INFORM January 2014 Volume 25 (1) 1–64

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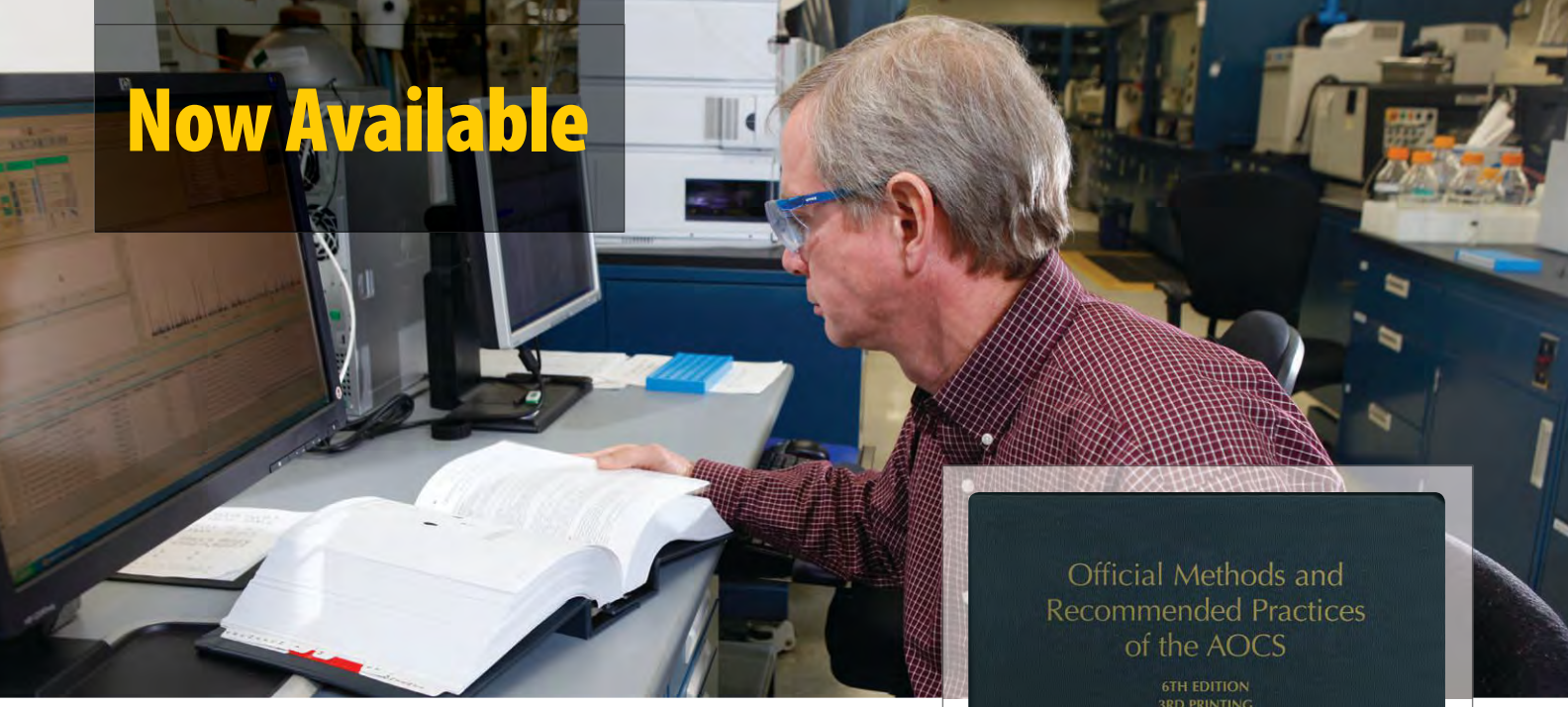


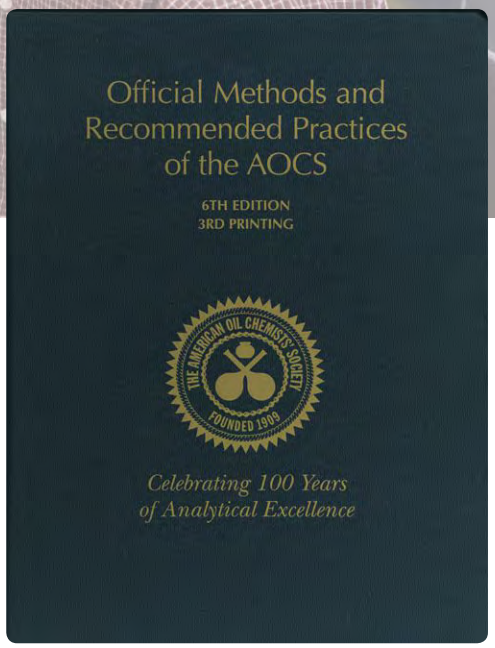
Official Methods and Recommended Practices of the AOCS, 6th Edition, 3rd Printing

The *Official Methods and Recommended Practices of the AOCS* contains currently recognized methodology required for proficiency testing in the Laboratory Proficiency Program (LPP), as well as AOCS Laboratory Certification. Additionally, AOCS methods are internationally recognized for trade, and several are listed by the Codex Alimentarius Commission. Worldwide acceptance has made the *AOCS Methods* a requirement wherever fats and oils are analyzed.

Having current AOCS Methods is one of the requirements for ISO 17025 accredited laboratories. Order the* Official Methods and Recommended Practices of the AOCS*, 6th Edition, 3rd Printing today to guarantee your lab is up-to-date!

The 3rd Printing has been updated with *2013–2014 Methods Additions and Revisions*, which includes several new methods, including three methods for testing 3-MCPD esters, and revisions to 25 previously approved methods.

Make sure you have the latest methods to stay accredited, preserve laboratory integrity, and achieve technical excellence.



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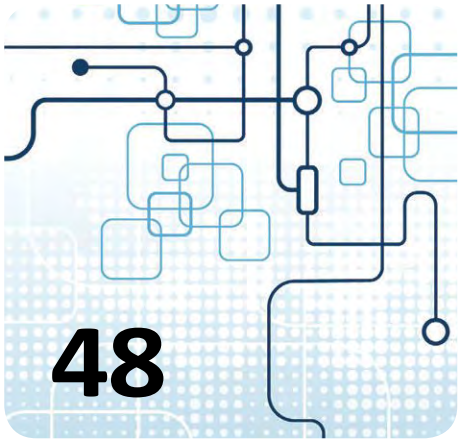

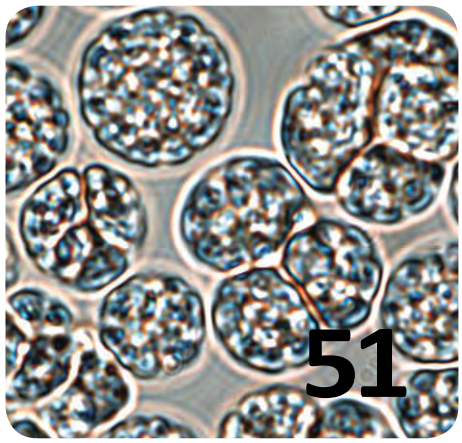

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Palm Oil

Production, Processing, Characterization, and Uses

Oi-Ming Lai, Chin-Ping Tan, and Casimir C. Akoh, Editors
Hardbound. 2012. ISBN: 978-0-9818936-9-3. 852 pages.
Product code: 264

Palm oil is the world's most widely produced vegetable oil and is consumed in over 150 countries. This book serves as a highly informative compilation of recent research and advances in various areas of the palm oil industry.

