning the question of the relative capacities of cholesterol and its oxidation products to participate in the atherosclerosis process. In addition, it will review literature on the content of cholesterol oxides in foods, including our positive identification of cholesterol oxides in dehydrated foods or fats processed at high temperatures. Finally, suggestions will be made for further experimentation on the toxicities of cholesterol and its autoxidation products based on our new knowledge of the content of cholesterol oxides in foods.

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DISTRIBUTION OF ^{14}C AFTER ORAL ADMINISTRATION OF (U-^{14}C)-LABELED METHYL LINOLEATE HYDROPEROXIDES AND THEIR SECONDARY OXIDATION PRODUCTS IN RATS. Motoko Orada, Tohoku University, Food Chemistry Department, Tsutsumidori Amamiyamachi 1-1, Sendai 980, Japan; Teruo Miyazawa and Kenshiro Fujimoto, Tohoku University, and, Takashi Kaneda, Koriyama Women's College.

Lipid oxidation products are known to be toxic to animals and it has been suggested that the low molecular weight (LMW) fussion products are more toxic than monohydroperoxide, the primary product of autoxidation. In the present paper, the metabolic fate of LMW compounds were studied in comparison with other major oxidation products i.e., methyl linoleate hydroperoxides and polymers. oxidation products prepared by further oxidation of methyl [u-^{14}C]-linoleate hydroperoxides (MLHPO) were orally administered to rats, and their radioactive distributions in tissues and excretions were compared. The polymeric fraction consisted mainly of dimers of MLHPO. In the LMW fraction, 4-hydroxy-2-nonenal, 8-hydroxy methyl octanoate and 10-formyl methyl-9-decenoate were identified as major constituents by gas chromatography-mass spectrometry. When LMW compounds were administered to rats, the excreted radioactivity in expired gas and urine in 12 hr was significantly higher than in rats administered polymers or MLHPO. Maximum $^{14}CO_2$ expiration appeared 2-4 hr after administration of the LMW compounds. Radioactivity of the upper part of the small intestines six hr after administration of the LMW compounds was higher than the values from administered polymers or MLHPO. The remaining radioactivity in the digestive contents and feces 12 hr after administration of LMW compounds was much lower than the values observed for administered polymers or MLHPO. Among the internal organs, the liver contained the highest concentration of radioactivity in all three groups. The highest level of radioactivity was found in liver 6 hr after the administration of LMW compounds. The levels in kidney, brain, heart and lung followed. These results suggest that among various lipid oxidation products, the LMW compounds are preferentially absorbed and transported in rat tissues where damage to biological systems occurs.

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DISCRIMINATION BETWEEN OXIDATION AND MAILLARD BROWNING BY MEANS OF PHOSPHOLIPID-DERIVED FLUORESCENCE IN MODEL SYSTEMS AND FOODS. William L. Porter, U.S. Army Natick R&D Center, Kansas Street, Natick, MA 01760-5020, and E.D. Black, Y-K. Kim, L. Hoke and J.G. Kapsalis, U.S. Army Natick R&D Center.

Energy-dense combat ration model systems containing lactose,

casein and stripped corn oil encapsulated in acetone-stripped soy lecithin have been monitored during storage at high temperatures for polymerization by means of chemical and spectrophotometric methods, including both adsorption and fluorescence. The objective was to develope rapid, labor-saving methods applicable to both soluble and heavily cross-linked, insoluble components of the heated model system. Systems to monitor sugar-amine (Maillard) browning include front-face fluorescence of acid-precipitated casein slurries, solution fluorescence and 410 nm absorption of the aqueous phase after pronase digest, and low intensity fluorescence of the chloroform-methanol extract of the browned material. Lipid oxidation methods developed include vapor phase detection by means of polyamide plate fluorescence in presence of oxidizing lipid and high intensity fluorescence of the chloroform-methanol extract. Excitation and emission wavelengths and intensity of the Maillard-derived fluorescence are much lower than those derived from lipid oxidation, although both are largely due to condensation products with amine-containing phospholipids. These differences and the capability for vapor phase detection of lipid oxidation by polyamide fluorescence permit separation of the two degradative processes. The chloroform-methanol method in both lipid oxidation and Maillard browning and the polyamide method in lipid oxidation are relatively free from interference arising from common food components. They are potentially widely applicable and susceptible to semi-automation.

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STUDY ON THE ANTIOXIDATIVE ACTIVITY OF 1,3-DIOXAINDAN DERIVATIVES. Tsugio Isobe and Hajime Seino, School of Hygienic Sciences, Kitasato University, 1-15-1 Kitasato Sagamihara-shi Kanagawa 228 Japan.

Sesamol has an excellent antioxidative activity. It also shows antimicrobial activities against some bacteria and fungi. These activities are considered to be caused by the phenolic hydroxyl group in its molecule, however, the methylenedioxy group is also supposed to have some influence on these activities. The authors have been studying this function by synthesizing compounds which have an alkylidenedioxy group and determining the antioxidant activities. In this report, 1,3-dioxaindan derivatives were synthesized by the condensation of catechol with several ketones and the antioxidative activities of these compounds were determined. In these compounds, 2-methyl-2-tert-butyl-1,3-dioxaindan showed high potency to prevent the autoxidation of lard although it has no phenolic hydroxyl group. Other 1,3-dioxaindan derivatives also showed antioxidative activities. Electron spin resonance measurements were also performed for the radicals orginated from these compounds by oxidizing them with lead oxide.

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STUDIES ON THE OXIDATION OF ANIMAL FATS. Allen J. St. Angelo and John R. Vercellotti, USDA-ARS-SRRC-New Oleans, LA, and Harold P. Dupuy, V.P.I., Blacksburg, VA.

The term "warmed-over flavor," or WOF, has been described as the rapid development of oxidized flavor in refrigerated cooked meats, in which a rancid or stale flavor becomes apparent within 48 hours at 40 C. (Tims and Watts, Food Technol. 1958). Whereas this original definition has been with us well into the third decade, there is some evidence accumulating that suggests reactions other than lipid oxidation type are involved in the formation of WOF. This presentation will discuss lipid oxidation reactions from the native fat in beef and poultry and chemical changes found in carbohydrate and amino acid precursors. A new method to isolate volatile flavor compounds for assessment of meat quality will also be discussed.

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NEW ASPECTS OF ANTIOXIDANTS FROM UNROASTED AND ROASTED SESAME SEED OILS. Yasuko Fukuda, Ichimura Gakuen Junior College, Uchikubo 61-1, Inuyama-shi, Aichi 484, Japan; Masauyasu Nagata and Toshihiko Osawa, Nagoya University, and Tatsuhiko Ozaki, Takemoto Oil and Fat Co., Ltd.

Sesame oil, usually roasted seed oil, has been preferred in the Far East because of its good roasted flavor and stability to oxidation and believed to have some medical benefits. But the antioxidative factor of the oil has not been investigated yet. Our investigation using HPLC analysis showed that the oil has considerable amount of tocopherol and small amount of sesamol. Furthermore, we demonstrated that a significant amount of sesamol (max. 0.12% in oil) was produced from sesamolin (a kind of lignan in sesame seed) during frying at over 160 C, thus increasing the stability of the oil and the fried foods. On the other hand, unroasted seed oil being commonly used in the world since ancient time has also been known to be resistant to oxidation but the reason of stability has remained obscure. This investigation showed that a main antioxidant in the oil was P3, which was the strongest one of newly identified lignan type antioxidants in the sesame seed. It was found that large amount of P3 in the oil was formed during the industrial bleaching process from sesamolin.

Session LL Saturday afternoon
Marine Oils

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LIPID PRODUCTION IN A MARINE ALGA USING CAGE CULTURE TURBIDOSTATS FOR CONTINUOUS AUTOMATED CULTURE WITH LIPID CLASS MEASUREMENT BY THE CHROMAROD-IATROSCAN (TLC/FID) SYSTEM. Christopher C. Parrish, Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada; Peter J. Wangersky, Department of Oceanography, Dalhousie University, and Robert G. Ackman, Canadian Institute of Fisheries Technology, Technical University of Nova Scotia.

Lipid production in stressed algal cultures is an almost virgin field for both pure and applied research. An understanding of factors affecting lipid production by algae in the laboratory should give insights in to the dynamics of the production of compounds with high calorific value at the base of food-webs in the oceans. This information could also be used to mamipulate the lipid content of algae grown in large scale cultures to provide optimal lipid production for commercial applications. Our analytical technology provides a unique approach to the detailed examination of lipid production by cultured algae. A new method for growing and studying algae has been used: the cage culture turbidostat; and a new analytical technique has been used for lipid class measurement: the Chromarod-Iatroscan (TLC/FID) system. In this study, the marine diatom, *Phaeodactylum tricornutum*, was grown with various supply rates of inorganic nutrients. Up to three culturing units were supplied with medium of known nutrient simultaneously, and the intracellular lipid content and the lipid content in the effluent media were monitored. It was found that the intracellular synthesis of a storage component, triglyceride, was clearly triggered by nitrogen-stress, while the synthesis of the membrane-associated polar lipid classes was reduced under these conditions. Differences were also found in the production rate of extracellular lipid classes: both the amounts and types of lipids produced nutrient-stressed and nutrient-replete conditions were significantly different.

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CIS-5-OLEFINIC UNUSUAL FATTY ACIDS AS COMMON LIPID COMPONENTS OF ECHINOIDEA FROM JAPAN WATERS. Toru Takagi, Department of Chemistry, Faculty of

Fisheries, Hokkaido University, Minato-cho, Hakodate, Japan; Masaki Kaneniwa and Yutaka Itabashi, Hokkaido University, and R.G. Ackman, Canadian Institute of Fisheries Technology, Technical University of Nova Scotia.

Open-tubular GLC analyses of fatty acids of the lipids from the whole animals of 12 species of Echinoidea collected on the Pacific coast of Japan showed that they all contained fatty acids having an isolated *cis*-5-olefinic bond (totalcontents 5.9-21.1%, mean 12.2% in the total fatty acids) similar to the composition reported for the Atlantic sea urchins by us in 1980. The 5-olefinic acids found were *cis*-5-eicosenoic acid (5-20:1), all-*cis*-5,11- and 5,13-eicosadienoic acids (5,11- and 5,13-20:2) as the major components, and 5-14:1, 5-16:1, 5-18:1, 5,11,14-20:3, and 5,11,14,17,-20:4 were undertaken by GLC and GC-MS of the ozonolysis products. ^{13}C-NMR analyses of the fatty acid fractions supported the occurrence of the *cis*-5-olefinic acids, but have not shown the presence of an isolated *trans*-5-olefinic bond, and *cis*- or *trans*-2- to 4- olefinic bonds in them. The contents of the 5-olefinic acids in the fatty acids of the polar lipids (PL) were generally higher than those in the neutral lipids. The 5-olefinic acid contents in the fatty acids from the egg PL were higher than those of the total lipids from the whole animals. The functions and origins of the 5-olefinic acids in the Echinoidea are discussed.

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METABOLISM OF WAX ESTERS IN FISH. Mitsumasa Mankura, Research Institute, Ikeda Tohka Industries Co., Ltd., 97 Minooki-cho, Fukuyama-shi, Hiroshima-ken, Japan 720, and Mitsu Kayama, Laboratory of Marine Biochemistry, Hiroshima University.

Hepatopancrease homogenates of carp, *Cyprinus carpio*, contained enzymes involved in the hydrolysis and esterfication of wax esters (WE). Synthesis of WE with 10,000 × g supernatant showed the optimal activities between 30-40 C and at pH 4-5. Although WE syntheses from fatty acids were promoted by the addition of fatty alcohol up to the concentration of 10 mM, the incorporation into phospholipids was inhibited by the addition of fattya alcohols. Fatty acid moieties of phosphatidylcholine (PC) and triacylglycerols (TG) were also incorporated into WE. The enzymes were salted out by 30% saturation with ammonium sulfate. This fraction also contined the highest activities of lipase and esterase. The anzyme for WE hydrolysis, together with lipase and sterase, was eluted in tube nubmers 20 to 22 (void volume) from a Sephadex G-200 column. On the other hand, the enzyme for WE synthesis was eluted in tube nubmers 33-35. It thus appears that the two activities belong to different enzyme proteins. The synthetic enzyme was activated by the addition of APT, CoA, and NADH. When the hydrolysis and synthesis of WE by the anzyme preparation from that the copepods have rather higher activity of WE hydrolysis and a relatively high value of synthesis, while the carp hepatopancreas shows higher values n lipase and esterase activities. Furthermore, force-fed WE ([1-^{14}C]cetyl alcohol) were also oxidized in intestine, and they behaved like the dietary fatty acids. The fates of dietary WE and fatty alcohols are discussed.

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EFFECTS OF DIFFERENT COOKING METHODS ON LIPID COMPOSITION OF FISH FILLETS. Yoshimi Ohno and Kumiko Fujii, Sanyo Gakuen Junior College, 1-14-1, Hirai, Okayama-city, Okayama Pref., Japan.

The effects of cooking by boiling, steaming and baking on the lipid content, cholesterols, phospholipids and fatty acid composition of five sea fishes (Spanish mackerel, Japanese sea bass, horse mackerel, sand borer and flying fish) were determined. A moisture loss resulted by cooking in all cases. The lipid content and amounts of total cholesterols and total phospholipids were not significantly changed by ten or 15 minutes cooking in all species of fish examined. There was a significant decrease in chloesterol and phospholipid contents of horse mackerel in 60 minutes cooking especially by steaming and baking rather than by boiling. Major fatty acids of the total lipids were C16:0, C16:1, C18:0, C18:1, C20:5 and C22:6. The concentration of polyenes varied with species of fish; however, cooking for 10 or 15 minutes did not significantly affect the fatty acid composition. Fatty acid composition of horse mackerel was changed by 60 minutes cooking. There seemed to be a decrease in polyenes and a little increase in monoenes by three different methods of cooking.

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COMPARISON OF SELECTED LIPIDS FROM TESTES OF IMMATURE HARP SEALS (*PHOCA GROENLANDICA*), MATURE AND IMMATURE GREY SEALS (*HALICHOERUS GRYPUS*), MATURE WALRUS (*ODOBENUS ROSMARUS*) AND MATURE BELUGA (*DELPHINAPTERUS LEUCAS*). M. Yurkowski, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba.

The testes of mature walrus, beluga and sexually-active grey seals (1.6-1.9% wet) contained less lipid than the immature harp (10-14 days old) and grey (1-4 days old) seal pups (2.9-8.2%). Sterol ester (SE) was a major neutral lipid in all testes except those of mature grey seal. The levels of triglycerides (TG), sterols and diglycerides were similar in all testes, but the levels of free fatty acids and monoglycerides were higher in the walruses and beluga testes. Mature grey seal testes had the highest level of phospholipids (PPL). Significant differences in the levels of various positional isomers of 16:1, 18:1, 20:1 and 22:1 in CE, TG and PPL from testes suggest that these animals consumed different diets. Small amounts of 24:4ω6, 24:5ω6, 24:5ω3 and 24:6ω3 were tentatively identified in CE, TG and PPL. The TG and CE of mature grey seal testes contained more ω6 acids and 22:5ω6 and less ω3 acids, and the PPL contained more 22:5ω6 and 22:6ω3 and less ω3 acids, 20:5ω3 and 22:5ω3, than the immature testes. The differences in the levels of these fatty acids in testes of all mammals studied may be linked to differences in their diets and reproductive status. These marine mammals tend to retain high levels of ω acids in their testes even though their natural diets usually contain higher levels of ω3 that ω6 acids.

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SLIME-ASSOCIATED LIPID-DERIVED VOLATILE AROMA COMPOUNDS RELATED TO THE LIFE CYCLE STAGES OF SALMON (*ONCORHYNCHUS SP.*). David B. Josephson, Department of Food Science, University of Wisconsin-Madison, 1605 Linden Drive, Madison, WI 53706, and Robert C. Lindsay and David A. Stuiber, Department of Food Science, University of Wisconsin-Madison.

Lipid-derived volatile aroma compounds from several species of freshly harvested salmon from the Pacific Ocean and the Great Lakes were quantitatively determined at various stages of the life cycle. During each of the stages of the life cycle, all of the salmon that were analyzed possessed eight carbon alcohols and carbonyls which contributed distinct plant-like aromas to the fish. Spawning-condition salmon in freshwater environments additionally possessed a group of nine-carbon alcohols and carbonyls that added cucumber-, or melon-like odor notes. Viewing the measurement of these enzymically-derived volatile compounds as a probe for biochemical and physiological processes provided a basis for the development of a hypothesis implicating leukotriene-related biochemistry in the regulation of mucus secretion by salmon and the biogenesis of the nine-carbon volatile aroma compounds as well as two 1,3,5-octatriene isomers.

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PROSTAGLANDIN SYNTHESIS IN FISH THROMBOCYTE. Mitsu Kayama, Laboratory of Marine Biochemistry, Faculty of Applied Biological Science, Hiroshima University, 2-17 Midori-

machi, Fukuyama-shi, Hiroshima-ken, Japan 720, and, Tetsuya Sado, Applied REsearch Laboratories I, Lion Corporation.

It is well known that mammalian platelets convert arachidonic acid (AA) to prostaglandins (PGs), especially to thromboxane A_2 (TXA_2), which is a potent inducer for blood aggregation. Although thrombocytes are platelets, the syntheses of PGs in fish thrombocytes still remain unclear. Therefore, the aim of present study is to clarify the capability of PG synthesis in washed carp and rainbow trout thrombocytes, and to compare with that in the washed human platelets. Potential precursors for PG synthesis in fish thrombocyte fractions were measured by a gas-liquid chromatography. The phospholipid fraction of docosahexanenoic acid (DHA) in the percentage of 9.9, 4.6, and 20.2, respectively. Phospholipid fraction of rainbow trout thrombocytes consisted of AA, EPA, and DHA in 6.4%, 4.1%, and 23.4%, respectively. The ^{14}C-labelled substrates ere examined for their conversion capability to PGs. DHA was not converted to PGs with any fish thrombocytes so far. Washed rainbow trout thrombocytes converted mainly AA to TXB_2 similar to washed human platelets. However, washed carp thrombocytes did not convert AA to TXB_2, but to PGE_2, $PGF_2\alpha$, and PGD_2. Washed human platelets converted EPA to TXB_3 with difficulty and washed carp thrombocytes were also slow to convert EPA to PGs. On the other hand, washed rainbow trout thrombocytes converted EPA to TXB_3. However, addition of AA to washed carp thrombocytes induced conversion of EPA to PGE_3, $PGF_3\alpha$, and PGD_3. Thrombocyte aggregation responses were recorded by using an electric aggregometer. Aggregation was inhibited with the addition of exogenous EPA or DHA in each gish thrombocytes. The addition of AA produced irreversible aggregation in rainbow trout thrombocytes similar to human platelets. However, irreversible aggregation response of carp thrombocyte was not induced by the addition of AA.