Palm Oil includes 25 chapters on various aspects of palm, including:

- Production, processing, characterization, and utilization
- Palm oil as a *trans* fat alternative
- Palm biomass, bioenergy, biofuels, and sustainability
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- Uses in food, nutritional, and oleochemical products

This book is ideal for researchers, food scientists, food manufacturers, and other scientists who want to better utilize palm oil and its components.

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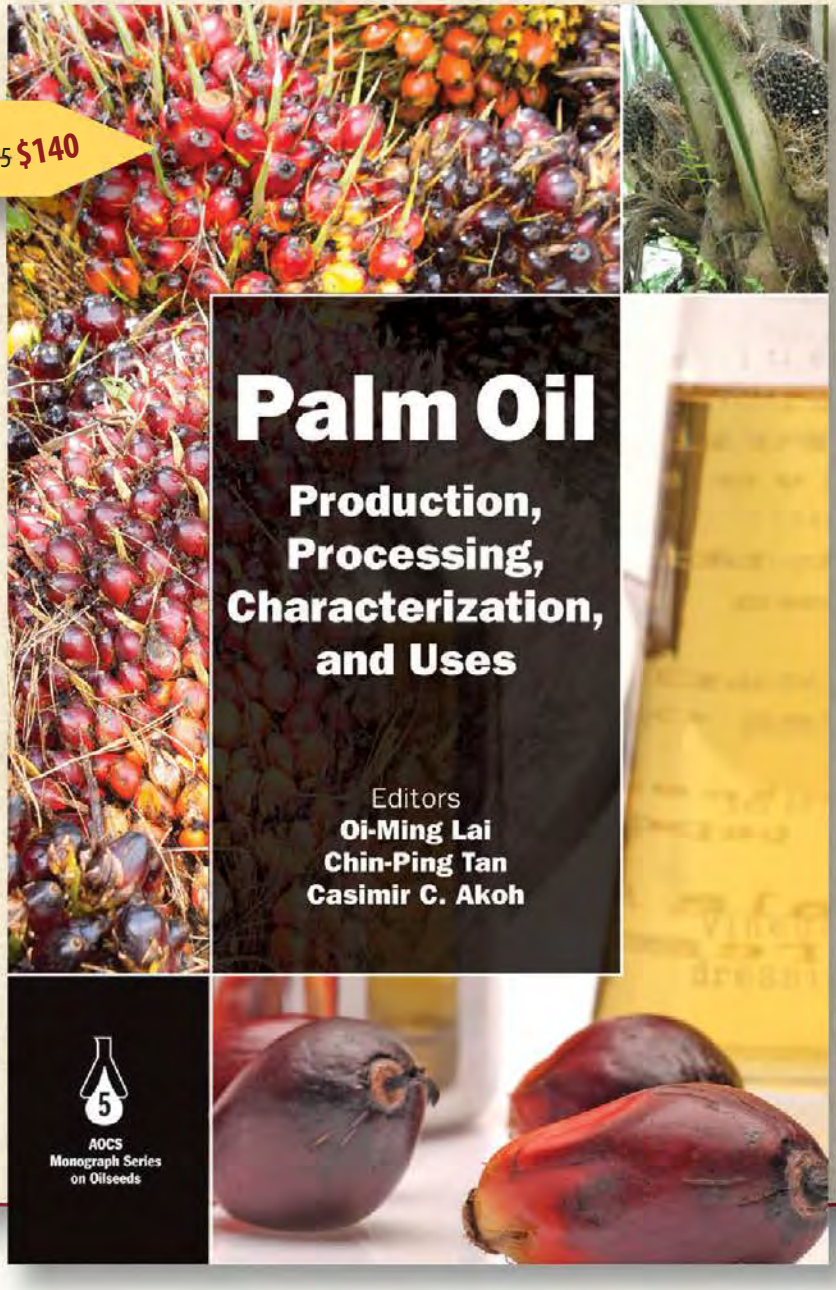

Oi-Ming Lai is a professor in enzyme technology from the Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. She is the coeditor of *Healthful Lipids* (AOCS Press), the author or co-author of more than 100 referenced SCI publications, more than 8 book chapters, and the holder of 8 patents. Oi-Ming's research program includes the development and improvement of structured lipids, upscaling and bioreactor design and kinetics, and product diversification of various oilseed components and their by-products. She has won numerous professional awards including the WIPO's Best Invention by Woman Award in 2008 in Brussels, Belgium. She also sits on the Editorial Board of the Food and Bioprocess Technology Journal published by Springer USA. *AOCS Member since 2001*.

Chin-Ping Tan is an associate professor in the Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia. He has a strong interest in physicochemical properties and changes in the quality of palm oil products. His current research interests center on lipid science and technology, food nanotechnology, food emulsion, food processing, flavor sciences, and thermal analysis. He has published 5 book chapters, over 120 refereed papers in international refereed journals, and presented more than 130 papers in various national and international conferences. To date, he has received more than 40 research awards by various national and international bodies. *AOCS Member 2000–2012*.

Casimir C. Akoh is a distinguished research professor in the Department of Food Science and Technology and an adjunct professor of foods and nutrition at The University of Georgia, Athens. His research interest is in improving human health through healthful lipids produced by enzymatic reactions. He is the co-editor of the book *Carbohydrates as Fat Substitutes* (Marcel Dekker, Inc.), coeditor of *Healthful Lipids* (AOCS Press), editor of *Handbook of Functional Lipids* (CRC Press), the author or co-author of over 210 referenced SCI publications, the holder of three U.S. patents, and is a member of the Institute of Food Technologists, the American Oil Chemists' Society, and the American Chemical Society. He has received numerous international professional awards for his work on lipids including 1998 IFT Samuel Cate Prescott Award, 2003 D. W. Brooks Award, 2004 AOCS Stephen S. Chang Award, 2008 IFT Stephen S. Chang Award, 2008 IFT Research and Development Award, 2009 AOCS Biotechnology Division Lifetime Achievement Award, and 2012 Supelco/Nicholas Pelick-AOCS Research Award. *AOCS Member since 2008*.

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AOCS advances the science and technology of oils, fats, surfactants, and related materials, enriching the lives of people everywhere.

INFORM
International News on Fats, Oils, and Related Materials
ISSN: 1528-9303 IFRMEC 25 (1) 1–64

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Inform (ISSN: 1528-9303) is published 10 times per year in January, February, March, April, May, June, July/August, September, October, November/December by AOCS Press, 2710 South Boulder Drive, Urbana, IL 61802-6996 USA . Phone: +1 217-359-2344. Periodicals Postage paid at Urbana, IL, and additional mailing offices. **POSTMASTER:** Send address changes to *Inform*, P.O. Box 17190, Urbana, IL 61803-7190 USA.

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LIPID OXIDATION IN DRY PET FOOD

- Dry pet food contains a relatively high level of oils/fats to increase the palatability of the products and to provide essential fatty acids, nutrients, and energy.
- Lipid oxidation in dry pet food is different from that in human food since dry pet food has a special composition, structure, and physicochemical properties.
- Preventing lipid oxidation in dry pet food requires new strategies that address the complex mechanisms and factors that are unique to these foods.

Dry pet food is composed of a number of ingredients that contain proteins, lipids, carbohydrates (polysaccharides, oligosaccharides, monosaccharides, and fibers), vitamins, minerals and preservatives (antioxidants and antimicrobials), which provide balanced or special nutrients for companion animals. However, lipid oxidation in dry pet food is closely related to only some of the ingredients, such as oils/fats and oils/fats-containing ingredients. Examples of oils /fats used in the pet food industry would be soybean, canola, sunflower, corn, flaxseed, palm, algal and fish oils, and chicken fat, pork fat or CWG (choice white grease), and beef tallow.

Dry pet food contains a relatively high level of oils/fats to increase the palatability of the products and to provide essential fatty acids, nutrients, and energy for pets. Examples of oils/fats-containing ingredients would be protein meals such as chicken, poultry, pork, beef, lamb, and fish meal as well as liquid/dry palatants that are added to pet foods to make them more palatable. Protein meals are subject to oxidation due to their high levels of fats and proteins, as well as their high surface area-to-volume ratios. In addition, liquid palatants are oil-in-water emulsions that are prone to oxidation. Therefore, oxidative stability and shelf life in dry pet food rely heavily on the initial quality of the oils/fats, protein meals, and palatants.

BAsIc s Tr UcTUr e OF Dr y PeT FOOD

Dry kibble is an extruded product characterized by a porous structure roughly divided into two parts: the kibble matrix (core) and the kibble surface coated with oils/fats and palatant(s). The kibble matrix is produced by mixing a number of formulated dry and liquid ingredients together. The mix is transferred to a pre-conditioner and then moves through an extruder at a defined temperature, pressure, and moisture level, being forced through a spiral-shaped screw and out the die of the extruder. After that, the extruded kibble is dried and cooled. The cooled, uncoated kibble is coated with oils/fats and palatants, then packaged.

When pet food ingredients are exposed to heat and moisture during extrusion, starch gelatinization helps the kibble to bind and causes expansion of the kibble through extrusion. Meanwhile, the protein denatures and the lipid in the ingredients, such as chicken meal, will interact with the starch and the protein. The gelatinization of starch, denaturation of protein, and the interaction of starch, protein, and lipid generate the porous and multiphase structure of the kibble matrix. Also, "air bubbles" can be distributed in the kibble matrix. The lipid in kibble matrix may be called the non-continuous lipid phase, while the lipid on the coating surface would be considered a continuous lipid phase. This would be the case when higher levels of oils/fats are applied to the surface of the kibble.

ch Ar Ac Ter IsTIc s OF LIPID OXIDATION IN Dr y PeT FOOD

While much is already understood about lipid oxidation in bulk oil, oil-in-water (o/w) emulsions, encapsulated oil powder, milk powder, and extruded human food, less is known about the mechanism of lipid oxidation within the kibble structure just

CONTINUED ON NEXT PAGE

Min Hu

Min Hu is a senior scientist working for DuPont Nutrition and Health in New Century, Kansas, USA. He has many years of academic and working experience in the food and pet food industry. The main focus of his research and development is on lipid chemistry, lipid oxidation and digestion, and antioxidants and bioactive compounds. He may be contacted at min.hu@dupont.com.

Jerry Erdmann

Jerry Erdmann is a member of the Innovation team within the Nutrition and Health Division of DuPont. He has served as the group manager–food protection at DuPont for the last eight years. Erdmann received both his B.S. in Microbiology and M.S. in Food Microbiology from Iowa State University. He can be contacted at jerry.erdmann@dupont.com.

described. The characteristics of lipid oxidation in dry pet food are, in fact, quite distinct from those in other foods and ingredients.

Lipid oxidation rates differ on the coated surface of the kibble and in the matrix (core) of the kibble. Because the kibble matrix is porous and the lipids there are non-continuous, lipid oxidation may occur at a higher rate in the matrix than on the coated kibble surface. Coated oils/fats, by contrast, are basically continuous, in particular when a high level of oils/fats is used for enrobing, and a high level of antioxidants may be added to the coated oils/fats to provide further protection against oxidation.

As one can see from Figure 1, hexanal levels in three commercially available dry dog foods were evaluated after being stored for 56 and 68 days. Data were collected from the kibble surface, in the core (matrix), and for the whole kibble. For dry pet food 1, the hexanal level of the core of the kibble was slightly higher than that of the surface. The core of dry pet foods 2 and 3 showed higher hexanal levels in comparison to the surface and whole kibble.

In addition, a commercial dry product containing fish oil was selected to evaluate oxidative stability of the kibble core (matrix) and kibble surface. The propanal level of the product was used as a marker for the evaluation of oxidative stability of the product. Figures 2a and 2b indicate that the peroxide value, hexanal, and propanal levels in the kibble core were higher than on the kibble surface. The data confirm that the kibble core (matrix) is more readily oxidized than the kibble surface.

Lipid oxidation in the kibble matrix could occur rapidly in the non-continuous lipid phase owing to the kibble's porous structure, inclusion of "air bubbles," and high ratio of surface area to volume of the non-continuous lipid. Thus, the lipid oxidation rate in the kibble matrix would be higher, particularly for those products coated with lower levels of oils/fats. The oxidation rate depends on matrix structure, diffusion of O_2, lipid location in the matrix, and the level of coated lipid on the kibble surface. Lipid oxidation on the kibble surface might be similar to bulk oil oxidation, in particular, when a high level of oil/fat and a low level of palatant are applied by enrobing. Accordingly, the rate of lipid oxidation on the kibble surface could be lower.

Lipid oxidation in dry pet food may be attributed to the oxidation of mixed oils/fats, since a dry pet food may contain different oils/fats for coating, such as chicken fat, canola and fish oils. In addition, the dry pet food may contain different protein meals, such as chicken, pork, fish, or lamb, in the kibble matrix.

Furthermore, different oil/fat-containing ingredients in a dry pet food may have varying oxidative stabilities and different degrees of freshness when used in producing dry

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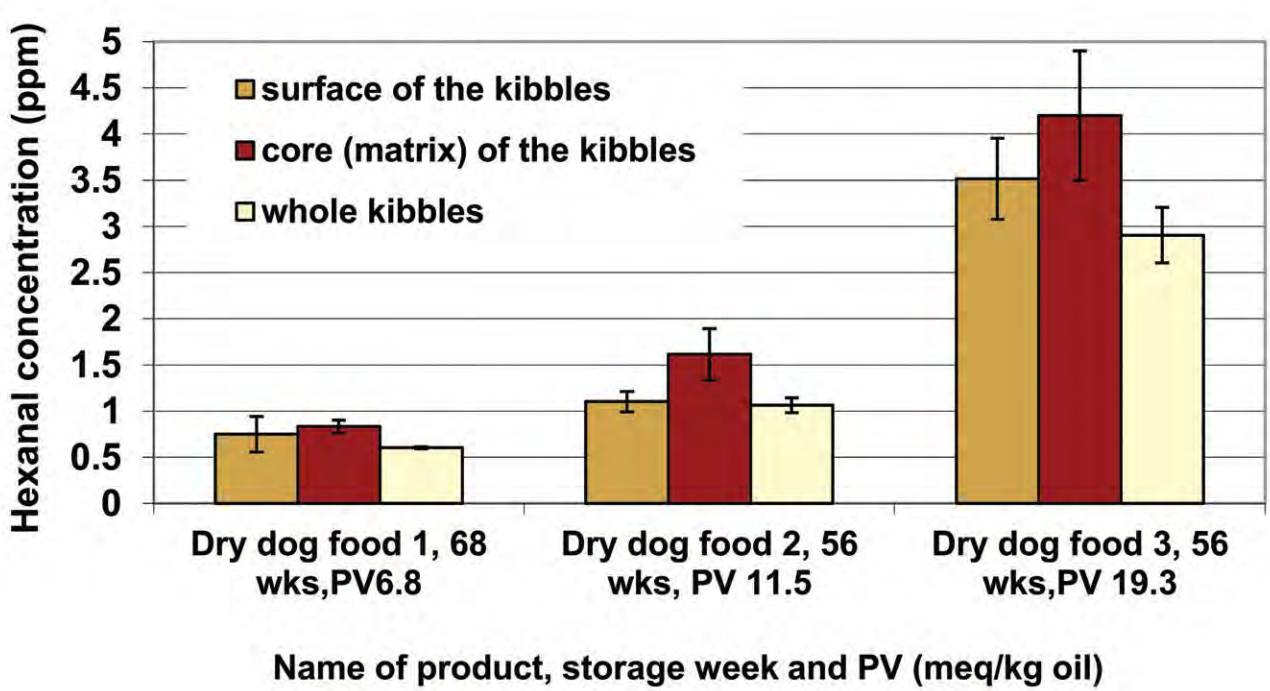