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FATTY ACID COMPOSITION OF COMMONLY CONSUMED MARINE AND LAND MAMMAL FATS, AND OF RED CELL PHOSPHOLIPIDS AND BREAST MILK FROM INUIT LIVING ON BAFFIN ISLAND. Sheila M. Innis, University of British Columbia, Department of Pediatrics, The Research Center, 950 West 28th Avenue, VAncouver, B.C. Canada V5Z 4H4; Harriet V. Kuhnlein, McGill University, and David Kinlock, Health and Welfare Canada.

High dietary intakes of ω-3 fatty acids have been shown to produce a number of important physiological effects related to the fatty acid composition and function of structural phospholipids and to prostaglandin pathways. Most work to date has emphasized fish as a source of ω-3 fatty acids and in experimental studies fish oil concentrates have been used to demonstrate, for e.g., alterations of platelet fatty acids and platelet function. In many northern Inuit groups marine mammals contribute a greater percentage to the daily fat and protein intake than fish, however, little information is available on their lipid composition. In September 1985, samples of blubber and muktuk from five species of marine mammal, and of adipose tissue from caribou and polar bear were obtained with the assistance of a small community of Inuit on Baffin Island. No fish were caught during this month. At the same time, blood samples were given by 185 of the Inuit aged one month to 76 years. Three samples of breast milk were given by woment in their second month of lactation. Comparable samples of blood and breast milk were obtained from Vancouver residents. Red cell membrane phospholipids were purified and separated into phosphatidylcholine, phosphatidylserine+-inositol, phosphatidyl-ethanolamine and shpingomyelin by TLC. Their acid composition, together with that of breast milk and animal fat were determined by capillary column GLC. Marine mammals had a predominance of monoenoic (16:1, 18:1 and 20:1) and 22:5 and 22:6 ω-3 fatty acids. In comparison to data for Northern fish these mammals were higher 16:1 (24%), low in 22:1, and contained higher 22:5ω3. The composition of polar bear fat was similar with 16%, 24% and 18% fatty acids as 16:1, 18:1 and 20:1, 8% each 22:5ω-3 and 22:6ω3. Caribou fat was more typical of a land mammal, being rech in 16:0, 18:0 and 18:1 and with all other fatty acids < 2%. In comparison to Vancouver samples Inuit milk had three × more 16:1, 10 » more of 20:5ω3 and of 22:6ω, 5 × 2:5ω3 and 33% less 18:1. Levels of all ω-6 fatty acids were reduced. Inuit red cell phospholipids showed a similar diet influence with increased long chain ω-3 fatty acids. Substantial quantities of ω-3 fatty acids are thus found throughout food-chain of traditional Northern foods ranging from fish and mraine mammals, to land mammals consuming a based-marine diet and to the human population and the milk produced by them for their own young. The cooperative help of the Inuit who participated in these studies must be recognized for without this these data could not have been collected.

Session MM Saturday morning Symposium on Oilseed Proteins-Breeding for Protein

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BREEDING FOR SEED PROTEIN CONTENT IN SOYBEANS. James R. Wilcox, USDA-ARS, Department of Agronomy, Purdue University, W. Lafayette, IN 47907.

Currently grown soybean cultivars average 40% protein in the seed. This value has remained relatively constant during 50 years of genetic improvement in soybeans. Production of soybean protein has increased due to increased seed yields rather than to increased seed protein content. Accession in the U. S. germplasm collection contain as much as 52 percent protein in the seed but are agronomically poor and very low in seed yield. The strong inverse relationship between oil and protein in the seed has been one deterrent to producing high protein cultivars. The inverse relationship between seed yield and seed protein content has prevented the development of high yielding cultivars with seed protein content in excess of 45 percent. Economic incentives to compensate for low seed yields would be needed to stimulate production of cultivars with high seed protein content.

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INHERITANCE OF LIPOXYGENASE ISOZYMES IN SOYBEAN SEEDS. Keisuke Kitamura and Akio Kikuchi, Faculty of Agriculture, Iwate University Morioka, Japan 020.

The lipoxygenase actions that generate grassy beany flavors are significant problems in the effective use of soybean seeds for human foods. Normal soybean seeds contain three lipoxygenase isozymes, called L-1, L-2 and L-3. Recently, three types of lipoxygenase lacking mutants, L-1-less, L-2-less, and L-3-less soybeans were found. Genetic studies have demonstrated that the absence of L-1, L-2 and L-3 from the seeds are under the control of single recessive alleles, lx_1, lx_2, lx_3, respectively. So far, two types of double mutant soybeans were identified to lack both L-1 and L-3, and both L-2 and L-3, respectively. F_2 seeds from the cross the L-1 L-3-less line X PI 86023(L-2-less) were examined for the presence or absence of the isozymes by the improved SDS-PAGE. The results showed that the Lx_3 locus is independent of Lx_1 and Lx_2 loci, respectively. The genetic analyses of F_2 and F_3 seeds from the cross the L-1-less X the L-2-less lines revealed that the Lx_1 locus tightly links with the Lx_2 locus. There was no significant difference in the activity levels of lead lipoxygenase(s) among Suzuyutaka (normal), and the near isolines lacking L-1, L-2 and L-3, respectively.

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QUANTITATIVE EXPRESSION OF BEAN SEED PROTEINS. Fredrick A. Bliss, Department of Horticulture, University of

Wisconsin-Madison.

The quantities of constituent seed proteins of common bean (*Phaseolus vulgaris* L.) contribute to both the relative amount (percentage) and the quality of protein. Skillful manipulation of genes that control the qualitative and quantitative expression of these proteins provides an opportunity for directed improvement of protein traits while increasing or at least maintaining yield levels of commercial cultivars. Phaseolin is the primary globulin seed storage protein of bean. Although there are a limited number of phaseolin electrophoretic variants among cultivars, extensive variation is present in the wild germplasm. Quantitative expression is controlled not only by non genetic factors and genes with small effects, but also mutant genes that show large direct or indirect effects on phaseolin. Presence or absence of seed lectins, controlled by a single gene, affects phaseolin content per seed by up to 15%, while different alleles at the lectin locus show smaller effects. The presence of arcelin, a novel protein, first found in wild beans and controlled by a multiple allele series, results in decreases in phaseolin of up to 60%. A mutant gene transferred from *P. coccincus* to *P. vulgaris*, results in the lack of any imunologically-detectable phaseolin. The introduction of these genes into horticulturally useful stocks allow quantitative and qualitative manipulation of phaseolin with concomitant increases in amino acid constant percentage total protein and total protein per hectase.

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BIOGENESIS OF PROTEIN BODIES IN MATURING PUMPKIN COTYLEDONS. Mikio Nishimura, Ikuko Hara-Nishimura, Makoto Hayashi and Takashi Akazawa, Research Institute for Biochemical Regulation, School of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan.

Numerous investigations have been carried out to reveal the biosynthesis and intracellular transport of storage proteins. However, there still exist missing links concerning the biogenesis of protein bodies where the storage proteins are localized, although electron microscopic observation indicate that rER or vacuoles play a role in the biogenesis. In order to disclose the functional role of vacuoles on the biogenesis of protein bodies, we isolated the vacuoles from three developmental stages of pumpkin cotyledons, harvested at 14- (early stage), 22- (middle stage) and 30- (late stage) day after anthesis. Isolated vacuoles were confirmed to be free from contamination by other organelles. A large amount of storage protein, 11S globulin, was present in the vacuoles in every stage. A light microscopic inspection showed that one to three crystalloids of 11S globulin appeared in a single vacuole isolated from the middle stage of maturing cotyledons and some big crystalloids (ϕ =4 μm) nearly identical in size to the matured crystalloids are discerned to be budding from vacuoles. Overall results strongly indicate that preprotein bodies containing a single crystalloid bud from vacuoles, give rise to protein bodies during the later stage of seed maturation.

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THE STRUCTURE AND EXPRESSION OF GLYCININ GENES FROM SOYBEAN. Niels C. Nielsen, USDA-ARS, Department of Agronomy-Lilly Hall, Purdue University, West Lafayette, IN 47907, and Liliane Floener, R. Paul Evans, Bernard J. Scallon, Craig Dickinson and Tae Ju Cho, Purdue University.

The glycinin subunits are encoded by a family of at least five homologous genes, designated Gy1, Gy2, Gy3, Gy4, and Gy5. This family is divided into two group on the basis of nucleotide sequence homology and subunit primary structure. Group-I genes (Gy1, Gy2, Gy3) have been sequenced and compared to the sequence for Group-II genes (Gy3, Gy4). Although common structural features are conserved (eg., placement of the three introns, typical eucaryote transcription control sequences, etc.) the size of the introns and the DNA sequence of the 5' and 3' non-coding regions vary considerably. Exons-1, -2 and -4 of the genes are highly conserved whereas exon-3 is quite variable. Homology between proteins from genes within the same group is high (80–90%), but is low (40–50%) for between group comparisons. Computer alignment of soybean and other legume 7 and 11 S storage protein primary sequences has revealed that they have extensive regions of secondary structure preferences in common, as well as a hypervariable region. Alteration of the coding sequence in this hypervariable region may have minimal detrimental effect on the structure of the proteins, and could be useful in efforts to manipulate seed quality. Experiments designed to begin exploring the effect of structural alterations in this region will be described.

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EXPRESSION OF SOYBEAN SEED PROTEIN GENES IN TRANSFORMED TOBACCO PLANTS. Robert B. Goldberg, Department of Biology, University of California, Los Angeles, CA 90024.

Soybean seed protein genes were transferred to tobacco plants using a Ti-plasmid vector. Regenerated plants contained one copy of each seed protein gene and the genes were inherited in a simple Mendelian pattern. The seed protein genes were expressed in transformed tobacco plants similar to that which occurs in soybean. For example, the β-conglycinin storage protein gene was only expressed during tobacco seed development while the seed lectin gene was expressed in developing seeds and in the mature tobacco root. Each seed protein gene was temporally regulated during tobacco seed development. Seed protein mRNAs increased and decreased during seed development; however, the time of these events followed physiological processes inherent in tobacco seed development. Together, these data show that soybean seed protein genes are correctly regulated in transformed plants and that sequences controlling their expression are recognized by regulatory factors present in tobacco cells.

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THE ACCUMULATION SITES AND MECHANISMS OF RICE STORAGE PROTEINS. Kunisuke Tanaka, Department of Agricultural Chemistry, College of Agriculture, Kyoto Prefectural University, Shimogamo Sakyoku, Kyoto 606, Japan; Masahiro Ogawa, Kyoto University, and Hiroshi Yamagata, Osaka Medical College.

Three kinds of proteinaceous particles are observed in rice endosperm. Aleuron grain(AG) in aleurone cells contains alubumin and globulin together with phytin. Protein body Type I(PB-I) and II(PB-II) in the starch endosperm contain prolamin(13 kDa polypeptide) and glutelin(22–23 and 37–39 kDa polypeptides), respectively. In the formation of AG, some proplastid-like structure is concerned. PB-I is formed from endoplasmic reticulum membrane and prolamin is synthesized by polysomes attached on the surface. PB-II originates from vacuole. Glutelin is synthesized on the rough endoplasmic reticulum as a precursor, then transferred to PB-II via Golgi body. During the transfer, the precursor is split into mature glutelin subunits. Some rice mutants show remarked changes in the prolamin and/or glutelin contents. Those mutants are useful for the investigation of the accumulation mechanisms of rice storage proteins.

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FACTORS CONTROLLING THERMAL GELATION AND GEL PROPERTIES OF SOYBEAN 11S GLOBULIN. Tomohiko Mori, Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan; Shigeru Utsumi, Kyoto University and Takashi Nakamura, Hohnen Oil Co. Ltd., Japan

The gel-forming ability as well as nutritional properties of soybean proteins are responsible for their quality. We report here some aspects on the relationships between thermal gelation and gel properties and protein-chemical properties of soybean 11S

globulin (glycinin). The thermal gelling process consists of three stages, i.e., aggregation of glycinin molecules to form strands, association of strands to form a gel network, and increase of gel hardness due to maturing of the intermolecular bondings within the strands of the gel network. The first stage requires 100 C of heating temperature, while the later stages proceed at below 100 C down to 80 C. The acidic subunit AS-IV, which is linked with a basic subunit to form an intermediary subunit through some bonds other than disulfide bonds, contributes to the rate of gelation. AS-III, which is the largest constituent acidic subunit, causes a significant increase in the hardness of the gel through affecting the elaborateness of the gel networks. The turbidity of the gels exhibits a tendency to increase with increasing content of sulfhydryl groups of glycinin.

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TRENDS AND PROSPECTS IN THE SURFACTANT INDUSTRY IN JAPAN. Fumikatsu Tokiwa, Kao Corporation, 14-10, Nihonbashi Kayabacho 1-chome, Chuo-ku, Tokyo 103, Japan.

In 1984, Japanese surfactant shipments for industrial uses reached 600,000 metric tons, worth 170 billion yen, at an annual growth rate of 3% during the last 5 years. Though losing its relative share, the textile industry remains the largest single outlet at 31% of the total. Few surface-active compounds of innovative features have appeared in recent years. However, developments in application technology of surfactants are seen in reflection of a changing marketplace and varying requirements of the end-use industries. Not merely being "additives," they are more widely applied as processing aids to help rationalize manufacturing processes and improve productivity of industries such as textile and steel as well as pulp and paper. Examples include dispersants of steel rolling oils for higher speed sheet output and de-inking agents for cost/performance effective use of recycled pulp. Also, surfactants are increasingly serving energy/fuel-related sues, such as stabilizers for coal/oil and coal/water mixtures; pour point depressants for diesel engine oils. Notable, further, is a trend in pursuit of synergestic effects by surfactant combinations. Beyond the scope of conventional application studies so far in practice, a more basic research appears to be in progress over the functional characteristics of surfactants forming structures of their own. It is anticipated that such studies will lead to development in biomimetics and molecular electronics.

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DEVELOPMENT OF DETERGENT AND TOILETRIES IN JAPAN ON THE POINT OF VIEW OF CONSUMER'S DEMAND AND ENVIRONMENT. Akira Mori, Lion Corporation, No. 13-12, 7-Chome, Hirai, Edogawa-ku, Tokyo 132, Japan.

After the war, the detergent and toiletry industry in Japan developed very rapidly by following after the progress of Western countries, especially the United States; it rapidly reached a mature stage. During the development detergents induced unexpected effects on the environment and consumers caused by the rapid increase of consumption caused by modernization of human living like the spread of washing machines. The industry spontaneously has made many efforts to improve its products to solve such problems, and succeeded in concentrating on those which produce better effects to sewage water, or human skin and hair. The author reviews the history of these improvements and recent trends of detergents and toiletries especially from the following points of view: (i) suitable performance for consumers' use; (ii) mildness to human skin and hair, and (iii) influence of drainage to rivers and lakes.

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REMOVAL OF TRIGLYCERIDE FROM COTTON FABRIC BY LIPOLYTIC ENZYME-SURFACTANT SYSTEM. Tomiko Fujii, Takako Hashimoto, Tokuzo Kawase and Motoi Minagawa, Osaka City University, 3-3-138, Sugimoto, Sumiyoshiku, Osaka, 558 Japan.

The effect of lipase on the removal of triglyceride from cotton fabric was evaluated in aqueous solutions of sodium dodecyl sulfate (SDS) as an anionic surfactant and monoalkyl deca(oxyethylene)ether (APE) as a nonionic surfactant. Seven kinds of lipases having different positional specificities on the hydrolysis of triglyceride were used. The removal of triglyceride from cotton fabric by the surfactant solution with lipase was higher than that without lipase, regardless of the type of surfactant. In the case of using SDS, the lipases with positional specificity, which accumulate di- and monoglycerides in the reaction mixture, were more effective than those without positional specificity which produce a considerable amount of free fatty acid. Further, the removal by the system of the former lipases and SDS is higher than the sum of the removal by lipase alone and that by SDS alone. Here the lipase having positional specificity seems to be synergistically effective because the hydrolysates such as di- and monoglycerides are preferentially removed by the interaction between these glycerides and SDS. On the other hand, in the case of using APE, no significant difference on removal was observed between lipases with and without positional specificity. The removal by the system of lipase and APE is found as the sum of the removal by lipase alone and that by APE alone. It means the effect of lipase is additive. Therefore, triglyceride and its hydrolysates are removed as a homogeneous single phase droplet by APE.

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ASSOCIATION BEHAVIOR OF POLYOXYETHYLENEDODECYLETHERPROPIOSULFONATES IN THE VICINITY OF CMC. Masahiro Fukuda, Takamitsu Tamura and Kazuo Ohbu, Applied Research Laboratories II, Lion Corporation, 7-13-12, Hirai, Edogawaku, Tokyo 132, Japan.

Association behavior of homogeneous polyoxyethylenedodecylether propiosulfonates, i.e., $C_{12}H_{25}O(CH_2CH_2O)_p(CH_2)_3SO_3Na$ (p = 1 to 8) was investigated especially in the vicinity of the critical micelle concentration (cmc). The cmc's of homogeneous surfactants were determined by the electric conductivity method. The surface tension curve for each surfactant solution showed a minimum below the cmc. It was also found that the apparent molal volume of each surfactant began to change even below the cmc. A light scattering study suggested that there existed a small aggregation of the surfactants before micelle formation began abruptly. Consequently, it is concluded that the change of molecular packing at the air/water interface is accompanied with that of association behavior in bulk phase. Moreover, it became clear by ESR measurements that the surfactant molecules interacted with a spin-labeled stearic acid molecule even below the cmc. It is noteworthy that the well known polyoxyethylenedodecylethersulfates do not possess a characteristic feature similar to that observed for the aforementioned surfactants. On the other hand, in a buffer solution (ionic strength 0.03, pH 11) these characteristic features of homogeneous surfactants were not recognized. Therefore, the structure of the solvated hydrophilic part of the surfactant appears to play an important role in the formation of small aggregates of the surfactants prior to micelle formation.

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INTERACTION BETWEEN LIPOLYTIC ENZYME AND DETERGENTS. Tokuzo Kawase, Wol-seun Kim, Tomiko Fujii and Motoi Minagawa, Osaka City University, 3-3138, Sugimoto, Sumiyoshiku, Osaka, 558 Japan.