FIG. 1. *Hexanal concentration of surface, core, and whole kibbles of three commercial dry dog foods without fish oil. PV, peroxide value.*

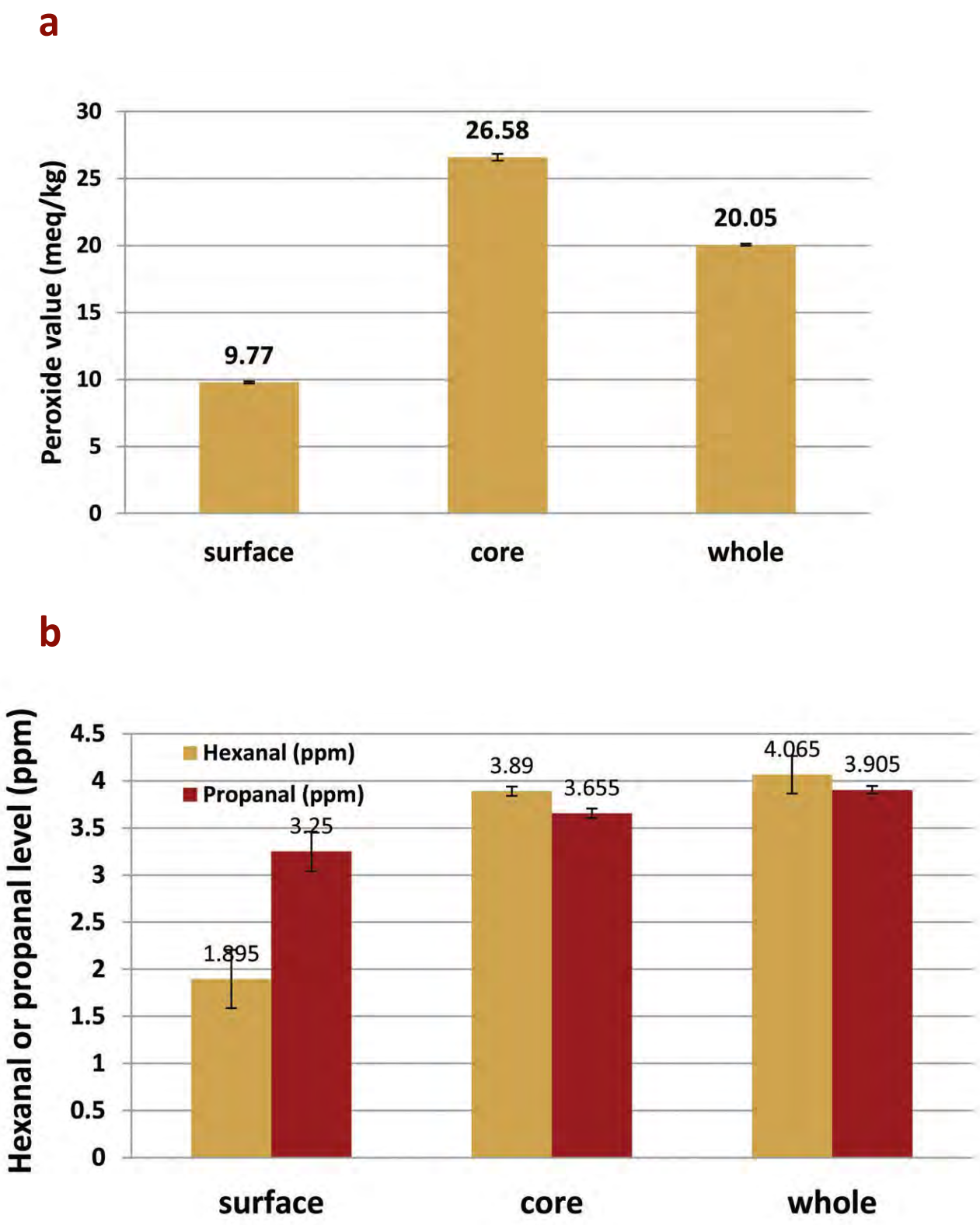


FIG. 2. *(a) PV and (b) hexanal and propanal levels of kibble surface, core (matrix), and whole kibble of a commercial dry pet food incorporating fish oil and stored for 125 days.*

pet food. These may have negative impacts on the oxidative stability of the finished dry product (kibble).

Finally, lipid oxidation reactions in solid phase, like dry kibble, may be different from that in liquid phase, like bulk oil and o/w emulsions. This is because the target molecules, such as oxygen, free radicals, lipids, and antioxidants, may have different diffusion rates in solid and liquid phases. The solid and liquid phases also differ in molecular structure and in physical and chemical properties. Thus, lipid oxidation in the solid and liquid phases may follow different oxidation mechanisms, which need to be studied further.

FAcTOrs AFFecTINg LIPID OXIDATION IN Dry PeT FOOD

Extrusion has an impact on lipid oxidation in dry kibble. For instance, extrusion may inactivate lipoxygenase in some of the ingredients, which would result in a positive impact on lipid oxidation and shelf life; extrusion may lead to the formation of starch-lipid complexes that may slightly protect the lipid in the kibble matrix from lipid oxidation; Maillard reaction products may be formed, which would increase the oxidative stability of the lipids since those Maillard reaction products might be antioxidants.

On the other hand, extrusion increases the porosity of the kibble, thus decreasing its oxidative stability; also, high temperature during extrusion may decrease oxidative stability of the extrudate (kibble). Extrusion can produce kibbles of different sizes, shapes, and surface areas, which can influence the rate of lipid oxidation. Water activity and water content also affect the oxidative stability and shelf life of a dry pet food. Finally, packaging, too, plays a role in the oxidative stability of dry pet foods, especially for those containing higher levels of fish oil.

Because lipid oxidation in dry pet food is complex, and because these products and their ingredients have different molecular structures and physicochemical properties compared with human foods and ingredients, unique strategies are required to effectively prevent lipid oxidation in dry pet food. ■

sPecIALTy LIPIDs IN PET NUTRITION

Jenifer Heydinger Galante

Jenifer Heydinger Galante is senior research manager for Stepan Lipid Nutrition, headquartered in Maywood, New Jersey, USA. She holds a Ph.D. in organic chemistry from the University of Wisconsin-Madison (USA). She can be contacted at jheydinger@stepan.com.

The health benefits offered by long-chain omega-3 fatty acids, medium-chain triglycerides (MCT), and conjugated linoleic acids (CLA) to humans are well documented. A growing number of studies have shown that these lipids offer many of the same health benefits to their pets as well.

FIsh OIL FATTy AcIDs

The long-chain omega-3 fatty acids, EPA (eicosapentaenoic acid, C20:5 n-3) and DHA (docosahexaenoic acid, C22:6 n-3) are found in oils from coldwater marine fatty fish such as tuna, sardines, and anchovies. Scientific research over the years has clearly established that EPA and DHA contribute to the normal function of the heart, while DHA contributes to maintenance of normal brain function and normal vision in humans.

Dietary supplementation with EPA and DHA provides benefits throughout a lifetime. When provided pre- and postnatally, they can accelerate the maturation of the brain and the visual system. In adults, they may protect against coronary heart disease, improve bone health, and reduce inflammatory processes, espe-

cially in rheumatoid arthritis. For the elderly, supplementation may improve cognitive function. Recent research has shown these omega-3 fatty acids provide value in the same health arenas for pets.

In 2006, work conducted by Bauer and colleagues demonstrated the positive effects on visual development of providing puppies with DHA supplementation. Zicker and coworkers published a study in 2012 investigating the effects on cognitive development of providing fish oil for the first year of a puppy's life. Puppies were fed a control diet low in DHA or a diet with moderate or high levels of DHA. Researchers monitored a number of parameters, including the ability of the puppies to learn new tasks. In the reversal task, pups were taught to navigate a positional (T) maze to access a food reward. Then they had to learn to reverse the direction traveled through the maze to find the reward. Puppies receiving moderate and high levels of DHA were able to learn this reversal task with significantly fewer errors than puppies receiving low DHA levels. In addition, puppies on the high DHA diet were able to complete the task successfully more times in a row than dogs on the other diets (Fig. 1, page 12).

In another study published in 2012, Moreau and coworkers provided adult dogs affected by naturally occurring osteoarthritis with either a control diet or a diet rich in long-chain omega-3 fatty acids for 13 weeks. At the beginning, middle, and end of the study, the force exerted on the dog's leg when contacting the ground was measured (the peak vertically oriented ground reaction force). Dogs receiving the omega-3 diet showed significant improvement in motor function as measured by ground reaction force. Perhaps more importantly, the owners of the dogs on the omega-3 diet reported a marked improvement in their dog's activities of daily living.

A review article published by Bauer in 2011 summarized the areas of research in omega-3 supplementation for both dogs and cats. In addition to those areas already mentioned, research has focused on other inflammatory conditions; neurological, cardio, and brain health; kidney health; and even cancer. While more research has been done with dogs than cats, the latter have been shown to benefit from supplementation as well.

MEDIUM-CHAIN TRIGLYCERIDES

MCT have been used in human medical nutrition for more than 50 years. MCT are the C8 and C10 fatty acid esters of glycerin. In comparison, the long-chain triglycerides (LCT) in most fats and oils are primarily made up of C16 and C18 fatty acids. This difference in fatty acid chain length is responsible for the contrasts in physical and metabolic properties between LCT and MCT. MCT are absorbed more quickly and digested more easily than LCT. Unlike LCT, MCT are hydrolyzed completely in the gut. The resulting

CONTINUED ON NEXT PAGE

- **Specialty lipids offer many of the same health benefits to pets as to humans.**
- **Conjugated linoleic acids and medium-chain triglycerides (MCT) both contribute to weight management; MCT can benefit cognition.**
- **Long-chain omega-3 fatty acids provide cardiovascular and neurological benefits.**

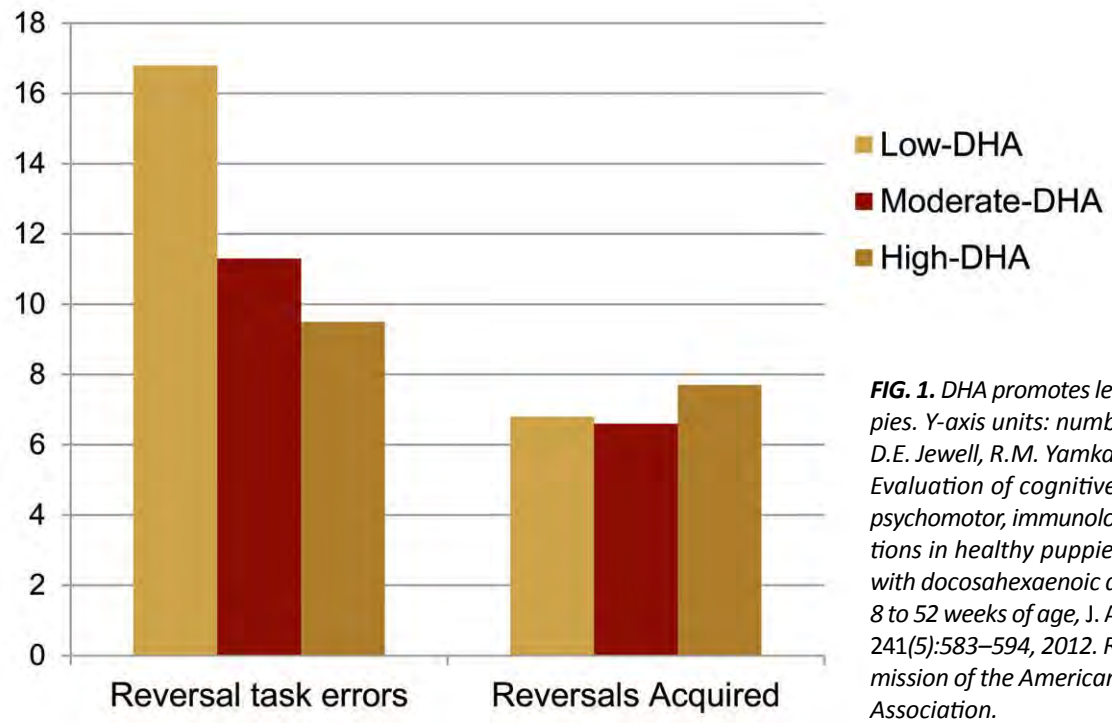


FIG. 1. *DHA promotes learning ability in puppies. Y-axis units: number. From S.C. Zicker, D.E. Jewell, R.M. Yamka, and N.W. Milgram, Evaluation of cognitive learning, memory, psychomotor, immunologic, and retinal functions in healthy puppies fed foods fortified with docosahexaenoic acid-rich fish oil from 8 to 52 weeks of age,* J. Am. Vet. Med. Assoc. 241*(5):583–594, 2012. Reproduced with permission of the American Veterinary Medical Association.*

AOCS methods for pet food analysis

A number of official methods and recommended practices of the AOCS are available for the analysis of the fat and fatty acid content of pet food ingredients and finished products.

Five of the most recently validated methods are:

- Ce 1h-05: *Determination of* cis-, trans-*, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC*—see http://tinyurl.com/Ce-1h-05-GLC.
- Ce 1i-07: *Determination of saturated,* cis-*monounsaturated, and* cis-*polyunsaturated fatty acids in marine and other oils containing long chain polyunsaturated fatty acids (PUFAs) by Capillary GLC*—see http://tinyurl.com/Ce-1i-07-GLC.
- Ce 1j-07: *Determination of* cis-*, trans-, Saturated, Monounsaturated, and Polyunsaturated Fatty Acids in Extracted Fats by Capillary GLC*—see http://tinyurl.com/Ce-1j-07-Cap-GL.
- Ce 2b-11: *Direct methylation of lipids in foods by alkali hydrolysis*—see http://tinyurl.com/Ce-2b-11-AH.
- Ce-2c-11: *Direct methylation of lipids in foods by alkali hydrolysis*—see http://tinyurl.com/Ce-2c-11-AH.

medium-chain fatty acids are able to enter the circulatory system directly via the portal vein. From there they are transported to the liver, where they are rapidly oxidized. Therefore, MCT can provide an important source of energy, especially for patients with impaired LCT metabolism, for premature infants, and for the critically ill.