In a series of the studies on the application of lipolytic enzyme to detergency, the interaction between lipase and surfactants was investigated by measuring residual lipase activities, surface tensions, specific conductivities, solubilizations of oil color and adsorption isotherms of lipase-sodium dodecyl sulfate (SDS) solution. The ac-

tivities of several microbial lipases, when contacted with surfactant, increased to a maximum and then remained constant over the wide range of surfactant concentration. The surface tensions of lipase-SDS solution decreased more effectively in comparison with those at the same concentration of SDS alone, and reached a plateau at about 1/4 concentration of cmc of SDS in phosphate buffer. At that concentration, the lipase activity also reached a maximum and the solubilization of oil color began. These results suggest that SDS interacts with lipase protein and, as a result of adsorption of SDS on lipase, a new lipase-surfactant complex (cluster), which would be more surface active than the original lipase, is formed, and oil color is solubilized at first in this cluster, not in the SDS micelle. After that, the surface tensions of lipase-SDS solution again decreased and became constant at minimum. The result of adsorption isotherms was consistent with those of surface tension, that is, the adsorption amount of SDS on lipase increased gradually and became constant at the concentration where the surface tension was in plateau, and then increased steeply with the increase of the concentration of SDS.

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KINETIC STUDIES ON OLEFIN CO-SULFONATION. Kyozo Kitano, Shizuo Sekiguchi and Toshiaki Ogoshi, Process Development Laboratory, Lion Corporation, No. 13-12, 7-chome, Hirai, Edogawa-Ku, Tokyo, Japan.

Inner olefin sulfonate (IOS) has a sulfonic group at the internal position of its alkyl chain. This IOS exhibits some characteristic properties in aqueous solution, namely, lower craft point, excellent wetting power and so on. In addition to EOR surfactant, we can expect IOS as an important active ingredient for household cleaners by utilizing the above properties. In this report, we refer to the sulfonation reaction of inner olefin from two points. One is the sulfonation mechanism, and the other is the kinetics. Sulfonation mechanism: From the analysis of IOS component, we suggest a carbyl sulfate as a main intermediate for inner olefin sulfonation. Kinetics: We tried to estimate the relative reaction rates of inner olefins through AO/IO co-sulfonation. Considering the side reaction (isomerization and polymerization of olefin), we estimated $K_{IO}/K_{AO} < 1$ for AO/IO = 50/50 co-sulfonation. And the order of reactivity among the double bond position is $\Delta^1 > \Delta^2, \Delta^3, \Delta^4$.

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CHAIN DYNAMICS IN LAMELLAR PHASES OF SELECTIVELY DEUTERATED NONIONIC SURFACTANTS BY ^2H NMR. A.J.I. Ward, M.A. Phillippi, J.C-L. Hsieh, C. Marie and H. Ku, The Clorox Company, P.O. Box 493, Pleasanton, CA 94566.

Nonionic surfactants in aqueous lamellar phases are capable of incorporating large amounts of oil. Deuterated analogues of n-alkyl polyoxyethylene glycol ether have been synthesized and studied by deuterium NMR spectroscopy. The order parameter profiles from both the alkyl and polyoxyethylene chains in the lamellar phase have been obtained as a function of water and oil content. Comparison of the degree of order in these nonionic surfactant systems with that found in ionic surfactant and phospholipid systems indicates a more flexible lamellar/water interface. Further, the position of maximum order is found not to occur at the alkyl/polyoxyethylene chain interface. Effects of solubilized N-alkanes upon the order of the surfactant molecules have been invvestigated as a function of alkane chain-length. Apart from a general change in the molecular ordering resulting from the presence of solubilized molecules, local variations may be observed, the nature of which depends upon the relative sizes of the alkane chains with respect to that of the surfactant.

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DEUTERIUM NMR STUDY OF HYDROTROPIC ACTION IN LYOTROPRIC LAMELLAR PHASES OF A NONIONIC SURFACTANT. A.J.I. Ward, M.A. Phillippi, J.C-L. Hsieh and R.J. Wiersema, The Clorox Company, P.O. Box 493, Pleasanton, CA 94566.

A simple way of monitoring hydrotropic action in aqueous nonionic surfactant systems has been devised. Use has been made of the deuterium NMR spectra observed for water in the lamellar phase of a nonionic surfactant to study the action of agents such as propylene glycol, iso-propyl alcohol, butanol, sodium xylene sulphonate etc. The observed quadrupolar splitting of the water molecules is a weighted average from molecules which are associated with the polyoxyethylene headgroups and those which are essentially isotropic. Changes in headgroup hydration, therefore, to a first approximation can be followed by observing the quadrupolar splitting. The overall mechanism of hydrotropic action involves the destabilization of the lamellar phase. Some hydrotropes were found to initiate this process primarily through changes in the state of surfactant headgroup solvation, whereas, others operate via a direct steric effect on the surfactant molecules packing arising from the location of their incorporation into the lamellar structure.

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AN INVESTIGATION OF MICROEMULSION ASSOCIATION USING FOURIER-TRANSFER PULSED FIELD GRADIENT NMR BASED SELF-DIFFUSION DATA. M.A. Phillippi, A.J.I. Ward, J.C-L. Hsieh and R.J. Wiersema, The Clorox Company, P.O. Box 493, Pleasanton, CA 94566.

Molecular association in the L_2 phase of nonionic ($C_{12}EO_4$) surfactant/hydrocarbon/H_2O microemulsions have been investigated by Fourier-Transformed pulse-field gradient NMR. The observed changes of self diffusion coefficients of each component in various compositions are associated with the change of molecular interactions and aggregation phenomenon. Addition of hydrotropic molecules such as propylene glycol modify the interfacial interactions. This modification affects the colloidal properties, such as the level of hydrocarbon incorporation and the phase stability with respect to the temperature variation. Results from anionic microemulsions will also be discussed. This technique is found to be useful in providing an overall picture of microemulsion with respect to association, structure and stability.

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STRUCTURAL ASPECTS IN AQUEOUS MICELLAR SOLUTIONS OF SURFACTANT MIXTURES. J.C-L. Hsieh, D.R. Scheuing, W.D. Turley, A.J.I. Ward and M.A. Phillippi, The Clorox Company, P.O. Box 493, Pleasanton, CA 94566.

The physicochemical and colloidal properties of mixed micelles have been a subject of interest. The mixed surfactant system can manifest differences in critical micellization concentration, size distribution of micelles, alkyl chain packing, head group interaction, counterion binding, microviscosity, surfactant monomer partition, solubilizate intake, etc. Studies of blends of a nonionic surfactant ($C_{12}EO_8$) and a cationic surfactant ($C_{16}TAC$) have been made. Results by Fourier-transformed infrared spectroscopy, fluorescence probe, gas chromatography, differential scanning calorimetry, dynamic light-scattering and nuclear magnetic resonance will be discussed. All these measurements reveal distinctive or directional changes of physical parameters as the molar ratio of two surfactants varies. The data are considered in light of theoretical models for mixed micelles.

Session OO Saturday afternoon Analysis of Lipids VI

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A CRITIQUE OF THE LIPOXYGENASE METHOD FOR THE DETERMINATION OF POLYUNSATURATED FATTY ACIDS. C. Szonyi, J.D. Craske and S. Haran, Unilever Australia Limited,

P.O. Box 9, Balmain, NSW, 2041, Australia.

The lipoxygenase method for the determination of polyunsaturated fatty acids (PUFA) has been acclaimed because the enzyme reacts only with the nutritionally important *cis*-methylene-interrupted isomers. It is now shown that existing lipoxygenase methods do not yield consistently reliable results. Both falsely high or low results can be obtained depending upon the relative qualities of standard and sample. Erroneous results are caused by unwanted autoxidation brought about by inadequate protection from oxygen, and the presence in the reagents and/or the sample of trace metals and of organic pro-oxidants in the sample. By decreasing the amount of available oxygen and of the trace metals, the accuracy of the method can be improved for those samples that contain little or no organic pro-oxidant, yielding a CV of about 2% at the 40-60% level. The accuracy of the method decreases with increasing amounts of organic pro-oxidants. As the analyst does not know the oxidative status of any sample, it is not possible to determine to what extent any result is in error. While alternative techniques such as GLC also have certain drawbacks, results obtained from the lipoxygenase method must be treated with caution.

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CHARACTERIZATION OF THE LIPIDS OF *METHANOBACTERIUM (M.) THERMOAUTOTROPHICUM*.J.K.G. Kramer and F.D. Sauer, Animal Research Centre, Research Branch, Agriculture Canada, Ottawa, Ontario K1A OC6.

M. thermoautotrophicum contained 1.7% total lipids on dry matter basis. Total lipids were separated into 20 components using a newly developed two directional TLC solvent system; chloroform/methanol/conc. NH_4OH (60/30/10) in the first direction, and chloroform/acetone/methanol/acetic acid/water (50/20/10/15/5) in the second direction. Eight phospholipids, 3 glycolipids, 3 phosphglycolipids and 2 phosphonolipids were identified. Seven of the 20 compounds were shown to have diphytanylglycerol ether or bidiphytanyldiglycerol tetraether, confirmed by FAB-MS. Glucose and arabinose were the only sugars identified in the glycolipids. Sulfur was shown to be present in these lipids. The proposed structures of these major lipid classes will be presented.

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DETERMINATION OF OCTADECENOATE AND OCTADECADIENOATE ISOMERS IN VEGETABLE OIL PRODUCTS. Mark S. Fraser and George Frankl, Beatrice Grocery Group, 1645 West Valencia Drive, Fullerton, CA 92634.

A multi-step analytical method has been developed for the measurement of octadecenoate and octadecadienoate isomers, positional and geometric, present in commercial vegetable oil products. The key feature of the method is ozonolysis of the unsaturated methyl esters in the presence of BF_3-MeOH to mono- and dimethylester cleavage products. This reaction possesses several advantages: (i) production of the characteristic chain length cleavage product in high yield (little chain degradation); (ii) production of dimethylmalonate from isomers containing the 1,4-diene structure, and (iii) production of commercially available methyl- and dimethylester products. Application of the ozonolysis procedure to fatty acid methyl ester fractions, prepared by reversed phase HPLC, having a given number and geometry of double bonds allows bot the position and geometry of the carbon-carbon double bonds to be determined. For octadecenoate isomers, either the monomethylester or the dimethylester products can be used to locate the position of the original carbon-carbon double bond. Dimethylester products were used to approximate the relative amounts of various octadecadienoate isomers. In the case of mono-*trans*-octadecadienoates, the geometry of each double bond remains unspecified. The influence of the severity of hydrogenation on the amounts and types of isomers will be discussed.

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EFFECT OF 3-ACYL CHAIN-LENGTH ON THE MOLECULAR PACKING OF 1,2-DIPALMITOYL-3-ACYL-*sn*-GLYCEROLS. Dharma R. Kodali, David Atkinson and Donald M. Small, Biophysics Institute, Boston University School of Medicine, Housman Medical Research Center, 80 East Concord St., Boston, MA 02118.

Thermal and structural changes of a series of 1,2-dipalmitoyl-3-acyl-*sn*-glycerols (PPX) (X = 2-16) were studied by DSC and x-ray diffraction. On quenching from the isotropic liquid the short 3-acyl chain compounds (X = 2-8) produced a hexagonally chain packed α-phase (d = 4.05Å) with a bimolecular (PP2 = 51Å) or a unimolecular (PP4 = 28Å, PP6 = 30Å and PP8 = 32Å) length structure. The stability of the α-phase markedly decreased as the 3-acyl chain length increased facilitating its conversion to an orthorhombic perpendicularly packed β'-phase. The long spacings (46-53Å), and the stability of the β'-phase increased as the 3-acyl chain lengthened, indicating a trilayered structure where the 3-short acyl chains form a middle layer between two different layers of 1,2-dipalmitoyl chains. If 'X' is >4 the trilayered structure is the most stable; if 'X' is 4 or less the trilayered structure is unstable and transforms to a stable bilayered β-phase (d = ~43Å). Quenching the long acyl chain compounds (X = 12, 14 and 16) from the isotropic liquid produced a bilayered (PP12 = 42.5Å, PP14 = 45Å and PP16 = 47Å) α-phase. Prolonged incubation below the melting point of the α-phase it transformed to a bilayered (PP12 = 40Å and PP16 = 42Å) β'-phase. However the β'-phase is also unstable and on incubation near its melting temperature, it is converted to a stable β-bilayer (d = ~40Å). The intermediate member of the series, PP10, showed five different polymorphic forms. Quenching PP10 from the isotropic liquid formed a bilayered (d = 39Å) α-phase, which after prolonged incubation below its melting temperature (22 C) produced a bilayered (37Å) β_1'- phase. Melting the β_1'-phase (34 C) produced another bilayered (37Å) β'_2-phase with slightly different chain packing. Incubating the β'_2-phase at 37 C for >45 hr produced a stable trilayered (d = 56Å) β-phase (m.p. = 46 C). If the α-phase was rapidly heated to 37 C and held at this temperature a β'-phase (m.p. = 43 C) with a hexalayer (d = 111Å) structure (two successive trilayers tilted in opposite directions) was obtained.

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DYNAMIC POLYMORPHISM OF *VERNONIA GALAMENSIS* TRIBLYCERIDES DURING COOLING-REHEATING CYCLES. Shu-Pei Chang, Kenneth D. Carlson and John A. Rothfus, U.S. Department of Agriculture, Northern Regional Research Center, 1815 N. University St., Peoria, IL 61604.

The dynamic polymorphism of *Vernonia galamensis* oil, whose major acid is *cis*-12,13-epoxy-*cis*-9-octadecenoic acid, was examined by DSC during cooling-reheating cycles. Vernonia oil remains a liquid well below its melting point (T_m = 283 K), and crystallizes in the range 208-238 K (T_f) with both T_f and ΔH_f inversely proportional to the cooling rate. The glass with disordered molecular structure obtained at cooling rates ≥80 K/min persistently liberated heat below T_f indicating continued nucleation and crystal growth in the solid state, which stops at the glass transition temperature, T_g. Below T_g, the molecular order is frozen in a variety of states dependent upon prior cooling history. On reheating, molecular ordering begins at T_g, with additional "crystallization" occurring at T_2 (191-215 K) via a solid-solid transition. T_2 and ΔH_2 are positively correlated to heating rates. Apparent perfecting of crystal structure occurs at T_3 and T_4 on further heating. Crystals resulting from these dynamic molecular ordering processes melted at 283 K (T_m), regardless of prior cooling history or subsequent heating rates. Exothermic transitions, T_f, T_2 and T_4, changed with cooling and heating rates according to the equation, $T = a + b°(X + c)$, where T is the transition temperature, X is the cooling or heating rate and *a*, *b* and *c* are constants for each transition. Limiting transition temperatures are obtained from the equation when X = 0.

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REASONS FOR THE HIGH PROPORTION OF PHEOPHYTIN IN EDIBLE PLANT OILS. Riichiro Usuki, Shokei Women's Junior College, 1-9-15, Hachiman, Sendai, 980 Japan; Yasushi Endo, Depart-

ment of Food Chemistry, Tohoku University, and Takashi Kaneda, Koriyama Women's College.

Our previous reports have shown that the four pigments of chlorophyll (Chl) and phophytin (Phy) are present, in general, in concentrations of 0.06 to 0.3 ppm in refined edible plant oils and that a high proportion of the pigments, over 80%, was observed to be Phy. In addition, Phy showed a higher prooxidant activity and higher stability through the photooxidation of triglycerides than Chl. Therefore, we concluded that the Phy content must be noted, when considering the oxidative stability of edible oils. In order to reduce the Phy level in oils, it is necessary to understand the reasons for the high proportion of Phy in refined edible oils. In this investigation, the compositional changes in Chl and Phy during the extraction and some refining processes were evaluated by photofluorometric analysis. When Canola seed oil was extracted with 6 kinds of solvents, the proportion of Phy was the highest (97%) in oil extracted with n-hexane, while it was 13% in that extracted with chloroform-methanol (2:1), and only 3% in that extracted with n-hexane followed by extraction with chloroform-methanol. These results show that the use of n-hexane as a solvent reduces the extraction of Chl, which is attached to proteins in the plant tissue. Next, Canola seed oil was extracted with n-hexane for different extraction times, and at different temperatures. Phy content in the extracted oil increased with increase in extraction time and/or temperature.

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RHYTHMS IN CHOLESTEROL, CHOLESTERYL ESTERS, FREE FATTY ACIDS AND TRIGLYCERIDES IN BLOOD OF LACTATING DAIRY COWS. Joel Bitman, D.L. Wood and A.M. Lefcourt, Milk Secretion and Mastitis Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705.

Intensive blood sampling was undertaken to determine whether circulating neutral lipids in lactating dairy cows exhibit rhythmic variations. Plasma neutral lipids were measured by quantitative thin-layer chromatography on every fourth integrated 15-min blood sample from 6 lactating dairy cows for 48 hr. The cows were: housed in an environmental chamber at 20 C with 16L:8D (lights on at 0700); fed daily at 0900; miled at 0830 and 2000. Variables monitored included: body temperature, ammonia-nitrogen, urea-nitrogen, glucose, T_3, T_4, growth hormone, insulin, cortisol and prolactin. During 24-hr periods, the lipids exhibited a dominant ultradian rhythm of 2.5 h. Mean levels of cholesterol (CHOL), cholesteryl esters (CE), free fatty acids (FFA) and triglycerides (TG) were 21.4, 175.4, 3.1 and 6.3 mg/dl, respectively. Amplitudes of the rhythms were: 60% of mean (FFA and TG) and 20% (CHOL and CE). There was no apparent circadian rhythm in these neutral lipid components. Feeding, light and dark, and milking did not appear to affect the ultradian rhythms. All of the other metabolic and hormonal variables exhibited circadian rhythms; most exhibited ultradian rhythms with periods of 90–120 min.

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OXIDATIVE STABILITY OF LOW-LINOLENIC ACID SOYBEAN OIL, HIGH STEARIC ACID SOYBEAN OIL, AND TWO COMMERCIAL SOYBEAN OILS. Lynne A. Miller, Pamela J. White, Walter R. Fehr and Earl G. Hammond, Iowa State University, 107 MacKay Hall, Ames, IA 50011.

It is generally agreed that the stability of soybean oil to oxidative flavor deterioration would be much improved if it contained less linolenic acid. In this study, low-linolenic soybeans (18:3 $\sim$ 3.2%) derived from mutagen-treated seeds were extracted and their oils refined by standard laboratory procedures with no additives. Two commercial strains of soybeans, BSR101 (18:3 $\sim$ 8%) and Pella (18:3 $\sim$ 6.1), were treated similarly. A high-stearic acid soybean line that was produced during the mutagen treatments was also extracted and refined similarly. The four oils were stored at 28 and 55 C and examined periodically for peroxide value and conjugated dienoic acid value. Sensory comparisons also were done by a 12-member panel, by using a 9-point scoring scale for intensity of rancid odor and flavor. Chemical and sensory analyses will be presented.

Session PP Saturday afternoon Biotechnology of Oils and Oilseeds

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IDENTIFICATION OF MUTANTS PREVENTING n-HEXADECANE UPTAKE AMONG 26 n-ALKANE NON-UTILIZING MUTANTS OF YARROWIA (SACCAROMYCOPSIS) LIPOLYTICA. John B. Bassel and Robert K. Mortimer, Department of Biophysics, Donner Laboratory, University of California, Berkeley, CA 94720.

Genetic analyses of n-alkane non-utilizing mutants of the yeast *Yarrowia (Saccharomycopsis) lipolytica* were continued. By analyses of inter-mutant complementation and recombination a total of 26 genetic loci have been identified. Mutations representing these loci have phenotypes characteristic of defects in substrate uptake or in one or more of the enzymatic activities making up the hydroxylase complex. Tests of 14C n-hexadecane uptake by a set of alkane-negative mutants representing the 26 loci show that 16 of the mutations cause a significant reduction in n-alkane uptake. N-alkane uptake by *Y. lipolytica* is shown to be inducible and to be inhibited by the metabolic poisons 2–4 dinitrophenol and KCN. The latter observation indicates that n-alkane uptake by *Y. lipolytica* is due to active transport.

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MICROBIAL PRODUCTION OF TRIGLYCERIDE-RICH FATS AND OILS. Bernard C. Sekula, Best Foods Research and Engineering Center, 1120 Commerce Ave., Union, NJ 07083.

Oleaginous microorganisms, i.e. those that accumulate >25% of their biomass as lipid, have long been regarded as potential commercial sources of fats and oils. Microbial oil production has generally focused on the utilization of carbohydrates or liquid oils and hydrocarbons. Little success has been demonstrated using fats, which are solid at ordinary fermentation conditions, primarily because of problems regarding substrate dispersion, uptake and/or assimilation. We have addressed this problem and have developed a process for the production of yeast lipids rich in triglycerides using stearic acid (C18:0) as the carbon source. This process allows the lipid yields to be increased and permits greater manipulation of the fatty acid composition in the yeast lipids. The efficient utilization utilization of stearic acid is dependent upon the successful preparation and maintenance of the substrate as an emulsion.

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GENETIC STUDY ON THE YEAST PHOSPHATIDYLETHANOLAMINE METHYLATION PATHWAY. Satoshi Yamashita, Department of Biochemistry, Gunma University, School of Medicine, Maebashi 371, Japan, and Tsutomu Kodaki, Yuko Tsukagoshi, Junichi Nikawa and Kohei Hosaka, Gunma University School of Medicine.

The yeast *Saccharomyces cerevisiae* possesses the phosphatidylethanolamine methylation pathway which forms phosphatidylcholine by the three-step methylation of phosphatidylethanolamine by *S*-adenosylmethionine. By selecting choline auxotrophs from various parents we isolated two classes of mutants with defects in the methylation pathway. One was defective in the phosphatidylethanolamine methyltransferase activity *(pem1)*, and the other was defective in the phosphatidylmonomethylethanolamine and phosphatidyldimethylethanolamine methyltransferase activities *(pem2)*. The two mutations segregated independently. These results show the involve-

ment of multiple methyltransferases in the phosphatidylethanolamine methylation pathway. We have shown that the enzymes of the methylation pathway are repressed by the co-existence of *myo*-inositol and choline. In a regulatory mutant designated as strain 172, the methyltransferases were fully repressed by *myo*-inositol alone. The regulatory alteration was due to a recessive nuclear gene mutation. Genetic analysis has suggested that this gene encodes a trans-acting positive regulator. Using a phosphatidylethanolamine methyltransferase mutant *(pem1)* we isolated two DNA clones from a wild-type yeast gene library. One clone complemented the *pem1* mutation alone, but the other could complement both *pem1* and *pem2* mutations. These clones are being sequenced.

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PERFORMANCE CHARACTERISTICS OF AN IMMOBILIZED LIPASE MEMBRANE REACTOR. Frank Taylor and Dennis J. O'Brien, Eastern Regional Research Center, USDA, 600 E. Mermaid Lane, Philadelphia, PA 19118.

Lipase enzyme catalysis of the hyrolysis of fats and oils has been 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 have been obtained for the hydrolysis at 50 C of bleached tallow by a thermostable lipase from *Thermomyces lanuginosus* immobilized in a microporous membrane. Flat plate membrane holders were compared with multi-layered pleated capsule filters. The retention of enzyme in the membrane cannot be fully explained by a simple adsorption mechanism. Immobilized activity of 0.2 micromoles/min per square centimeter of membrane and a half-life of over two months have been demonstrated. Higher flux of tallow through the membrane produced a higher activity but shorter half-life. Half-life was longer at low flux, high enzyme loading, and when partially hydrolyzed tallow was recycled through the reactor. Data were interpreted by a model in which the reaction rate was limited by diffusion.

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DEVELOPMENT OF A MEMBRANE BIOREACTOR SYSTEM FOR LIPASE-CATALYZED PROCESSING OF OIL AND FATTY ACID. Tsuneo Yamané, Mohammad Mozammel Hoq and Shoichi Shimizu, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464, Japan.

Conventionally, lipase-catalyzed synthesis of glycerides and hydrolysis of triacylglycerides have been carried out in emulsion systems where reactions take place at the interface of oil droplets. Such an emulsion system for industrial processes has certain drawbacks. Therefore, we have developed a microporous hydrophobic membrane bioreactor system based on the principle of nonemulsion. Two bioreactor configurations, i.e. flat membrane and hollow fiber module, both of which were made of microporous polypropylene, were tested. With these bioreactor systems, continuous production of glycerides and hydrolysis of triacylglycerides by lipase were carried out. For continuous synthesis of glycerides (flat membrane bioreactor), *Chromobacterium viscosum* and *Mucor miehei* lipases were used. The product, glycerides, was obtained at the outlet, in a single phase with no other phase from oleic or linoleic acid and glycerol. Highest conversion (ca. 90%) was obtained when water content of the glycerol solution was 3-4%. The reaction could be continued at least for one month yielding conversions over 70%. The main components of glycerides formed was almost equimolar amounts of mono- and diglycerides. For continuous hydrolysis of triacylglycerides (flat and hollow fiber bioreactors), olive oil and *Candida cylindracea* lipase were used as triacylglyceride and lipase. The lipase could be easily adsorbed onto oil-impregnated membrane from its aqueous solution. Fatty acid was obtained in a single phase unmised with another phase. The lipase was stabilized significantly by glycerol added to the buffer solution. The countercurrent flow mode was superior to cocurrent one. An unvaried half life of 2 weeks of the adsorbed enzyme was observed when increasing amounts of the enzyme were loaded. The used hollow fiber module was demonstrated to be regenerable. (The amount of fatty acid produced) divided by (amount of lipase used × time) of the adsorbed lipase in the hollow fiber module was 26 times that of gel-entrapped lipase.

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IMMOBILIZED LIPASE CHARACTERISTICS IN ESTER SYNTHESIS AND EFFECTS OF WATER AND TEMPERATURE IN VARIOUS REACTIONS. P. Eigtved, Novo Industri A/S, Novo Alle, DK-2880 Bagsvaerd, Denmark; T.T. Hansen, Novo Industri A/S, and H. Sakaguchi, Novo Industri (Japan) Ltd.

Characteristics of immobilized lipase from *Mucor, Candida* and *Pseudomonas* spp will be presented. Investigations on the hydrolytic and synthetic activity at different temperatures and water concentrations are summarized. Water dependence, thermal stability, and specificity will be illustrated in various reactions: hydrolysis, alcoholysis, acidolysis, acidolysis, interesterification, and ester synthesis. Immobilized *Mucor* and *Pseudomonas* preparations are useful in synthetic reactions above 60 C. The non-specific *Candida* lipase has good hydrolytic properties at low temperatures, but thermal stability was not improved by immobilization and the synthetic activity low. Examples given will focus on the application of immobilized lipases to synthesize esters. Fatty acid esters from methyl to fatty alcohol can be prepared by direct esterification or alcoholysis. Flavors and waxes constitute some of the interesting products. Results include the batch synthesis of wax ester in 98% yield using immobilized *Mucor* lipase for 3 hr at 70 C.

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ENZYMATIC REACTION OF OILS AND FATS BY BIOREACTOR. Tadashi Funada, Jiro Hirano and Yukihisa Tanaka, Nippon Oil and Fats Co. Ltd., Tsukuba Research Laboratory, Tokodai 5-10, Toyosato-Machi Tsukuba, Ibaraki 300-26 Japan.

The authors developed a new bioreactor which performs efficiently oil-water heterogeneous enzymatic reactions. The column is filled with enzymatic solution to effect reactions by bubbling dispersively small drips up from the bottom. Without preparatory emulsification nor using emulsifying agent, enzymatic reactions can be made efficiently for finer droplets of fat with 0.1–2 mm diameter, generated through the newly developed disperser. A set of the device consists of a cylinder of 30 mm diameter and 70 mm high, and a disperser which are interconnected to form a multi-stage bioreactor. Oily substances react while floating up dispersively through a stage, then aggregate at the upper part of the stage thus forming an oily layer. At the next stage, the new surface of the layer contributes to further reactions by its redispersion. Since this procedure is repeated in each stage, the reaction efficiency is greatly improved. The characteristics of the reactor are described as follows: (i) continuous reactions are possible; (ii) no emulsifying agent is needed; (iii) no large driving power is required, and (iv) there is no engulfing of air. By using the reactor, the authors experimented hydrolysis of olive oil. Eight stages were piled up for reactions by using *C. cylindracea* lipase (500 U/ml-water) under the conditions of pore size of oil (0.1 mm) and flow rate of oil (0.5 g/min). After one pass (2 hr) of the experimentation, the authors obtained a yield of 96%.

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ENZYMATIC SYNTHESIS OF CARBOHYDRATE ESTERS OF FATTY ACID (V). REACTION PRODUCTS OF SORBITOL WITH FATTY ACIDS. Tsuyoshi Uchibori, Hajime Seino and Toshiyuki Nishitani, School of Hygienic Sciences, Kitasato University, Kitasato 1-15-1, Sagamihara-shi, Kanagawa 228, Japan, and Isamu Morita, Dai-Ichi Kogyo Seiyaku Co. Ltd.

Esterification of sorbitol with fatty acids using the lipase obtained from *Candida cylindracea* was carried out by mixing the lipase and the substrates in a buffer solution and incubating at 40 C. After freeze-drying the mixture, the products were extracted and subjected

to high performance liquid chromatography (HPLC). It was observed by HPLC that mono- to tetraesters of sorbitol were produced by the enzyme reaction, and that di- and monoesters were predominent. These products were isolated through preparative HPLC, and their structures were examined by infrared (IR) and ^{13}C nuclear magnetic resonance (NMR) spectroscopies. It was confirmed that inner ether formation of sorbitol had not occurred and the obtained products were uncyclized sorbitol esters. The surface tension of the aqueous solution of mono and diesters was determined. The critical micelle concentration of sorbitol monooleate was found to be of the order of 10^{-4} mol/l. The emulsifying properties of the sorbitol esters were examined by finding their capability to emulsify water into soybean oil or soybean oil into water.

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GENETIC AND MOLECULAR FEATURES OF LIPOXYGENASE ISOLINES. Corinne S. Davies, Purdue University, Department of Agronomy, W. Lafayette, IN 47907, and Niels C. Nielsen, USDA-ARS and Purdue University.

Soybean lipoxygenase has long been implicated in the generation of off-flavors in soy products. Germplasm screening has resulted in the identification of nullalleles (lx_1, lx_2, and lx_3) for each of the three seed lipoxygenase isozymes. Our genetic results indicated tight linkage between gene loci Lx_1 and Lx_2 and independent segregation of the Lx_3 locus. Near-isogenic lines for various combinations of the lx_1, lx_2, and lx_3 null-alleles have been developed. This material is being used to definitively test the effect of lipoxygenase on seed quality. Molecular characterization of null-alleles lx_1 and lx_3 indicated the presence of lipoxygenase-1 and -3 mRNA in both mutant phenotypes, although the level of lipoxygenase-3 mRNA was greatly reduced in plants lacking the lipoxygenase-3 protein. Genomic southern blot hydrbidization experiments demonstrated that a restriction fragment length polymorphism was present in the near-isogenic lines bearing the Lx_1 vs. lx_1 alleles.

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SEEKING THE MOLECULAR GENETIC BASIS FOR THE REGULATION OF OIL CONTENT IN SOYBEANS. Prachuab Kwanyuen, USDA-ARS, 4114 Williams Hall, North Carolina State University, Raleigh, NC 27695-7620, and Joseph W. Burton and Richard F. Wilson, USDA-ARS.

The oil concentration of soybean seed is a quantitative trait which is maternally influenced, i.e. determined by the genotype of the plant on which the seed are borne. Genetic variability for this trait among soybean genotypes may range from 12 to 27% of the seed dry weight. Although such genotypes have been used to develop adapted germplasm exhibiting a high-oil or low-oil, concentration little is known about the molecular genetic regulation of the trait. Because triacylglycerol accounts for ca. 90% of soybean oil, genetic regulation of the enzyme diacylglycerol acyltransferase (DGAT) may be a factor which governs the expression of the trait. This investigation will test the hypothesis that genetic variability for oil content in soybeans is affected through biochemical/biophysical changes in DGAT. However, certain impediments must be overcome before it is feasible to test that hypothesis. First, germplasm exhibiting high-oil and low-oil traits must be developed with a common genetic background. Second, there must be an economical means to obtain a large quantity of hybrid seed from reciprocal crosses of high-oil and low-oil parents. Third, the membrane-bound enzyme which catalyses triacylglycerol synthesis (DGAT) must be purified to homogeneity. The accomplishment of these objectives and the research approach to establish a molecular basis for the genetic regulation of oil content in soybeans will be shown.

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GENETIC CONVERSION OF LINSEED OIL FROM INDUSTRIAL TO EDIBLE QUALITY. Allan G. Green, C.S.I.R.O. Plant Industry, GPO Box 1600, Canberra City, ACT 2601, Australia.

High levels of linolenic acid (45–65%) currently preclude the use of linseed as an edible oil because of associated flavor reversion problems. A genotype having greatly improved oxidative stability has been obtained through reduction in linolenic acid content by mutation breeding. Following EMS treatment of cv. Glenelg seeds (45% linolenic), two mutants, M1589 and M1722, having reduced levels of linolenic acid (28–30%) were isolated using the TBA test. The mutations are in unlinked genes and exhibit additive gene action. By recombining the M1589 and M1722 mutations into a single genotype linolenic acid content was further reduced to around 1%, a level which is lower than that in soybean and rapeseed oils. The reduction in linolenic acid content is associated with an equal increase in linoleic acid to between 50% and 70%, depending on temperature during seed maturation. Proportions of other fatty acids remain unaltered. The mutations thus prevent the formation of linolenic acid by desaturation of linoleic acid. Laboratory-scale refining, bleaching and deodorizing yielded a bland oil of light color. Oxidative stability measured by the AOM method was equivalent to that of sunflower oil and many times better than high-linolenic linseed oil. The low-linolenic linseed oil should therefore be suitable for use as a polyunsaturated edible vegetable oil.

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THE EFFECT OF BODY SIZE AND POSTPARTUM WEIGHT CHANGE ON FATTY ACIDS IN HUMAN MILK. Kenneth E. Hundrieser, Richard M. Clark and Robert G. Jensen, University of Connecticut, Nutritional Sciences Department, Roy Jones Bldg. Box U-17, Storrs, CT 06268.

Twenty mothers donated milk at 2, 6, 12 and 16 wk postpartum. Dietary information and maternal weights were obtained at the time of milk collection. Total calories and percentages of protein, lipid and carbohydrate in the diet did not change significantly during the study. Total lipids were extracted from the milk. Lipids were transesterified and the wt percentages of the fatty acid methyl esters were determined by GLC. Least squares regression equations were obtained between each fatty acid, body size and weight change. Three categories of wt were used: 80-100%, 100-120% and 120-210% ideal wt. Three categories of wt change between collection periods were used: a loss of more than 1% ideal wt, no wt change and a gain of more than 1% ideal wt. The fatty acids most affected by body size and wt change were 12:0 and 14:0. These fatty acids are thought to be synthesized by the mammary gland and their percentages in milk incrased with body size. The percentages of 12:0 and 14:0 also increased with wt loss and decreased with wt gain. A significant interaction was observed between body size and wt change. The highest percentages of 12:0 and 14:0 were found in overwtight donors losing wt while the lowest values were found in milk from underweight donors gaining wt. We conclude that body size and wt change through 16 wk of lactation are maternal variables that potentially affect the mammary gland synthesis rate of 12:0 and 14:0.

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EFFECTS OF DIETARY n-3 FATTY ACIDS ON POSTPRANDIAL SERUM LIPIDS. Reginald Saynor and Timothy Gillot, Sheffield Cardiothoracic Unit, Northern General Hospital, Herries Rd, Sheffield S5 7AU England.

The role of serum triglyceride in coronary heart disease is not yet fully understood. Remnants of the triglyceride-rich lipoproteins can become incorporated in to the vessel intama, thereby contributing to the formation of atheroma. It has been suggested that atherosclerosis may be a post-prandial disease, developing

during the period after a meal when the circulating triglyceride levels are high. Further work has demonstrated that the rate at which re-occlusion of coronary atery bypass grafts occurs correlates with serum triglyceride and cholesterol but not with cigarette smoking or hypertension. The standard technique for determining the lipid status of an individual is to take blood samples on three occasions after a 12-14 hr fast and to measure the serum cholesterol, triglyceride and lipoprotein electrophesis. It has been shown that up to 25% of patients haveing suffered a myocardial infarction (MI) have a normal lipid profile. However, a quite distinctive post-prandial triglyceride pattern occurred in the subjects with proven coronary disease suggesting that this approach was required in order to identify individuals at risk from this disease. The present study was disigned to attempt to develop a test to identify those at risk, by the post-prandial triglyceride pattern. At the same time it was decided to test the effectiveness of n-3 fatty acids on modifying the triglyceride response to a fatty meal. Subjects having suffered an MI and those with no evidence of heart disease were given a meal containing 80 g fat after a fsting blood sample had been taken. Further blood samples were taken at 1.5, 3, 4.5 and 6 hr after the meal. The triglyceride response in the MI group increased to a mean of 3.8mmol/l at 4.5 hr after the meal. However, in the "normal" group, the post-prandial triglyceride curve was much flatter with a mximum mean concentration of 2.4mmol/l at 3 hr. Although no significant difference was apparent in the total cholesterol concentrations, the high density lipoprotein cholesterol (CHDL) decreased in a pattern which followed the increase of triglyceride but not of the same magnitude. After 4 wk of a dietary supplement of n-3 rich fish oil (MaxEPA), the test was repeated. There was a significant fall in all the triglyceride samples when compared wiht the pre-oil test and in fact, the MI group mean triglyceride was "normalized" to give a post-prandial curve similar to that of the normal group. Although there was a slight and insignificant increase in the total chlesterol, this was probably accounted for by the increase in CHDL. These results suggest that it may be possible to identify individuals at risk from coronary disease by using the serum triblyceride as a risk marker, but further work needs to be undertaken.