The ultimate products of oxidation of MCT are the ketone bodies acetoacetate and β-hydroxybutyrate. These ketones are the only alternative source of energy for the brain. This is important in the case of starvation or in diseases involving impaired glucose metabolism, such as Alzheimer's disease. Research has demonstrated that providing MCT to these patients can help to improve cognitive function.

Research has also shown that dogs can receive the same benefits to cognitive function. In 2010, Pan and coworkers subjected aging beagles to a battery of cognition tests to compare the effects of an MCT-supplemented diet to a control diet. In almost all test protocols, those animals receiving MCT showed significantly better performance than the control group. In addition, the more difficult the task, the more pronounced was the effect of the MCT. In one example, the dogs were taught to identify a landmark indicating a food reward. When asked to repeat this task one month later, the test group receiving MCT was able to complete the task with significantly fewer errors than the control group (Fig. 2). Additionally,

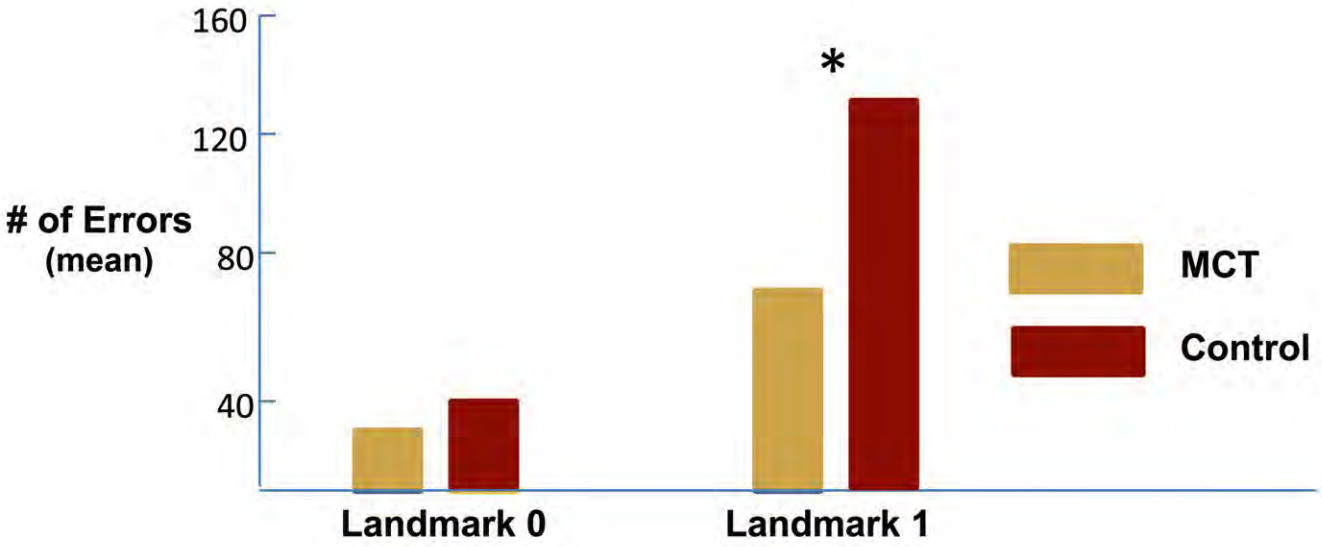


FIG. 2. *Medium-chain triglycerides (MCT) improve cognition in adult dogs. From Pan* et al., Br. J. Nutr. 103*(12):1746–1754, 2010. Copyright © 2010 Yuanlong Pan, Brian Larson, Joseph A. Araujo, Winnie Lau, Christina De Rivera, Ruben Santana, Asa Gore, and Norton W. Milgram. Reprinted with the permission of Cambridge University Press.*

the MCT test group was shown to have significantly higher levels of β-hydroxybutyrate in their bloodstreams, confirming that the MCT were able to increase blood ketone levels to serve as an alternate fuel for the brain.

Another feature of MCT is their ability to increase energy expenditure and fat oxidation compared to consumption of LCT. This can lead to decreased body fat. In 1982, Baba and colleagues fed rats 150% of their daily caloric requirements, with 50% of calories coming from either MCT or LCT. While both groups gained weight, the MCT-fed group gained significantly less weight than the group receiving LCT.

A similar effect has been shown with dogs. In a study conducted by Miller and coworkers (2004), two sets of dogs, one obese and the other lean, were fed either a diet containing MCT or a control diet with LCT. Both groups were fed fewer calories than maintenance to ensure weight loss. Both lean and obese dogs receiving the diet lost significantly more weight than dogs on the control diet (Fig. 3, page 14). In fact, the lean dogs were losing so much weight on the MCT diet that the study had to be stopped early.

CONJUGATED LINOLEIC ACIDS

CLA were first identified by researchers (Ha *et al.*, 1987) at the University of Wisconsin-Madison (USA) as potent anti-

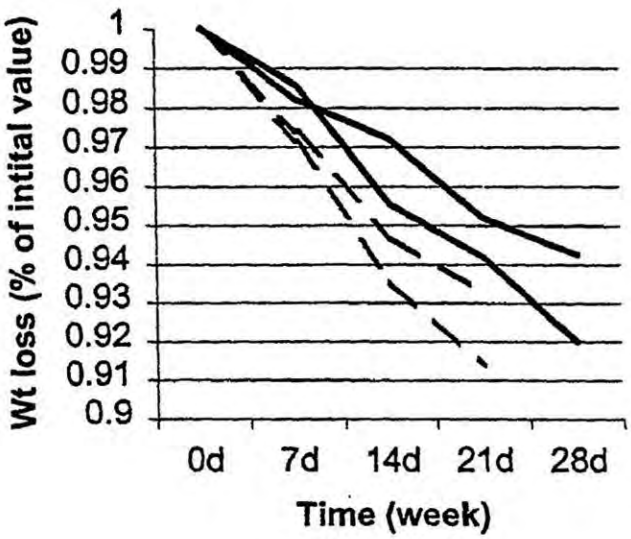
CONTINUED ON NEXT PAGE

Global pet food market to reach $75 billion by 2017

According to a market report published by Transparency Market Research, "Pet Food Market—Global Industry Size, Market Share, Trends, Analysis and Forecast, 2011–2017" (published August 2012, see http://www.transparencymarketresearch.com/pet-food-market.html), the global pet food market was worth $58.6 billion in 2011 and is expected to reach the value of $74.8 billion in 2017, growing at a compound annual growth rate (CAGR) of 4.2% from 2011 to 2017.

North America is expected to remain the largest regional segment for the pet food industry in terms of revenue generation, accounting for about 40% of the total revenue. Asia Pacific will be the fastest growing segment due to a growing trend toward and increasing importance of pets in homes.

The North American market for pet foods was valued at $21.7 billion in 2011 and was expected to grow to $22.4 billion in 2012.With the number of market drivers and product innovations in pet foods, the North American pet foods market is forecasted to reach $26.6 billion in 2017 at the CAGR of 3.5% from 2011 to 2017. The European market, being the second-largest consumer of pet foods, is expected to grow at a CAGR of 4.4% from 2011 to 2017.