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DEFICIENCIES OF ARCHIDONIC ACID AND OTHER POLYUNSATURATED ACIDS IN HUMAN DISEASE. Ralph T. Holman and Susan B. Johnson, The Hormel Institute, University of Minnesota, 801 16th Ave. N.E., Austin, MN 55912.

Disease and abnormal physiological state have profound effects upon the patterns of polyunsaturated acids (PUFA) in tissue and serum lipids. The PUFA most strongly affected are 20:3ω6, 20:4ω6, 20:5ω3 and 22:6ω3. Divergent disease states sometimes have similar effects upon PUFA pattern. Examples involving deficiency of dietary precursors and of metabolically derived PUFA will be shown.

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CORTISOL-CHOLESTEROL RELATIONSHIPS IN CORONARY ARTERY DISEASE, PREGNANCY AND STRESS. H.A. Schwertner, L. Torres, W.G. Jackson, H.A. Maldonado and J.D. Whitson, USAF School of Aerospace Medicine, Brooks AFB, TX 78235-5301.

Serum cholesterol concentrations are elevated in a number of diseases, including coronary artery disease, IN addition, elevated cholesterol levels are associated with a number of non-disease states such as pregnancy and some forms of stress. The mechanisms responsible for the increases in cholesterol are not known; however, we have recently found a significant association ($P < 0.05$) between cortisol and cholesterol in individuals with minimal coronary artery disease (20 to 49% narrowing) and in individuals with significant coronary artery disease (> 50% narrowing). In this study, we sought to determine if a similar association between cortisol and cholesterol occurs during pregnancy and during labor and delivery. For the study, cholesterol and cortisol concentrations were measured from early gestation through delivery in 33 normal pregnant women. During the course of pregnancy, cholesterol increased from 145 to 219 mg/dl, plasma cortisol increased from 8.6 to 19.1 μg/dl, and urinary cortisol increased from 0.10 to 0.17 μg/mg creatinine. Pooled correlations between plasma cholesterol and plasma and urinary cortisol wer all significant ($P < 0.001$). Further significant increases in cholesterol (256 mg/dl; $P < 0.005$) and cortisol ($P < 0.001$) occurred during labor and delivery and both dropped markedly after delivery. Plasma estradiol increased during pregnancy; however, it did not increase during labor. The results suggest that the increases in cholesterol associated with coronary artery disease, pregnancy and the stress of labor and delivery could be due, in part, to increased cortisol concentrations.

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THE EFFECT OF LIPID DEPLETION AND REPLETION ON THE METABOLISM OF A STEROL AUTOTROPH. J. Martyn Gunn, Gary W. Williams and Roberto de Antueno, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843-2128.

The role of membrane fluidity on metabolic processes has been examined in a sterol auxotrophic mutant (S_2) of LM mouse fibroblasts. compared to wild-type (wt) cells, the S_2 mutant had lower rates of protein synthesis and higher rates of intracellular protein degradation indicative of a slower growing, more catabolic cell type. Exposure to a medium containing 10% (v/v) cholesterol-free (lipid-depleted) fetal calf serum increased rates of intracellular protein degradation and decreased rates of protein synthesis. This response could be equated to growth inhibition of the cells because it was more noticeable in sparse cultures 48-72 hr after lipid starvation. supplementation with cholesterol decreased rates of protein degradation measured in the presence or absence of 10 mM NH_4Cl, indicating an inhibition of autophagocytosis. The EC_{50} for this effect of cholesterol on autophagocytosis was 25 μmM. Rates of protein synthesis and degradation could be restored to normal by growth in lipid-depleted serum supplemented with 50-100 μM of either cholesterol or palmitate (16:0) or linoleic acid (18:2). The effect of cholesterol plus 18:2 was approximately additive. Rates of 2-deooxyglucose (2-DOG) transport were slightly higher in S_2 compared to wt cells. Rates of 2-DOG transport wer depressed in lipid-depleted medium but were restored to normal by supplementation with cholesterol and 16:0. However, both wild-type and S_2 cells had low rates of glucose transport when grown in 18:2-supplemented media. The generally anabiolic effects of insulin and catabolic effects of dexamethasone were not altered overtly by lipid supplementation or depletion.

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MEMBRANE STRUCTURE OF LIPOSOME-TYPE ARTIFICIAL RED BLOOD CELLS STABILIZED WITH CARBOXYMETHYL CHITIN. Atsushi Kato and Tamotsu Kondo, Science University of Tokyo, Faculty of Pharmaceutical Sciences, 12, Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan.

Liposome-type artificial red blood cells (ARBC) stabilized with carboxymethyl chitin (CM chitin) were prepared by a two-step emulsification technique. The mean diameter of the ARBC was estimated to be about 310 nm by scanning electron microscopic observations. Various electron microscopic techniques (freeze-fracturing, freeze-substitution and negative-staining) revealed that the structure of the ARBC is not homogeneous but contained both uni- amd multilamellar vesicles. CM chitin did not yield a continuous layer but occurred in patches over the entire membrane surface. In order to investigate the membrane structure of the vesicles in an aqueous phase, disintegration tests on the ARBC using enzymes (lysozyme,, chitinase and phospholipase C) for the selective digestion of membrane components were carried out. The results suggest that CM chitin molecules are likely to cover the

lecithin layers of the ARBC rather coarsely forming a mesh-like structure with openings large enough to allow phospholipase C molecules to make easy access to, and to act on the lecithin molecules on the ARBC surface.

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CHEMICAL SIMULATION OF Pωω-CONTAINING AMINE OXIDASE BY MICELLAR REACTION. Yoshiki Ohshiro, Sinobu Itoh, Yutaka Kitamura, Nobuyuki Kato and Toshio Agawa, Department of Applied Chemistry, Osada University, Yamada-oka 2-1, Suita, Osaka 565, Japan.

Studies on chemical functions of coenzyme Pωω-containing amine oxidases are discussed. Under aerobic conditions, primary amines are oxidaized to the corresponding carbonyl compounds by the reaction with a catalytic amount of Pωω at around neutral pH. Strecker-type cedarboxylic deamination is observed in the reaction of α-amino acids. These reactions are first-order in the appearance of reduced Pωω and in the amine concentration. The cationic micellc provides a good environment for the reaction. The large isotope effect and low activity for secondary and tertiary amines indicates that the reaction proceeds via the covalent addition of the amine to the quinone followed by rate-limiting α-proton removal. The details and the mechanism of the reaction will be presented.

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REGULATION OF DOLICHYL PHOSPHATE AVAILABILITY IN GERMINATING SOYBEANS. Kothapalli Ravi, Jack W. Rip and Kenneth K. Carroll, University of Western Ontario, Department of Biochemistry, London, Ontario, N6A 5C1 Canada.

The availability of dolichyl phosphate (Dol-P) is a major factor in the rate of formation of N-linked glycoproteins in mammalian cells. Recent studies in our laboratory suggested that glycoproteins required for seed germination and early plant development are formed via the Dol-P pathway. Soybean microsomes contain dolichol kinase and Dol-P phosphatase that regulate Dol-P levels by interconversion of Dol-P and dolichol. In the present study, soybean microsomes were fractionated into rough (RER) and smooth (SER) endoplasmic reticulum and Golgi, and the activity of dolichol kinase and Dol-P phosphatase was measured in each. Submicrosomal fractions were obtained using a procedure developed for rat liver and were characterized by marker enzymes, RNA content and electron microscopy. This method proved to be superior in terms of yield and purity to methods designed specifically for isolation of plant cell components. The site of N-glycosylateion, the RER, in soybean contained high levels of both dolichol kinase and Dol-P phosphatase. This makes possible a mechanism of regulation of the dol-P content of RER by means of cycles of phosphorylation and dephosphorylation. The level of Dol-P pohsphatase in RER of soybean was much higher than that observed previously in rat liver RER. this may relate to the proposed biosynthetic role for dol-P phosphatase in soybean an dother species where Dol-P is formed by rephosphorylation of preformed dolichol rather than by direct de novo synthesis.

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THE ROLE OF FRAGRANCES (VOLATILE LIPIDS) IN THE LIVES AND IDENTITIES OF ORCHIDS. Ralph T. Holman, The Hormel Institute, University of Minnesota, 801 16th Ave. N.E., Austin, MN 55912.

Floral fragrances, volatile compounds of low molecular weight, are generated by floral parts in lipid-rich glandular areas, are derived from lipids and are associated with them. Floral odors are inset attractants and may be necessary dietary components for synthesis of insect pheromones. Because orchids require certain insects for their pollination mechanisms, the attractant of those pollinators is an identifying character of the orchid to the insect. Thus, composition of floral odor should be a good taxonomic tool in identifying and characterizing the plant. The odor is turned on and off according to a betabolic cycle tuned evolutionarily to the activity of the insect, so day-might composition may vary significantly. Diurnal variations in total odor of orchid flowers are measurable, and widely divergent daily cycles indicate that odor production responds to different external stimuli. Odorous compounds may also be used by the plant for defense mechanisms. Examples of these phenomena will be given.

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SYNTHESIS OF 3,11-DIMETHYL-2-NONACOSANONE, A CONTACT-COURTING PHEROMONE OF THE GERMAN COCKROACH (*BLATTELLA GERMANICA* L.). Masato Nomura and Yoshihito Fujihara, Kinki University, Hiro-machi, Kure-shi, Hiroshima, Japan.

This paper is concerned mainly with the synthesis of 3,11-dimethyl-2-nonacosanone (5), well known as a collective pheromone of *Blattella germanica* belonging to a quasi-social insect. The preparation of 5 is shown in a scheme. 1-Hexadecanal (2) as a starting material was obtained from the dehydration of 1-hexadecanol (1) using Cu-Zu catalyst. 2-Icosanone (2) was obtained from Grignard reaction of 2 with 4-chloro-2-butanone followed by hydrogenation of double bond, formed by the elimination of OH group, with Raney nickel in 76% yield. On the other hand, 6-methoxyhexanal (CH_3O-$[CH_2]_5$-CHO) was obtained from 6-bromohexanoic acid by the $LiAlH_4$ reduction, oxidation and methoxidation (CH_3OHa), in this order. Grignard reaction of 2-methyl-3-oxobutanol with 6-methoxyhexanal gave 8-nethyl-9-oxodecanal (4) in 69% yield. Finally, 5 was obtained from the reaction of 3 with 4 similar to that described in the synthesis of 3. All of the compounds prepared here were identified by means of IR, NMR and MS spectra.

Poster Session I Friday afternoon

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THE RELATIONSHIP OF MATURITY TO 2-METHYL PROPANAL IN HEAD-SPACE VOLATILES OF PEANUTS. T.H. Sanders and R.L. Greene, USDA, ARS, National Peanut Research Laboratory, 1011 Forrester Dr. S.E., Dawson, GA 31742.

Accurate quality evaluation of peanuts is difficult and although peanuts are generally marketed on a size basis, relative lot-to-lot equivalency is not assured. Head-space volatile analysis techniques have been used to estimate peanut flavor and flavor potential. In this study head-space volatiles were examined from ground peanuts of different maturity held 30 min at 130 C before sampling. An inverse relationship between 2-methyl propanal and maturity was observed. Considering this relationship, the volatile composition of specific screen sizes of peanuts grown in three soil temperature regimes were compared. Previous evaluations of peanuts in the three treatments indicated an increased rate of crop maturation as soil temperature increased; however, seed size distribution was skewed toward larger peanuts in the cooled soil treatment and toward smaller peanuts in the heated soil treatment. Peanuts of the same screen size from cooled, ambient, and heated soil contained respectively decreasing levels of 2-methyl propanal. This data indicates that although the peanuts were the same size, maturity levels were different and various quality potentials were different. This data/technique may prove useful in determining lot-to-lot quality for directed manufacturing end use or as an early indicator of quality in the market grading system for peanuts.

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THE RELATIVE ANTIOXIDANT EFFECTIVENESS OF WOOD-SMOKE PHENOLS IN SOYBEAN OIL AND LARD UNDER

ACCELERATED CONDITIONS. S.L. Cuppett and D. Winters, University of Nebraska, Food Science and Technology, 134 Filley Hall-East Campus, Lincoln, NE 68583-0919.

The antioxidant activity of woodsmoke has been shown in the literature; however, little data has been reported on the relative antioxidant effectiveness of the individual phenols that have been identified in woodsmoke condensates and in liquid smokes. The higher boiling point phenols (syringol and its derivatives) have been reported to have greater antioxidant activity than the lower boiling point phenols, i.e. cresols, phenol, guaiacol. The purpose of the study being reported was to determine the relative antioxidant activity of a series of phenols reported to occur in woodsmoke and liquid smoke preparations. The phenols were tested relative to each other and to BHS for their antioxidant effectiveness in both a refined soybean oil and in lard. The results indicate that the ability of the phenols to prevent autoxidation is related to both the level and the media used for the test. In addition, some combination effects were seen.

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TRANS DIENE FORMATION DURING CATALYTIC HYDROGENATION AND ISOMERIZATION OF SOYBEAN OIL. Richard E. McDonald and David J. Armstrong, Food and Drug Administration, 1090 Tusculum Ave., Cincinnati, OH 45226.

There has been considerable controversy in the past several years regarding adverse physiological effects of fatty acid isomers derived from the hydrogenation of vegetable oil. Formation of the major *trans* dienes resulting from catalytically isomerized and hydrogenated soybean oil was studied to determine the effect of reaction parameters on their formation. Normally, hydrogenated vegetable oils contain much higher levels of *trans* monoenes than *trans* dienes. However, when a sulfur-poisoned nickel catalyst was used at 200 C, 10 psig hydrogen pressure, 0.02% nickel, and 30 min reaction time, the sum of the *cis-trans* and *trans-cis* dienes concentrations was 10.6% and the *trans* monoenes concentration was 5.1%. There was only a trace of *trans-trans* dienes. After a reaction time of 90 min, 16% *cis-trans* plus *trans-cis* dienes, 5% *trans-trans* dienes and 20% *trans* monoenes were present. When fresh nickel catalyst was used under the same conditions less than 3% total *trans* dienes and more than 35% *trans* monoenes were formed. When hydrogen was omitted from the reaction mixture, there was twice as much *trans* dienes formed with sulfur-poisoned catalyst as with fresh nickel. Evidence from this study indicates the mechanism for *trans* diene formation using poisoned catalysts is different than hydrogenation using fresh nickel catalyst. The formation of *trans* dienes can be minimized by avoiding the use of old or poisoned nickel catalyst for hydrogenation.

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THE CAROTENOIDS AND TOCOLS OF CORN GRAIN. Evelyn J. Weber, USDA-ARS, University of Illinois, S-320 Turner Hall, 1102 S. Goodwin Ave., Urbana, IL 61801.

The various forms of carotenoids and tocols have different vitamin and antioxidant activities. The ranges of variability and potentials for genetically altering the levels of these nutrients in feedstuffs such as corn were unknown. An HPLC procedure has been developed that permits determination of carotenoids and tocols in the same sample preparation from corn grain. For 16 corn inbreds, the total carotenoids ranged from 0.01 to 72 μg/g dry wt and the total tocols from 28 to 102 μg/g dry wt. Among four inbreds, the proportions of the kernel parts by weight were horny endosperm 67.7 ± 1.0% (SE), starchy endosperm 17.7 ± 1.0%, and germ plus tip cap 14.6 ± 0.5%. Total carotenoids were concentrated in the horny endosperm (82.6 ± 2.3%) and total tocols (76.8 ± 6.0%) in the germ. After storage of the whole grain for nine months, 57.1 ± 3.8% of the original total carotenoids were lost at room temperature and 19.3 ± 3.4% even at −10 C. Zeaxanthin was the individual carotenoid that was lost most rapidly. Loss of carotenoids may be particularly serious in poultry feeds because not only are the carotenes precursors of vitamin A but the xanthophyll pigments impart desirable yellow color to egg yolks and the skin of poultry. The tocols appear to be more stable with recoveries of 96.9 ± 3.2% after nine months storage at room temperature.

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IDENTIFICATION OF LINOLEIC ACID GEOMETRICAL ISOMERS IN FRYING OILS. J.L. Sebedio, A. Grandgirard, J. Prevost and C. Septier, INRA, Station de Recherches sur la Qualité des Aliments de l'Homme, 17 rue Sully B.V. 1540, 21034 Dijon Cédex, France.

A sunflower oil was heated in a commercial fryer at 200 C for 42 hr using 2-hr daily cycles. This oil was also heated at 275 C for 12 hr under nitrogen in order to study the influence of temperature and oxygen. The identifications were carried out using the oil heated under nitrogen. The total fatty acid methyl esters were fractionated into a polar and a nonpolar fraction by column chromatography on silicic acid. The nonpolar fraction which contained the cyclic fatty acid monomers and the straight chain fatty acids was further fractionated using urea. The urea adduct fraction was submitted to HPLC on a C18 reverse phase column using MeOH as solvent in order to isolate the C18:2 isomers. These were fractionated in five different classes by $AgNO_3$-TLC. The least mobile group was the 18:2Δ9c,Δ12c followed by the 18:2 ct + tc; then the 18:2 tt, and the c,t conjugated dienes. The most mobile group was the t,t conjugated dienes. Each class was submitted to hydrazine reduction and the resulting monoenes to $AgNo_3$-TLC. The resulting *cis* and *trans* fractions were submitted to ozonolysis in BF_3-MeOH in order to determine the position of the ethylenic bonds. The 18:2 (ct + tc) were the 9,12 isomers. Minor amount of 18:2Δ9t,12t was also detected. The *trans,trans* 18:2 conjugated dienes were the Δ9,11, Δ10,12 and Δ11,13. The *cis,trans* and *trans,cis* conjugated dienes had ethylenic bonds in the Δ9, Δ10, Δ11, Δ12 and Δ13 positions. All these isomers were also detected in a soybean oil used for commercial deep fat frying operations.

345P

IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS IN EDIBLE OILS BY DYNAMIC HEADSPACE GC/MS. B.D. Kirk, Tekmar Company, P.O. Box 371856, Cincinnati, OH 45222-1856.

The flavor quality of any oil can be highly dependent upon the presence of volatile organic compounds. Profiles of volatile compounds have been shown to correlate well with flavor quality. Most of the volatiles present will be a result of thermal, oxidative and photooxidative breakdown, and will be indicative of the age of the oil. Measurement of the volatiles can be accomplished by several methods. This paper details the use of dynamic headspace GC/MS analysis of oil volatiles. The sample, which is usually heated, is purged with an inert gas to remove the volatiles. The volatiles are retained on a short adsorbent column during this purge step. Purging in this manner enables large quantities of volatiles to be collected. The adsorbent is heated to release the collected volatiles and backflushed to sweep the sample onto the GC column. Separation is carried out on a fused silica column under temperature spectrometer. The advantages of this method include outstanding sensitivity, specificity and reproducibility. Topics to be presented include discussion of all required equipment and operating parameters, methods of calibration, and identification and quantitation of the volatile contents of several oil samples.

346P

PREPARATION AND CHARACTERIZATION OF DEUTERATED BEHENIC ACID. August V. Bailey and Ray H. Liu, USDA-ARS Southern Regional Research Center, P.O. Box 19687,

New Orleans, LA 70179.

Combined deuterium exchange and catalytic procedures are used to synthesize position-specific behenic acid-13,14-d_2 and behenic acid-12,12,13,14,15,15-d_6, a saturated 22 carbon fatty acid typically found in the SN-3 position of peanut oil triglycerides. Methyl esters of these compounds are analyzed by a gas chromatography/mass spectrometry system for their chemical and isotopic purities. Single ion display of reconstructed total ion chromatograms allows the quantitation of -d_0, -d_2 and -d_6 compounds. The capillary column used for this study resolved the methyl behenate-d_0 partially from the -d_2 and completely from the -d_6 counterparts. Series of characteristic ions are used to identify the positions of deuterium atoms in these compounds. Integrations of characteristic ions indicated that the methyl behenate-d_2 yield is about 95%, and the methyl behenate-d_6 yield is about 63% in these synthesis products.

347P

STABILITY OF THE CHARACTER FOR AFLATOXIN PRODUCTION BY *ASPERGILLUS FLAVUS* OBTAINED FROM ARIZONA COTTON. Louise S. Lee, Alan R. Lax, Jay E. Mellon and Maren A. Klich, Southern Regional Research Center, USDA/ARS, P.O. Box 09687, New Orleans, LA 70179.

The loss of aflatoxin-producing ability of *Aspergillus flavus* is poorly understood. Ten toxigenic isolates of *A. flavus* obtained from cottonseed grown in Arizona were transferred on a weekly basis on potato dextrose agar. In order to maintain culture homogeneity, a deliberate attempt was made to transfer a massive number of spores. Secondary metabolism of each transfer was monitored by quantitation of aflatoxins and kojic acid; shifts in protein biosynthesis were monitored by electrophoretic and enzyme kinetic techniques. After 12 transfers, all isolates had lost the aflatoxin-production character, but levels of kojic acid remained constant and a highly fluorescent oxidation product of kojic acid became evident. However, single spore isolations from these non-toxin producing populations identified some isolates with toxin producing ability. These results indicate a competitive advantage for non-toxin producing spores during development on laboratory media. Inoculation of developing cotton bolls with laboratory-derived non-toxin producing populations provided subsequent spore aggregates with restored capacity for aflatoxin production. Constituents of cotton must mediate the genetic expression of toxin production in populations of *A. flavus*.

348P

AFLATOXIN IN ARIZONA COTTONSEED: SIMULATION OF INSECT VECTORED INFECTION OF COTTON BOLLS BY *ASPERGILLUS FLAVUS*. Louise S. Lee and Wilton R. Goynes, Southern Regional Research Center, USDA/ARS, P.O. Box 19687, New Orleans, LA 70179, and Pauline E. Lacey, University of Arizona.

Insects have been implicated as a vector of *Aspergillus flavus* infection of cotton, but no experiments have been reported that simulate insect damage and subsequent aflatoxin formation. Carpel walls of bolls of varying ages of maturity were punctured to simulate damage of sucking insects, or drilled to simulate the hole caused by exit of the pink boll worm larva. *A. flavus* was applied at these wound sites and at natural suture openings. Optical and scanning electron microscopical studies were carried out to show fungal progression. Aflatoxin levels on individual seed and the distribution of such seed in locks were compared for inoculated bolls and bolls naturally contaminated with *A. flavus*. Bolls drilled at the carpel wall 35 days after flowering most closely resembled naturally contaminated bolls. No toxins were detected in seed following inoculation of bolls at suture openings. Mycelial penetration from wound site to seed took 4 to 5 days. Similarity of both toxin levels and pattern of toxin to non-toxin seed in naturally infected bolls and bolls wound inoculated 35 days after flowering, and lack of infection of seed following suture inoculation indicates that infection is primarily of unopened bolls close to maturity but prior to opening. These findings reinforce existing knowledge that control of insects lowers aflatoxin potential.

349P

ADSORPTION OF SURFACTANTS ON MODIFIED CONTROLLED POROSITY GLASSES. Andrzej Dawidowicz and Jerzy Szczypa.

Controlled porosity glasses (CPGs) are very often used as catalyzers, adsorbents, ion exchangers and supports of adhesively or chemically bonded stationary phases. Their strong adsorption properties result from the presence of boron atoms on the surface and the silicous structure as well. Surface hydroxyl groups and small amounts of surface sodium ions are responsible for ion exchange properties of the CPG. In this paper the thermally modified CPGs were used as adsorbents of sodium laurylsulphate in aqueous solutions of different pH. The results obtained were discussed in terms of electrochemical properties of the surface. The above experiments were carried out in order to test whether CPG with SDS bonded phase can be used as sorbent of hydrocarbon compounds.

350P

A SIMPLE METHOD FOR DETERMINING THE CRITICAL MICELLE CONCENTRATION OF SURFACTANTS IN SOLUTION. Steven C. Goheen and Robert S. Matson, Bio-Rad Laboratories, 1414 Harbour Way South, Richmond, CA 94801.

Surfactants were prepared as aqueous suspensions at several times their critical micelle concentration (CMC). Bio-Rad's Gradient Module and UV Monitor were used to analyze changes in optical density of a continuous series of solutions from water to the concentrated detergent solution. At the CMC, the absorbance of each of the detergent solutions changed to reflect the associated phase transition. Detergents that were studied included sodium dodecyl sulfate (SDS), sodium cholate, Triton X-100 and reduced Triton X-100. Both Triton X-100 and reduced Triton X-100 absorbs less UV light. The absorbance of these detergents changed only slightly at the CMC with no other apparent structural changes near this concentration. Sodium cholate gave similar results with a weak shift in the absorbance at the CMC. In contrast, SDS also demonstrated a dramatic change in UV absorbance above this concentration. This additional change may have been due to a shift in micellular size or chape. The analysis of each surfactant was accomplished in approximately 30 min. This simple analytical technique for studying the CMC of surfactants may become useful for rapidly determining optimal solubilization conditions for a variety of compounds.

351P

WATER AND COBALT-CATALYZED LIPID AUTOXIDATION. J.L. Kahl and E.G. Schanus, Washington State University, Department of Food Science and Human Nutrition, Pullman, WA 99164-6330.

The rate of cobalt-catalyzed (as 1 ppm cobaltous laurate) autoxidation of methyl linoleate was determined manometrically in a model system at six relative humidities (RH). At 0% RH, cobalt accelerated hydroperoxide decomposition (autoxidation), compared to the decomposition rate in a non-catalyzed pure lipid control. Water appeared to bind preferentially to the metal, inhibiting catalysis, since cobalt-catalyzed oxidation at all RH's tested (11–100%) exhibited rates approaching those of non-catalyzed autoxidation. This suggested that possibly the metal-hydroperoxide complex did not form at RH of 11% or greater. Previous studies in a pure lipid system indicated that water accelerated the decomposition rate between 11% and 50% RH. These results indicate that water inhibits cobalt-catalyzed lipid autoxidation, but accelerates autoxidation in a pure lipid system.

352P

A PROCEDURE FOR IMPROVING PARTICLE SIZE ANALYSIS DATA FOR SOYBEANS AND OTHER GRAINS. Marlowe L. Iverson and John M. Walls, USDA, Federal Grain Inspection Service, R-G AFB, Bldg. 221, Grandview, MO 64030.

Particle size distribution is an important factor in achieving reliable analytical results in near infrared reflectance analysis, oil analysis by solvent extractions, and in other analytical procedures. Separation of granular grain by particle size using sieves is a common procedure for arriving at indices of particle size but the separations are not always reproducible. An additional step for particle size analysis has been developed which first separates the granular material into two fractions or types of particles which are called discrete and agglomerate particles. More reproducible particle size determinations of the total granular material can be made by sieving and combining the data of the two fractions. It was found that the average mean particle sizes for the agglomerates (201 ± 7.4 microns) and the discrete particles (268 ± 8.6 microns) remained relatively constant for soybeans when the same grinding conditions were used; however, the percentage of agglomerates ranged from 28 to 77%. The mean particle size of the ground material could be determined by simply measuring the percentage of agglomerates since the distributions followed closely that of a log-normal distribution. Because an agglomerate particle consists of many small particles or is a very irregular particle, the total surface area per gram is much greater for the agglomerate fraction. Applications of the procedure for separating granular material into agglomerates and discrete particles has led to a better understanding of particle size reduction processes. Since the percentage of the agglomerate fraction varies and this fraction has a higher protein and oil content, e.g., in soybeans, it is necessary to take this grinding variable into account in NIR and other analysis procedures.

353P

THE USE OF SUPERCRITICAL FLUID CO_2 TO FRACTIONATE FATTY ACID ETHYL ESTERS DERIVED FROM MENHADEN OIL. William B. Nilsson, John Spinelli, Virginia F. Stout and Joanne Hudson, National Fish Meal and Oil Association, c/o Utilization Research Division, NWAFC, 2725 Montlake Blvd. E., F/NWC8, Seattle, WA 98112.

Several recent reports have suggested that fish oils contain one or more fatty acids of the Ω3 class which are physiologically active agents in the prevention of cardiovascular disease. Most of the clinical work has focused on eicosapentoenoic acid (EPA) and docosahexaenoic acid (DHA). Studies to establish the physiological activity of EPA and DHA require concentrated samples of these two fatty acids. Currently there is no economical method of providing them. We have investigated the use of carbon dioxide supercritical fluid extraction to fractionate fatty acid ethyl esters derived from menhaden oil. Starting with an ester mixture containing 16.3% EPA and 10.5% DHA, we have obtained separate fractions containing 45.4% EPA and 62.8% DHA. At this writing, we are working to substantially improve on these results. Both our latest findings and the basic conepts of this technique will be discussed.

354P

PIGMENT REMOVAL FROM CANOLA OIL USING CHLOROPHYLLASE. W.L. Levadoux, M.L. Kalmokoff and M.D. Pickard, CSP Foods Ltd., P.O. Box 190, Saskatoon, Saskatchewan, Canada S7K 3K7, and J.W.D. Groot Wassink, Plant Biotechnology Institute, National Research Council, Saskatoon, Saskatchewan, Canada.

Frost-damaged or prematurely harvested canola seeds (rapeseed) may yield oil with a high chlorophyll content (50–60 ppm). Enzymatic hydrolysis of chlorophyll, added to buffer/surfactant, buffer/acetone or buffer/acetone/canola oil, to produce water soluble chlorophyllide (green pigment), was studied using a crude chlorophyllase preparation (acetone-dried chloroplasts) from 15–20-day-old sugar beet seedlings (*Beta vulgaris* vari. salohill). In buffer/surfactant, the optimum pH for enzyme activity was temperature dependent. At 30 C and 0.25% Triton X-100 chlorophyllase showed maximum activity toward a crude chlorophyll preparation at pH 8–10. At 60 C, the activity was more than twofold higher , with a sharp maximum occurring at pH 8. Mg^{2+} enhanced the activity with an optimal concentration to 50 mM. At pH 7.5, 50 C and in the presence of only 6–8% acetone, the enzyme showed high affinity for chlorophyll (Km = 15 μM, 13.6 μg/ml) suggesting that the natural chlorophyll concentrations found in green canola oils might facilitate high enzymatic efficiencies. The crude enzyme was stable in buffer/acetone at pH 7.5 and 50 C for at least 2 hours. With acetone concentrations as low as 6%, maximum enzyme activities in buffer and buffer/canola oil required intensive mixing (homogenization) of the substrate, enzyme and liquid phases. In general, the rate and extent of chlorophyll hydrolysis were greater in buffer than in buffer/oil. In both reaction systems, chlorophyll hydrolysis slowed down with time due to accumulation of phytol, which proved to be a competitive inhibitor (K_i = 11 μM; 3.3 μ/ml). The other hydrolysis product, chlorophyllide, did not affect enzymatic activity. Crude canola oil used in the reconstitution of green oil did not support enzymatic chlorophyll hydrolysis, it required prior degumming and desoaping. The optimum buffer/oil ratio of the reaction mixtures was 2:1 (v/v).

355P

MECHANISTIC CONSIDERATIONS OF POLYMORPHIC TRANSFORMATIONS OF TRISTEARIN IN THE PRESENCE OF FOOD EMULSIFIERS. Nissim Garti, Casali Institute of Applied Chemistry, School of Applied Science, The Hebrew University of Jerusalem, Israel.

It has long been recognized that fatty acids and polymorphs of fats can influence the quality of some food and cosmetic products Emulsifiers have traditionally been added during the melting process to retard undesired polymorphic transformations. The present study is an attempt to understand the role of selected emulsifiers on such transformations. Tristearin was heated or aged under controlled conditions. Using DSC and x-ray techniques, $\Delta H\alpha$ and $\Delta H\beta$ were evaluated in view of the possible pathways of α transforming into β. Temperature regime (rate of heating) controls the extent of mobility of the fat molecules, the local crystal imperfection, and the degree of liquification and, as a result, dictates kinetically the rate of polymorphic transformation. The surfactant, added as an impurity, does not have a straightforward effect, as thought previously, but tended to vary with the kinetic conditions. It has been shown that certain solid surfactants will retard the α-β transformation while other will enhance it. The molecular structure and its crystalline organization dictates the effect of either creating imperfections in the α form, retarding its transformation to the β form or promoting dislocations which will enhance the transformation. Liquid surfactants create the local dislocations which facilitate the mobility of triglyceride molecules.

356P

THE ROLE OF WATER ADDITION IN THE BLEACHING OF FATS AND OILS. D.B. Shaw, R.B.J. Wright and P.K. Stemp, Laporte Industries Ltd., Moorfield Rd., Widnes, Cheshire, England.

During the refining of triglyceride oils water plays an important role in several processing stages. Of greatest significance we must cite recent major advances in degumming of fats and oils with citric and phosphoric acid solutions to produce oils suitable for physical refining. The addition of water before and during the bleaching stage has been proposed to be beneficial by several workers and is currently practiced by some refiners. We believe from our own work that the benefits are less clear cut. We find them dependent on oil

type and processing procedure. By using a system in which exact moisture levels can be monitored and held at known concentrations, we have examined the influence water has upon bleaching earth performance and, more significantly, final oil quality. We show how water addition influences the major performance characteristics of bleaching earths in removing soaps, pigments, phospholipids and metals. We also demonstrate how the formation of free fatty acid during bleaching is influenced by water content. Differences in behavior between the major oils are described. We conclude by making recommendations governing the addition of water during bleaching to achieve the best performance from the bleaching earth used.

357P

D. Urosevic, Buss Ltd.,

Poster will announce some new data for automatic filtration unit concept from controls and instrumentation combined with Buss hydrogenation and heat recovery system.

358P

CAPILLARY GAS LIQUID CHROMATOGRAPHY OF SOME CHOLESTEROL OXIDES. Chih-shang J. Shen and Alan J. Sheppard, Food and Drug Administration, 200 C St. S.W., Washington DC 20204.

Cholesterol oxidation products have been shown to be biologically active in many studies. The reported activities include angiotoxicity, atherogenicity, cytotoxicity, carcinogenicity and effects on some specific enzymes. In the present study, gas liquid chromatography (GLC) of some biologically important cholesterol oxides using highly efficient capillary columns and flame ionization detector is reported. Ten cholesterol oxides that include both isomeric pairs of cholest-5,6-epoxy-3β-ol and 7-hydroxycholesterol and side chain hydroxycholesterols that differ only in the position of the hydroxy group were studied. Some of these cholesterol oxides and their butyrate derivatives were found to be unstable at high temperature. Therefore, cholesterol and the oxides were derivatized to form the stable trimethylsilyl (TMS) ethers at room temperature for GLC analysis. Using an SE-30 (polydimethyl siloxane) flexible capillary column (50 M $\times$ 0.25 mm), baseline separation of most cholesterol oxides was obtained at a temperature of 300 C. Complete baseline separation of all cholesterol oxides studied can be achieved on a shorter SE-54 (polydiphenyldimethyl siloxane) fused silica column (30 M $\times$ 0.25 mm) at the following conditions: column temperature, 290 C; injector and detector temperature, 300 C; helium carrier flow rate, 0.74 ml/min; split ratio, 81/1.

359P

PRODUCTION AND UTILIZATION OF TEMPEH. F.G. Winarno, FTDC-IPB, Bogor, Indonesia.

Tempeh is one of the popular fermented foods of Indonesia. Tempeh has been produced and consumed in Indonesia for centuries, but there are no written records of its origin. It is consumed by all socioeconomic groups. The total annual production of tempeh is about 500,000 tons. In Indonesia, tempeh production is still a household art. Most of the 41,000 small cottage industries that make fresh tempeh daily are family run and together employ about 128,000 workers. The technology of traditional tempeh making is simple with extremely low cost of production. The major steps for preparation of tempeh are the same, regardless of the places and countries of production, that is similar to that of the Indonesian traditional method. The soybeans are soaked, dehulled, steamed, inoculated with tempeh starter, wrapped and incubated for 48 hr. The tempeh should be harvested as soon as the bean cotyledons have been completely overgrown and knitted into a compact cake. Fresh tempeh has only two-day shelf-life if stored at room temperature. A well-made tempeh by definition is a compact cake completely covered and penetrated by the white mold mycelium of *Rhizopus sp.* Tempeh is an excellent source of protein (19.5%), vitamins (vitamin B complex including vitamin B12) and minerals (calcium, phosphorus and iron). About 100 g of fresh tempeh supplies 10.9 g of available protein, or more than 25% of the daily adult male requirement of protein. The digestibility coefficient of tempeh is about 86.2%, which is lower than tofu (95%). The mold species responsible for tempeh fermentation does not produce aflatoxin. Further, there have been no reports on toxins found in foods prepared from soybean. Cooking of soybeans and subsequent fermentation with *R. oligosporus* decreases trypsin and chymotrypsin inhibitors in tempeh.