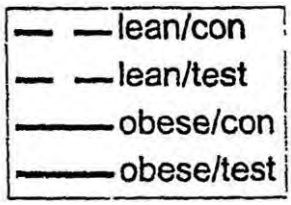


FIG. 3. *MCT-associated increase in weight loss in dogs. Abbreviations: MCT, medium-chain triglycerides; CON, control. From Miller et al. (2004).*

cancer agents in lab animals. Since their discovery, they have been investigated extensively, particularly for the ability of CLA to reduce fat mass, increase lean body mass, and improve fat oxidation. Research has indicated that CLA may also help to reduce inflammation, increase bone mineralization, and improve exercise endurance, all of which mimic the effects of exercise on physiology.

Commercial sources of CLA are 50:50 mixtures of the two active isomers, *cis*-9,*trans*-11- and *trans*-10,*cis*-12-CLA . They are derived from linoleic acid (*cis*-9,*cis*-12 C18:2) commonly found in safflower, sunflower or soybean oils.

While humans respond quite well to CLA supplementation for body fat loss, some animals, especially mice, dogs, and pigs, respond even better, resulting in dramatic effects. Research conducted by Hargrave and coworkers (2002) showed that a 1:1 mixture of the two CLA isomers caused a significant decrease in abdominal fat in mice. This work also helped to establish that it is the *trans*-10, *cis*-12 CLA isomer that is responsible for body fat reduction (Fig. 4).

CLA's ability to increase endurance capacity has been demonstrated in several trials. Kim and colleagues (2010) fed mice for 10 weeks on a CLA diet; the mice significantly increased their time to exhaustion on a treadmill compared to mice on the control diet. Similarly, Mizunoya and coworkers (2005) showed that mice fed a CLA diet for only one week significantly increased their time to exhaustion in swimming trials.

Ten years prior to the CLA swimming endurance trial, Fushiki and coworkers fed mice an MCT diet for six weeks and found that their swim time to exhaustion was significantly increased compared to the LCT-fed control group. This finding leads to the question, what effect would a combination of MCT and CLA have? Although few studies on the interactions of MCT and CLA have been done to date, a study by Hargrave and colleagues in 2005 showed that animals fed a combination of CLA and MCT lost significantly more body fat than animals fed on either diet alone.

While a great deal of research has been performed, ongoing work continues to demonstrate the health benefits that specialty lipids such as the omega-3 fatty acids, MCT, and CLA can provide pets, to help them lead healthier lives and bring continued joy to their owners. ■

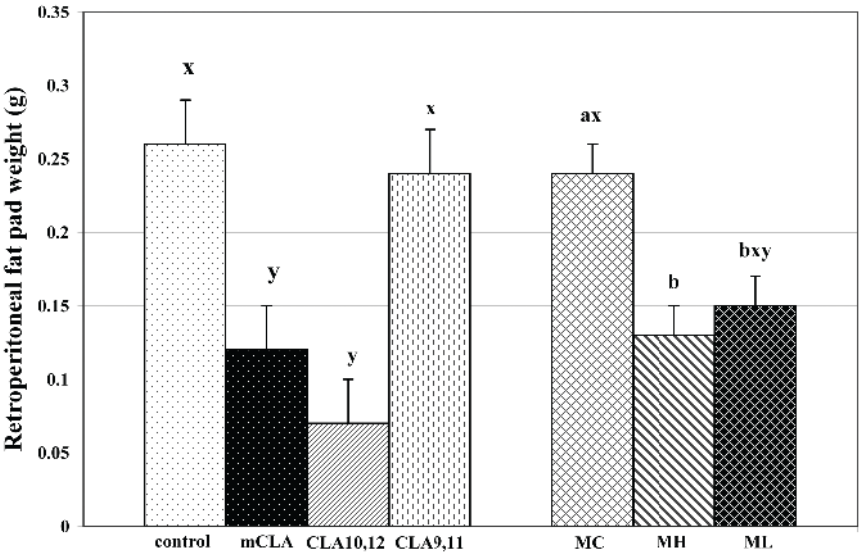


FIG. 4. *CLA promotes body fat loss. Abbreviations: CLA 10,12,* trans-*10,*cis-*12 CLA; CLA 9,11,* cis-*9,*trans-*11 CLA; mCLA, mixture of CLA isomers. From Hargrave et al.,* Obesity 10*(12):1284–1290, 2002. Copyright © 2002 Kimberly M. Hargrave, ChangLong Li, Brett J. Meyer, Stephen D. Kachman, Diane L. Hartzell, Mary Anne Della-Fera, Jess L. Miner, and Clifton A. Baile. This material is reproduced with permission of John Wiley & Sons, Inc.*

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FsmA UPDATe: ANIMAL FOOD/FEED REGULATION RELEASED

On October 26, 2013, the US Food and Drug Administration (FDA) released its long-awaited proposed rule on the production of pet food and animal feed. This latest proposed regulation mandated by the Food Safety Modernization Act (FSMA) of 2010 filled 405 pages and is officially known as the "Current Good Manufacturing Practices and Hazard Analysis and Risk-Based Preventive Controls for Food for Animals."

FDA estimates the proposed rule has a one-time compliance cost to industry of $100.74 million. "Discounting the one-time cost over 10 years at a 7% discount rate and adding the annual costs results in a total annualized compliance cost estimate of $128.75 million," the agency said in a news release.

Industry has 120 days (until February 26, 2014) to comment on the rule. But because the animal food/feed regulation must be examined and commented on in light of two previous FSMA regulations on imported food (the rule on Foreign Supplier Verification Programs, or FSVP) and third-party accreditation, several industry groups asked for an extension of the comment periods for those rules, which were originally set to close on November 26, 2013. On November 19, FDA extended the comment period for FSVP and third-part accreditation to January 27, 2014.

FDA and industry both have a great deal of work to do in very little time: A lawsuit by consumer groups heard by a federal court in California resulted in August 2013 in an amended order to FDA to complete all FSMA comment periods by March 2014. The court also ordered FDA to release the final proposed rules under FSMA on intentional adulteration in foods and sanitary transport of food and feed by December 20, 2013, and January 31, 2014, respectively. Further, the agency must—per the court ruling—publish all FSMA final rules by June 30, 2015. (See Table 1.)

Catherine Watkins
Catherine Watkins is Inform*'s associate editor and can be reached at cwatkins@aocs.org.*

- **In late October 2013, the US Food and Drug Administration (FDA) released the animal food and feed safety regulation under the Food Safety Modernization Act.**
- **The oil processing industry was hard at work analyzing the regulation for its implications as *Inform* went to press.**
- **FDA estimates the one-time cost to industry of compliance at $100.74 million.**

"These rules will have a major impact on our members," said Richard Sellers, vice president of feed regulation and nutrition with the American Feed Industry Association (AFIA), who was also speaking on behalf of the National Grain and Feed Association. Both organizations said they plan to make "extensive public comments" on the rules, as did the National Oilseed Processors Association (NOPA).

The regulation, in FDA's words, "focuses on preventing problems in order to improve the safety of these products. The preventive controls provisions of the proposed rule, which are required by the FDA Food Safety Modernization Act, would apply to domestic and imported animal food, including pet food, animal feed, and raw materials and ingredients." In essence, FSMA will change the regulatory system underpinning food safety in the United States from one that has been largely reactive to one that focuses on prevention.

Under the latest proposed regulation, facilities producing animal feed and food would be required to have written plans that:

- Identify hazards;
- Specify the steps that will be put in place to minimize or prevent those hazards;
- Identify monitoring procedures and record monitoring results; and
- Specify what actions would be taken to correct problems that arise.