360P

UNCONVENTIONAL OILS AND THEIR NUTRITION POTENTIALS. C. Rukmini, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

There is a great interest in exploiting unconventional oils of high potentials, to bridge the gap of edible oil shortage in the country. Unconventional oils like Mango kernel oil, *Hibiscus sabdariffa* oil, *Cleome viscosa* oil, *Terminelia bellirica* oil, neem oil, rice bran oil, mahua oil and kapok oil have been studied in detail for their chemical composition, nutritional quality and toxicological safety. Of these oils, mango kernel oil, *Cleome viscosa* oil and rice bran oil are found to be safe for edible purposes with added nutritional significance. *Cleome viscosa* oil and rice bran oil have a high hypocholesterolemic activity, and the latter also has antimutagenic activity and may have a role in fighting diseases like atherosclerosis and cancer. Mango kernel oil is found to be a good substitute for the highly priced cocoa butter fat which is used in confectionery. The significance of these results will be discussed.

361P

COMPOSITION AND NUTRITIVE VALUE OF NORTHERN WILD RICE, *ZIZANIA PALUSTRIS.* Krystyna Sosulski, Saskatchewan Research Council, 15 Innovation Blvd., Saskatoon, Saskatchewan S7N 2X8, and Frank W. Sosulski, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0.

The northern species of wild rice, *Zizania palustris*, is relatively popular because of its large grain size. However, most of the analytical data has been obtained on southern wild rice, *Z. aquatica.* The northern wild rice, after curing and parching, was found to be rich in protein and essential amino acids. The starch content of wild rice was comparable to domestic rice but lipid, fiber and ash contents were very low. The fatty acid composition of wild rice indicated a high level of polyunsaturation in the triglyceride and polar lipid components. Data on the sugars, phytin, fiber components, vitamins and essential minerals also are provided. Because wild rice is consumed as the whole grain, it represents an excellent nutritional package as well as a gourmet meal.

Poster Session II Saturday afternoon

362P

CATALYZED DEAMIDATION OF OILSEED PROTEINS. Frederick F. Shih and Agnes D. Kalmar, Southern Regional Research Center, New Orleans, LA 70179.

The effects of sodium dodecylsulfate (SDS) on the acid hydrolysis of cottonseed and other oilseed proteins have been investigated. Under relatively mild acid (0.2 N HCl) and temperature (70 C) conditions, small amounts of SDS (0.03–0.05 M)

catalyzed the hydrolysis of amide groups (deamidation) in cottonseed protein in preference to the hydrolysis of peptide bonds. High degrees of deamidation could be achieved with minimal peptide bond degradation, and functional properties of the protein were improved. The SDS-catalyzed deamidation of other oilseed proteins has also been investigated.

363P

SIMULTANEOUS ENERGY MINIMIZATION OF SEVEN TRIGLYCERIDE α-FORM MOLECULES IN VARIOUS PACKING ARRANGEMENTS. James W. Hagemann and J.A. Rothfus, Northern Regional Research Center, USDA-ARS, 1815 N. University St., Peoria, IL 61604.

A computer modeling procedure has been developed to minimize simultaneously the packing energy of seven triglyceride molecules in theoretical α-form conformations. This procedure, termed the Zero Edge Crystal Technique, varies translation values for the X-, Y- and Z-axes, and vertical rotation angles of six molecules surrounding a centralized stationary molecule and manipulates these parameters in such a manner that each molecule is always surrounded by six other molecules during interaction energy calculations. Most interactions to molecules outside the basic seven are duplications of interactions within the basic seven molecules except for changes in translation and rotation angle values. The program performs calculations to these outside molecules by temporarily placing a molecule in a position specified by parameter values from within the basic group and eliminates the molecule when interaction calculations are completed. This approach reduces memory requirements and eliminates crystal edge effects. Results thus far show that when rotation angles are incremented by more than 5 degrees, molecules assume best-fit positions that contain voids in hydrocarbon chain packing, while very small angle increments permit closer packing with subsequent increases in total packing energy.

364P

BILAYER ASYMMETRY IN LYSOPHOSPHATIDYLCHOLINE/CHOLESTEROL VESICLES: A ^{31}P NMR STUDY. Wolfgang J. Baumann and V.V. Kumar, University of Minnesota, The Hormel Institute, 801 16th Ave. N.E., Austin, MN 55912.

Sonication of lysophosphatidylcholine (lysoPC)/cholesterol (1:1) in Tris/HCl buffer for 3 hr yielded unilamellar vesicles of uniform size (28 nm d.). Phosphorus-31 NMR (32.20 MHz) of the vesicles (20 μmol lysoPC/ml) gave rise to a single peak (40.4 ppm, $\upsilon_1/_2$ 9.4 Hz, T_2* 0.034 sec). Upon addition of paramagnetic ions (1.2–12 mM), the signal was split: an additional, more intense signal appeared downfield (4.04 ppm) due to Pr^{3+} (1.2 mM); an intense upfield (4.01 ppm) signal appeared due to Yb^{3+} (2.5 mM). The more intense signal responsive to paramagnetic ions was assigned to lysoPC located in the outer vesicle shell; the unshifted signal represented inside lysoPC. Based on the peak intensities measured under proton noise decoupled or proton coupled conditions, an outside/inside lysoPC ratio ($R_o/_i$) of 6.5–7.0 was determined. When sonication was done in the presence of Pr^{3+} and outside ions were removed by dialysis, only the less intense (inside) signal remained shifted ($R_o/_i$ 6.8). Addition of Yb^{3+} (8 mM) to vesicles sonicated in the presence of Pr^{3+} (2.4 mM) caused an upfield shift (2.58 ppm) of the outside signal, while the inside signal retained its downfield shift (9.07 ppm; $R_o/_i$ 6.4). By comparison, the $R_o/_i$ value determined for phosphatidylcholine vesicles was only 2.2. Our data show that lysoPC/cholesterol vesicles (1:1) are drastically asymmetric with an outside-to-inside lysoPC ratio of about 6.7 $\pm$ 0.3. This is likely to reflect the geometric requirements of the lysoPC molecule.

365P

KINETICS OF LIPASE-CATALYZED ESTER EXCHANGE REACTIONS IN ORGANIC MEDIUM UNDER CONTROLLED HUMIDITY CIRCUMSTANCES. H.L. Goderis and P.P. Tobback, Catholic University of Leuven, Leuven, Belgium.

Rhizopus arrhizus lipase (E.C. 3.1.1.3.) immobilized by adsorption on Celite 535 (BDH) was used in n-hexane for the study of the interesterification reactions of triolein with palmitic acid at various relative concentrations. In this study, all elementary reaction steps involved in the interesterification are quantitatively studied separately. The equilibria between the various glycerides are quantitated. From comparison of experimental data on fatty acid exchange and theoretical calculations, inferences can be made concerning the mechanism of the enzymatic reaction at the enzyme-substrate-complex level. Kinetics are discussed as a function of some important reaction parameters such as the amount of water present in the system, the concentrations of substrates, the influence of the type of fatty acids used. These data are treated in conjunction with adsorption phenomena of reaction components at the interface between the immobilized enzyme phase and the substrate solution.

366P

EFFECT OF DEUTERATION ON PHASE TRANSITIONS AND POLYMORPHISM OF UNSATURATED ACIDS AND ESTERS. Shu-pei Chang and John A. Rothfus, U.S. Department of Agriculture, Northern Regional Research Center, 1815 N. University St., Peoria, IL 61604.

Deuterated methyl oleates, methyl linoleates and 12,15-octadecadienoic acids and methyl esters have been investigated by DSC. They contain 2,3 or 4 deuteriums along the chain, at the *cis*-9 double bond, at the ω-methyl end, or at the ester methyl end. Melting points (Tm) are lower for all deuterated compounds, especially those deuterated at either end. Compared to undeuterated compounds, enthalpies and entropies of fusion are higher for methyl-d compounds, the same for methyl oleate-9,10-d_2, and lower for compounds with deuteriums along the chain, indicating that the effects of shorter C-D bonds on packing of chain ends, *cis* double bonds, and the chains are different. For 12,15-octadecadienoic-9,10-d_2 acids and methyl esters, Tm decreases in the following order: 12t,15t > 12t,15c > 12c,15t > 12c,15c. One to two exotherms generally occurred upon reheating oleates and linoleates, but they were not encountered with the 12t,15c and 12c,15t acids or the 12t,15c methyl ester, which exhibited well-separated exotherms only when cooled below their freezing points. The results evidence distinct effects of double bond position and geometry on lipid crystallization properties. Polymorphism during reheating differed little for the other deuterated compounds, except for very small transitions before and beyond Tm.

367P

ESSENTIAL FATTY ACID DEFICIENCY (EFAD) AND LIPID SUPPLEMENTATION BY TOTAL PARENTERAL NUTRITION (TPN) IN STRESSED AND SEPTIC PATIENTS. Bruce A. Svingen, Ralph T. Holman and Susan B. Johnson, Hormel Institute, University of Minnesota, 801 16th Ave. N.E., Austin, MN 55912, and Peter B. Alden and Frank B. Cerra, Department of Surgery, University of Minnesota.

The fatty acid profiles of serum phospholipids in stressed and in septic patients on TPN were utilized to assess the development of EFAD. At the start of the study, patients were already minimally EFAD. The patients had moderate elevation of 16:0, 16:ω7, 18:0, 18:1ω9, 18:1-isomers and ω3, 20:3ω9. Levels of some of the major fatty acids of the ω6 family were lower than control, including 18:2ω6 and 20:4ω6. One group of patients was continued on TPN without benefit of lipid supplementation. Over the 7 to 11 days of the study, levels of 18:2ω6 decreased farther and 18:1ω9, 18:1-iso and 20:3ω9 increased. The triene/tetraene ratio increased to 0.15, borderline EFAD. A second group of patients received 500 ml/day of Intralipid in addition to their TPN. This soybean oil emulsion

served as a source of dietary ω6 and ω3 polyunsaturated fatty acids. During the relatively short time of the study, these patients showed an improvement in their essential fatty acid status. There was an increase in 18:2ω6 and 20:3ω6 while 18:1ω9, 18:1-iso and 20:3ω9 all decreased. However, 20:4ω6 did not show a positive response to dietary supplementation. In fact, the percentage of 20:4ω6 in the phospholipid fraction decreased. This decrease in 20:4ω6 may be due to its increased metabolism in the stressed and septic patients. The increases of other ω6 metabolites beyond 20:4ω6 indicate that the synthesis of 20:4ω6 from dietary precursors is not blocked.

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A NEW METHOD FOR FATTY ACID-SUGAR ESTER SYNTHESIS AND PURIFICATION. Sandra Cassina Kea and C.E. Walker, University of Nebraska, Department of Food Science and Technology, Lincoln, NE 68583-0919.

A new method of synthesis and purification of fatty acid-sugar esters has been extremely successful. A variety of fatty acids has been used, including stearic, palmitic, lauric and caprylic. The sugars used have been sucrose, glucose, fructose, maltose and corn sugars of various dextrose equivalence. Spectral analysis has been done which indicates that there are several different levels of substitution of the fatty acid onto the sugar moiety. The yields are roughly 80–95%. Some baking analyses have been done which indicate that the products have emulsification properties superior to those shown by presently available sugar esters and commonly used monodiglycerides.

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EFFECTS OF DIETARY FATTY ACIDS ON FATTY ACID RELEASE FROM A PERFUSED RAT VASCULAR BED. J.P. Mtabaji, M.S. Manku and D.F. Horrobin, Efamol Research Institute, P.O. Box 818, Kentville, Nova Scotia B4N 4H8 Canada.

It is now well recognized that polyunsaturated fatty acids have protective effects in cardiovascular disease. These beneficial effects seem to depend on the release of essential fatty acids. We have analyzed free fatty acids in the effluent of isolated perfused mesenteric vessels of rats fed diets of varying fatty acid composition to investigate the effects of dietary manipulation on free fatty acid release using a system with intact cells. Four-wk-old Sprague Dawley rats were fed one of five diets, a regular diet and four other diets containing per 100 g 2.5, 5.0, 7.5, 10.0 g of evening primrose oil (EPO) and 7.5, 5.0, 2.5, 0.0 g of fish oil (FO containing 18% EPA and 11% DHA), respectively. Isolated mesenteries were perfused in vitro and 30-min collections of the effluent were made. Albumin was added to the perfusing medium to act as a trap for free fatty acids. The effluent was extracted with 1.5 vol of chloroform/methanol. The free fatty acid fraction, separated by TLC, was methylated and analyzed by gas chromatography. The release of 18:2n-6 (LA) and 18:3n-6 (GLA) increased linearly with the increase in the proportion of EPO in the diet. Though release of 20:3n-6 (DGLA) showed the same trend much higher quantities of DGLA were noted with the diet containing 7.5% EPO and 2.5% FO and not with the 10.0% EPO diet. FO appeared to blunt the release of 20:4n-6 (AA). The release of 20:5n-3 and that of 22:6n-3 were also strongly affected by the diet, and increased linearly with the increase in the proportion of fish oil in the diet. The results indicate that release of EFA can clearly be manipulated by changes in diet and that release of DGLA can be enhanced, whereas that of AA can be blocked by FO.

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EFFECT OF TYPE OF DIET ON DOCOSAHEXAENOIC ACID LEVEL IN STREPTOZOTOCIN-INDUCED DIABETIC RAT. Y-S. Huang, R. Takahashi, K. Fujii and D.F. Horrobin, Efamol Research Institute, P.O. Box 818, Kentville, Nova Scotia, Canada, B4N 4H8.

Diabetes suppresses the activities of fatty acid desaturation. Thus, an increased 18:2 level and a decreased 20:4n-6 level are frequently demonstrated. However, many reports have also shown an elevated level of 22:6n-3 in diabetic rats suggesting that the metabolism of 18:3n-3 may be different from that of 18:2n-6. Our recent studies have indicated that diabetes suppressed both 18:3n-3 and 18:2n-6 metabolism. Hence, the nonparallel changes of long chain n-3 and n-6 fatty acids in diabetic rats may be a result of low utilization of dietary 22:6n-3. In order to clarify this paradox, we have examined in streptozotocin-induced diabetic rats the effect of different dietary regimens on liver phospholipid fatty acid composition. Sprague-Dawley rats (6–7 weeks old) were induced diabetic by a single intraperitoneal injection of streptozotocin (75 mg/kg) and were fed beef tallow (contained no essential fatty acids), safflower oil (contained 80% 18:2n-6), marine oil (contained 9.6% 20:5n-3 and 11.2% 22:6n-3) and linseed oil (contained 20.4% 18:2n-6 and 48.8% 18:3n-3). After 3 wk of feeding, the fatty acid compositions of liver phospholipids were examined. The levels of 22:6n-3 were significantly increased in diabetic rats fed regular chow (4.5% extractable fat contained 1.8% 20:5n-3 and 1.1% 22:6n-3) with any oil supplementation or fat-free diet supplemented with marine oil. On the other hand, when linseed oil supplemented FF diet was given to diabetic rats, the levels of 22:6n-3 were significantly decreased. These results indicated that the accumulation of 22:6n-3 in liver PL of chow or marine oil-fed diabetic rats did not result from an increased conversion of 18:3n-3, but was due to a low utilization of dietary 22:6n-3 in diabetic rats.

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LIVER AND PLASMA PHOSPHOLIPID FATTY ACIDS IN RATS FED WITH MARINE AND EVENING PRIMROSE OILS. B.A. Nassar, Y-S. Huang, M.S. Manku and D.F. Horrobin, Efamol Research Institute, P.O. Box 818, Kentville, Nova Scotia, Canada B4N 4H8.

Linoleic acid (LA) and its metabolites are known to lower plasma cholesterol. There is also an inverse correlation between the levels of LA in adipose tissue, and more of dihomogammalinoleic acid (DGLA), and the risk of coronary heart disease (CHD). Fish oil rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), also known to lower the plasma triglycerides and cholesterol, is associated with the low incidence of CHD in Eskimos on a traditional diet. In the present study, we examined the interrelation between LA metabolites and fish oil fatty acids. Sprague-Dawley rats (150 g) were fed for 2 wk a semisynthetic fat-free diet supplemented with 10% by weight of different combinations of evening primrose oil, rich in LA and gamma-linolenic acid, the immediate precursor of DGLA, and Polepa, a marine oil rich in EPA and DHA. The fatty acid compositions of liver and plasma phospholipids were then analyzed. Results showed that animals fed higher proportions of Polepa consistently contained higher levels of DGLA ($p < 0.05$), and lower levels of arachidonic acid (AA) ($p < 0.01$). A highly significant inverse correlation between EPA levels and AA/DGLA ratio was found in both plasma ($r = -0.77$) and liver ($r = -0.79$). However, no such correlation was found between DHA levels and AA/DGLA ratio. This result suggests that EPA, but not DHA, in fish oil exerts an inhibitory effect on the conversion of DGLA to AA, and confirms the previous reports that n-3 fatty acids suppress the desaturation of n-6 acids.

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ISOLATION BY HPLC OF FREE MALONDIALDEHYDE AS A PRODUCT OF THE XANTHINE OXIDASE DEPENDENT, IRON-PROMOTED PEROXIDATION OF CARDIAC MEMBRANE PHOSPHOLIPID. David R. Janero and Barbara Burghardt, Hoffman-La Roche Inc., 340 Kingsland St., Building 76, Room 801, Nutley, NJ 07110.

Free radical formation by xanthine oxidase (XO) in the presence of tissue iron may help establish myocardial infarction through membrane phospholipid peroxidation and resultant formation of cyclic fatty acyl endoperoxides. Malondialdehyde (MDA) is recognized as one of several breakdown products of cyclic endoperoxides and, as such, is often taken as a quantitative measure of lipid peroxidation by virtue of its forming a colored adduct with thiobarbituric acid (TBA) upon heating at low pH. But since TBA-reactive substances other than MDA and the conditions of the "TBA test" itself influence both the stability of lipid endoperoxides and the formation of MDA-TBA chromogen, TBA number need not be indicative of actual MDA mass, and, therefore, of the incidence of lipid peroxidation. We report a quantitative kinetic study of MDA formation from liposomes comprised of native rat heart cardiac membrane phospholipids by XO acting on hypoxanthine in the presence of 100 μM iron at pH 7.4 (Hepes-KCl buffer). Kinetics of the brakdown of lipid endoperoxide were determined in two ways: (a) as TBA-reactivity of the reaction mixture under newly defined conditions whereby system components do not interfere with the development of MDA-TBA chromogen, and (b) as free MDA isolated by aminophase-HPLC. Throughout the course of the reaction, the MDA molar-equivalents as determined in the TBA test were identical to the actual molar amoutns of free MDA isolated chromatographically: both reached a maximal value of $\sim$45 μmol MDA/L l in $\sim$120 min. Since when analyzed by HPLC the reaction mixture is not subjected to the acidification or high heat of the TBA test, we conclude that the free MDA generated in this system is entirely produced during the peroxidation reaction itself as a bona fide product of the XO-induced peroxidation of cardiac membrane phospholipid and is not an artifact of the TBA test. It appears that the presence of iron in the enzymatic peroxidation reaction promotes the conversion of fatty acyl endoperoxide to free MDA.

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ANOXIA-LIKE INJURY IN THE NEONATAL RAT HEART MUSCLE CELL INDUCED BY THE FATTY ACID ANALOG 5-(TETRADECYLOXY)-2-FUROIC ACID (TOFA). David R. Janero and Charles Burghardt, Hoffmann-La Roche Inc., 340 Kingsland St., Building 76, Room 801, Nutley, NJ 07110.

The hypolipidemic agent TOFA alters the balance between free fatty acid oxidation and glucose oxidation, accelerating the former and thereby imposing feedback inhibition upon the latter. We have investigated the influence of TOFA on the heart muscle cell (myocyte) of the neonatal rat; this cell depends more upon the rather inefficient glycolytic pathway for its energy and less upon fatty oxidation. Myocytes in primary monolayer culture were labeled for 24 hr with [^{3}H]arachidonic acid and [^{14}C]palmitic acid to tag newly synthesized acyl lipids (mainly membrane phosphoglycerides) metabolically. The myocytes were subsequently washed free of unassimilated radioactivity and were then incubated for up to 17 hr with 0.1-1.0 mM TOFA (final concentrations). HPLC/TLC with radiochemical scanning was used to monitor the fates of the cellular acyl lipids and the myocyte high-energy nucleotide pools. Even with oxidizable serum lipid and glucose substrates present in the culture medium, myocytes incubated with TOFA for 5-6 hr stopped beating and evidence a granular cytoplasm containing numerous refractile lipid vesicles. By 7 hr exposure to TOFA, the myocytes displayed a dose-correlated loss in ATP ranging from $\sim$5% in the presence of medium (i.e., serum) glucose to >25% in its absence. Myocytes in both glycose-containing and glucose-free media were virtually depleted (>90% loss) of high-energy nucleotides by a 17 hr exposure to TOFA at >0.1 mM; these cells displayed deformities in several membranous organelles. By 7 hr, TOFA also elicited a dose-correlated release of [^{3}H]- and [^{14}C]-lipid label from myocyte membrane phosphoglycerides, largely as free fatty acid. By 17 hr at TOFA concentrations >0.3 mM, the release of cellular lipid radioactivity into the medium approached 50% of that which had been incorporated into newly synthesized membrane phosphoglycerides. In those cases where ATP depletion and lipid loss were $\lesssim$20%, the cells were able to recover metabolic function as shown by vigorous steady beating and ATP repletion to pre-TOFA levels within 12-24 hr after TOFA removal. Thus, TOFA does not lead to a more efficient energy metabolism in these cells. Rather, the response of neonatal myocytes to TOFA resembles the effects of oxygen deprivation (anoxia) on the heart cell; under both circumstances, the myocyte may be placed in an energy state detrimental to its normal physiology and the maintenance of the molecular integrity of its membrane phospholipids. If not reversed, the consequent structural and metabolic alterations can lead to heart muscle cell death.

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DIETARY CARBOHYDRATE AND ALCOHOLIC FATTY LIVER. G. Ananda Rao, Diana E. Riley and Edward C. Larkin, V.A. Medical Center, 150 Muir Rd., Martinez, CA 94553.

We have observed earlier that the fatty liver produced in rats by the consumption of Lieber-DeCarli ethanol diet for four weeks was prevented if the diet was supplemented with pyruvate (*Lipids* 19, 583 [1984]). It was not known whether the decrease in the percent contribution of alcohol calories (33% from 36%) in the diet due to the inclusion of pyruvate had a role in abolishing fatty liver. Hence, in the present study, we maintained the caloric content of alcohol at 36% in spite of the addition of pyruvate. Even when rats were fed such a diet for four weeks, the liver triglyceride content was comparable to that in controls which were pair-fed an isocaloric diet in which alcohol was replaced by dextrins. We also observed that when rats were maintained on a Lieber-DeCarli alcohol diet supplemented with glycine, fructose or galactose, fatty liver was not produced. The carbohydrate content of Lieber-DeCarli alcohol diet is very low (11% of total calories). Therefore, when rats ingest this diet, the liver glycogen level is markedly decreased. However, when rats consumed the alcohol diets supplemented with pyruvate, glycine, fructose or galactose, the hepatic glycogen content increased significantly. Our results suggest a strong association between the level of dietary carbohydrate and the development of alcoholic fatty liver.

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CALIBRATION HUMAN SERA LOW DENSITY LIPOPROTEIN (LDL) AND HIGH DENSITY LIPOPROTEIN (HDL) GRADIENT GEL ELECTROPHORESIS SUBFRACTIONS BY ANALYTIC ULTRACENTRIFUGATION. Frank T. Lindgren, A.V. Nichols, G.L. Adamson, M.A. Austin, L.A. Glines, V. Martin and R.M. Krauss, Lawrence Berkeley Laboratory, Donner Laboratory, 1-315, University of California, Berkeley, CA 94720, and P.D. Wood, Stanford University School of Medicine.

In a population of 90 normal adult males, total LDL d < 1.063 g/ml and total HDL d < 1.20 g/ml fractions were studied by both gradient gel electrophoresis (GGE) and analytic ultracentrifugation (AnUC). LDL GGE was on Pharmacia 2/16 gels stained with Coomassie Blue-R (555 nm). HDL GGE was on Pharmacia 4/30 gels stained with Coomassie Blue-G (603 nm). LDL densitometric integration within the R_f ranges 0.22-0.30-0.36-0.38-0.41-0.45-0.49 (relative to apoferritin) corresponded to S_f intervals of total intermediate density lipoprotein (S_f 10-20), S_f 8-10, S_f 6-8, S_f 4-6, S_f 2-4 and S_f 0-2, respectively. Mean correlation coefficients between such corresponding GGE and AnUC intervals were 0.62 ($p < 0.0001$). HDL densitometric integration within R_f ranges 0.45-0.63-0.71-0.84-0.95 (relative to albumin) corresponded to HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b} and HDL_{3c}, respectively. Mean correlation coefficients were $r = 0.84$ ($p < 0.0001$). GGE component concentrations were calculated from % total area under each component and the total LDL and HDL concentrations, obtained independently of the AnUC. GGE areas were assumed proportional to concentration, although LDL subfractions ranged from ca. 18-30% wt% protein and HDL ranged from ca. 40-60% wt% protein. Calibration refinements require experimental evaluation of the relative dye uptake per unit mass of each GGE subfraction. Correlations between AnUC total LDL and LDL determined by

LCL-cholesterol and quantitative microagarose electrophoresis were 0.900 and 0.870 ($p < 0.0001$), respectively. Correlations between AnUC total HDL and HDL determined by HDL-cholesterol and electrophoresis were 0.845 and 0.882 ($p < 0.0001$), respectively.

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ABSOLUTE CONFIGURATION OF PENTAHYDROXYL BILE ALCOHOLS EXCRETED BY PATIENTS WITH SITOSTEROLEMIA AND XANTHOMATOSIS(SX): CIRCULAR DICHROISM AND C^{13}-NMR STUDIES. B. Dayal, UMDNJ-New Jersey Medical School, 100 Bergen St., Newark, NJ 07103, and G.S. Tint, B. Toome and G. Salen, UMDNJ-New Jersey Medical School and VA Medical Center.

The absolute configuration of the C_{26}- and C_{27} pentahydroxy bile alcohols present in the urine and feces of a patient with sitosterolemia and xanthomatosis(SX), a rare inherited lipid storage disease, were determined from the lathanide-induced circular dichroism Cotton effects and C^{13}-NMR measurements. Under anhydrous conditions CD spectra of 26(or 27)-nor-5β-cholestane-3α,7α,12a,24S ,25ξ-pentol and 5β-cholestane-3α,7α,-!°α,-25(s),26-pe ntol in the presence of Eu(fod)$_3$, exhibited large induced split Cotton effects at 310 and 320 nm, respectively. Based on a model (Secondary-Secondary and primary-tertiary α-diols) in which R compounds have positive Cotton effects and S compounds have negative Cotton effects at 310 or 320 nm, it was concluded that the C_{26}- and C_{27} bile alcohols have the 1,2 glycol structures with carbons 24 and 25 having the S-configurations. These assignments were based upon comparison with model compounds, 5-cholestene-3,24(R),25-triol and 25(R),26-dihydroxycholesterol, whose single-crystal x-ray structure and C^{13}-NMR studies have been determined. The importance of these data is to establish a structural mechanism for the conversion of 5β-cholestane-3α,7α,-12α,-24S,25-pent ol rather than 5β-cholestane-3α,7α,12α,-24R,-25-penc ol into cholic acid in man as well as animals.

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IS AUTOCLAVING NECESSARY FOR AN ADEQUATE GROWTH PERFORMANCE OF RATS FED ON WINGED BEAN SEED FLOUR? Sachi Sri Kantha and J.W. Erdman Jr., University of Illinois, Urbana-Champaign, Department of Food Science, 567 Bevier Hall, 905 S. Goodwin Ave., Urbana, IL 61801.

The potential of winged bean (*Psophocarpus tetragonolobus*) as an oil and protein source as reviewed previously by our laboratory (*JAOCS* 61, 515 [1984]). In the present study, the growth performance of weanling rats fed one of three test diets of winged bean seed flour (WBSF) prepared by using village scale processing methods was studied. Five groups, each consisting of six animals, were fed the following diets: 10% casein, 15% untreated WBSF, 10% dry-heat treated WBSF, 10% wet heat-treated WBSF or no protein. Untreated WBSF and dry heat-treated WBSF diets were not conducive for growth. The NPR values calculated for casein-fed rats and wet heat-treated rats were 3.27 and 0.76 respectively. PER values for the same two groups were 3.16 and 2.17 respectively. In comparison to the previously published reports, this study infers that the protein quality of WBSF prepared form 30 min boiled seeds is comparable to that of WBSF prepared by autoclaving, a method not available at the village level.

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PURIFICATION AND SUBSTRATE SPECIFICITY OF RABBIT HEART LIPOPROTEIN LIPASE. John E. Bauer and Gary J. Sciscent, University of Florida, J-144 JHMHC, Department of Physiological Sciences, Gainesville, FL 32610.

Lipoprotein lipase (E.C. 3.1.1.34) was purified via affinity chromatography from rabbit heart. The enzyme was characterized by salt inhibition, an alkaline pH optimum and the need for a serum cofactor. Fatty acyl chain substrate specificity studies were performed comparing equimolar amounts of triolein (18:1Ω9, *cis*) with one other mono-acyl triglyceride in a diheptadecanoyl phosphatidyl choline stabilized, glycerol emulsion substrate. Hydrolyzed fatty acids were determined after extraction, derivatization to methyl esters and identification via gas chromatography with internal standard. Results were normalized based on rates of triolein hydrolysis at 60 min determined concurrently in the same reaction mixture. In addition, 1-palmitoyl, 2-oleyl, 3-stearate and 1,3 distearoyl, 2-oleate were used in separate experiments to measure acyl chain specificity of these mixed acyl triglycerides under similar conditions. The fatty acyl specificity of mono-acyl triglycerides was observed to be: !ç0°Ω6, all *trans* > 18:1Ω9, *cis*> 14:0 > 18:2Ω6, all *cis* > 18:3Ω6, all *cis* > 16:0 > 18:3Ω3, all *cis* > 18:0. For 1,3 distearoyl, 2-oleate, the preferred sequence was: 18:0 > 18:1Ω9, *cis*, while 1-palmitoyl, 2-oleyl, 3-stearate yielded specificities in the order: 16:0 > 18:0 > 18:1Ω9, *cis*. These data show that the interaction of LPL with these artificial substrates involves not only fatty acyl chain but positional specificity as well.

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A COMPARATIVE STUDY OF ADRENATE CYCLOOXYGENATION BY RAM AND SWINE VESICULAR MICROSOMES AND IN WHOLE TISSUE HOMOGENATES. Aldo Ferretti and Vincent P. Flanagan, USDA, ARS, Beltsville Human Nutrition Research Center, Bldg. 308, BARC-East, Beltsville, MD 20705.

We present conclusive mass spectrometric evidence for the synthesis of 1a,1b-dihomoprostaglandin E_2 and 1a,1b-dihomoprostaglandin F_2 by ram and swine vesicular microsomes and in whole tissue homogenates. Comparison of the conditions under which vesicular cyclooxygenase (CO) can utilize exogenous adrenate leads to the following conclusions: (i) swine seminal vesicles are enzyme-poor compared to the ram counterpart, and (ii) the swine CO, for reasons which are not readily apparent, undergoes complete inactivation during microsomal preparation. Cyclooxygenase activity could be demonstrated, however, when porcine vesicles were homogenized in the presence of the substrate. Furthermore, we observed an apparent age effect on the PGE/PGF production ratio in the ram. The objective of this study was to demonstrate the ability of mammalian systems to convert certain 22-carbon fatty acids into prostaglandins. On the basis of our findings, as well as analogous results previously reported, a biological role of the C22 fatty acids as prostaglandin precursors appears probable.

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AN IMPROVED METHOD FOR MASS SPECTROMETRIC QUANTIFICATION OF PGE-M. ITS APPLICATION TO A HUMAN DIET STUDY. Aldo Ferretti, Joseph T. Judd and Mary W. Marshall, USDA, ARS, Beltsville Human Nutrition Research Center, Bldg. 308, BARC-East, Beltsville, MD 20705.

A method previously developed in our laboratory for the assay of PGE-M, the terminal urinary catabolite of E prostaglandins (PGE), has been extensively improved and applied to a human diet study. The modifications, which concerned the multistep cleanup stage, resulted in prolonged life for the chromatographic columns and enhanced reproducibility of data. The interassay coefficient of variation dropped from 11% to 4.5%. The method thus revised was used in a pilot human diet study designed to explore systemic responses of PGE production to changes in dietary linoleate intake. Volunteers were fed two controlled diets containing 35% of energy from fat, with either 10 or 30 g linoleate/day, 30 to 50 g saturated fatty acids/day, and the balance mainly monounsaturates. Changes in the PG system were assessed by measuring PGE-M in 24-hr urine pools. Results indicate that changes in dietary levels of 18:2Ω6 of the described magnitude do not significantly influence total body turnover of PGE.

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EFFECT OF DIETARY PALM OIL AND ITS FRACTIONS ON RAT BLOOD LIPIDS AND HDL LIPOPROTEIN LEVELS. Kalyana Sundram and Augustine S.H. Ong, Palm Oil Research Institute of Malaysia (PORIM), 6 Persiaran Institusi, Bandar Baru Bangi, P.O. Box 10620, Kuala Lumpur, Malaysia, and Khor Hun Teik, Biochemistry Department, University of Malaya.

Male Sprague-Dawley rats were fed a high fat semisynthetic purified diet for a total of 15 wk. Fat content was provided by corn oil, soybean oil, palm oil, palm olein and palm stearin at the 40% energy level. Animals fed the various experimental diets showed no significant differences in organ weight, body weight or organ to body weight ratios. Plasma cholesterol content was lowest in animals fed soybean oil diet, whereas there was no significant difference between the corn oil and palm oil, palm olein and palm stearin groups. The free cholesterol content remained constant for all groups, but variations were evident in the esterified cholesterol content. Total plasma phospholipid content was significantly increased in the palm oil, palm olein and palm stearin groups as opposed to the corn and soybean oil groups. Increased HDL-cholesterol content was evident in the palm stearin and palm olein-fed animals. Only soybean oil group with the lowest HDL-cholesterol content was significantly different from the other four dietary groups. The antithrombotic prostacyclin PGI_2 measured by RIA was unexpectedly highest in palm oil and palm stearin-fed animals. Measurement of the platelet cholesterol/phospholipid molar ratio as a crude indication of membrane fluidity showed no significant difference among the groups. These results indicate that the nutritional properties of palm oil, palm olein and palm stearin are unlike those properties predicted from their respective fatty acid compositions.

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IMPROVED METHOD FOR THE SYNTHESIS OF 1 OR 3-ACYL-*sn*-GLYCEROLS. Dharma R. Kodali, Biophysics Institute, Boston University School of Medicine, Housman Medical Research Center, 80 E. Concord St., Boston, MA 02118.

Optically active 1 or 3-acyl-*sn*-glycerols were synthesized from 1,2 or 2,3-isopropylidene-*sn*-glycerols, respectively. The 1,2 or 2,3-isopropylidene-*sn*-glycerols were condensed with an appropriate long saturated fatty acid (C_{16}-C_{24}) in the presence of DCC and 4-dimethyl aminopyridine and the resulting compounds were treated with dimethylboron bromide at -50 C to give the title compounds. The acetal cleavage reaction by dimethylboron bromide to produce the long saturated 1 or 3-acyl-*sn*-glycerols was very effective and gave good yields (75-90%). The reaction conditions were mild and there was no apparent acyl migration (as evident from optical rotation). The enantiometric 1,2 and 2,3-isopropylidene-*sn*-glycerols were synthesized from D-mannitol and L-arabinose, respectively. The synthesis of 2,3-isopropylidene-*sn*-glycerol was improved to give an overall yield of 40% from L-arabinose. L-arabinose was converted to its diethyl mercaptal derivative by the treatment of ethanethiol and zinc chloride. L-arabinose diethylmercaptal was condensed with 2-methoxypropene in the presence of a catalytic amount of *P*-toluenesulfonic acid to give 4,5-isopropylidene-L-arabinose diethylmercaptal. Oxidation of this compound with sodium periodate followed by sodium borohydride reduction under alkaline conditions yielded 2,3-isopropylidene-*sn*-glycerol $[\alpha]_D^{22} = -14.80$ (neat). The dimethylboron bromide reaction for the acetal hydrolysis of isopropylidene-*sn*-glycerol esters of C_{16}-C_{24} fatty acids described, is very mild, efficient and devoid of all the drawbacks associated with the different acid hydrolysis procedures described earlier.