In addition, the proposed rule would—for the first time—establish Current Good Manufacturing Practices (CGMP) that specifically address animal food and feed.

FDA has proposed that these requirements be effective 60 days after the final rule is published in the *Federal Register*. Recognizing that small and very small businesses may need more time to comply with the requirements, FDA is proposing tiered compliance dates based on facility size (see www.fda.gov for more details).

CONTINUED ON NEXT PAGE

TABLE 1. Current schedule for promulgation of FSMA regulations (as of November 8, 2013)[a]

Preventive controls for human food [FSMA §103(a) and 103(c)]
January 16, 2013—draft rule published
November 15, 2013—deadline for public comments
June 30, 2015—deadline for publication of final rule
Produce safety standards [FSMA §105(a)]
January 16, 2013—draft rule published
November 15, 2013—deadline for public comments
June 30, 2015—deadline for publication of final rule
Foreign supplier verification program [FSMA §301(a)]
July 29, 2013—draft rule published
January 27, 2014—deadline for public comments
June 30, 2015—deadline for publication of final rule
Accreditation of third-party auditors (FSMA §307)
July 29, 2013—draft rule published
January 27, 2014—deadline for public comments
June 30, 2015—deadline for publication of final rule
Preventive controls for animal food [FSMA §103(a) and 103(c)]
October 29, 2013—draft rule published
February 26, 2014—deadline for public comments
June 30, 2015—deadline for publication of final rule
Intentional adulteration [FSMA §106(b)]b
December 20, 2013—deadline for draft rule
March 1, 2014—deadline for public comments
June 30, 2015—deadline for publication of final rule
Sanitary transport of food and feed (FSMA §111)b
January 31, 2014—deadline for draft rule
May 31, 2014—deadline for public comments
June 30, 2015—deadline for publication of final rule

[a]United States District Court for the Northern District of California ruling, accessed online on November 11, 2013 (see http://tinyurl.com/FSMA-ruling).

HIGHLIGHTS OF THE PROPOSED RULE

The proposed animal food CGMP address similar safety requirements as those contained in the proposed rule to update the human food CGMP regulations. These areas include:

- Hygiene practices for and training of personnel;
- Facility operations, maintenance, and sanitation;
- Equipment and utensil design, use, and maintenance;
- Processes and controls; and
- Warehousing and distribution.

However, the CGMP provisions in the proposed rule for animal food are not identical to those required by the proposed rule on preventive controls for human food. The animal food CGMP, for example, would not address certain practices that do not pertain to animal food, such as allergen cross-contact.

HAZARD ANALYSIS AND RISK-BASED PREVENTIVE CONTROLS

Under the proposed rule, each owner, operator, or agent in charge of a facility (those required to register with FDA under Section 415 of the Food, Drug & Cosmetics Act), with certain exceptions, would be required to comply with the hazard analysis and risk-based preventive controls.

The proposed hazard analysis and risk-based preventive control requirements are similar to Hazard Analysis and Critical Control Points (HACCP) systems, which were pioneered by the human food industry and are required for juice and seafood. They would require operators of a facility to understand the hazards that are reasonably likely to occur in their operation and to put in place preventive controls to minimize or prevent the hazards. "Although this proposed rule aligns well with HACCP," FDA notes on its website, "it differs in part in that preventive controls may be required at points other than at critical control points and critical limits would not be required for all preventive controls."

Each facility would be required to prepare and implement a written food safety plan, which would include the following:

- A hazard analysis that would identify and evaluate known or reasonably foreseeable hazards for each type of animal food manufactured, processed, packed, or held at the facility.
- Preventive controls, which would be identified and implemented to provide assurances that hazards that are reasonably likely to occur would be significantly minimized or prevented. These preventive controls would need to be appropriate for the facility and the animal food being produced, and could address, for example, animal food processing, prevention of cross-contamination, and sanitation affecting animal food safety.

  A recall plan for animal food for which there are hazards that are reasonably likely to occur would be required. It is unlikely that all possible prevention measures and verification procedures would be applied to all animal foods at all facilities. "FDA believes a supplier approval and verification program [that] is a risk-based and appropriate control to significantly minimize or prevent hazards from raw materials and ingredients [. . .] is consistent with current scientific understanding of food safety practices," the agency noted in written materials about the proposed regulation. "Although it is not included in the proposed requirements, it is discussed in the preamble and FDA is seeking comment on such a program."
- Monitoring procedures that would provide assurance that preventive controls are consistently performed and that there are records to document the monitoring.
- Corrective actions that would be used if preventive controls are not properly implemented. Facilities would be required to correct problems and minimize the likelihood of recurrence, evaluate the animal food for safety, and prevent affected animal food from entering commerce. If specific corrective action procedures are not established for the problem, or if a preventive control is found to be ineffective, the facility would also be required to re-evaluate the food safety plan to determine if modifications are needed.
- Verification activities to ensure that preventive controls are consistently implemented and are effective. Verification activities might include records review of monitoring, correction actions, or instru-

"This is the big one," said William Kanitz, president of Scoring Ag (Venice, Florida, USA), a provider of site-specific recordkeeping systems for the agricultural industry.

ment calibration. Preventive controls would also be required to be validated to ensure they are effective in controlling the hazard. In addition, the food safety plan must be reassessed at least every three years and otherwise when necessary. FDA recognizes that product and environmental testing programs are science-based verification activities that are commonly accepted in many sectors of the food industry. As above, although such activities are not included in the proposed requirements, FDA is seeking comment on these programs as well as using the review of customer and other complaints as part of verification.

- Recordkeeping activities, including a written food safety plan, including the hazard analysis. Companies would also be required to keep records of preventive controls, monitoring, corrective action, and verification procedures.

ASSISTANCE TO INDUSTRY

FDA will provide within six months of the publication of the final rule a guidance document that lists the requirements in plain language to help businesses, particularly small businesses, comply with the hazard analysis and preventive controls requirements. In addition, FDA has established a Food Safety Preventive Controls Alliance, with a specific subcommittee dedicated to animal foods, to disseminate information on hazards and controls to help industry, particularly small and very small businesses, comply with the new requirements.

IMPLICATIONS FOR THE OILSEED PROCESSING INDUSTRY

"This is the big one," said William Kanitz, president of Scoring Ag (Venice, Florida, USA), a provider of site-specific recordkeeping systems for the agricultural industry.

"Some companies are struggling to get up and running," he said about the fact that all recordkeeping must be tied to batch numbers and include sanitation activities along with quality control and mycotoxin testing. As onerous as that sounds, Kanitz stressed that in the end, it will cut companies' risk. "Once you get it going, FDA can't come in and order you to throw out a whole year's production. They can only order the destruction of the affected lot number."

Implementation of the FSMA regulations in general and the animal food, feed, and raw grains/ingredients regulation in particular likely will affect smaller facilities more than larger—as most often is the case with regulation. Kanitz said the cost of implementing a computerized recordkeeping system for food safety and HACCP would range from about $1,000 to $5,000, depending on the size of the facility.

Industry groups such as NOPA had yet to file comments on the proposed regulation as *Inform* went to press. Future issues of the magazine will discuss the rule in more detail. ■

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In September 2013, the US Environmental Protection Agency (EPA) withdrew plans to propose two new chemical safety regulations. One would have forbidden chemical manufacturers from claiming the identity of a substance as confidential business information in health and safety data they submit to EPA. The second would have placed bisphenol A, polybrominated diphenyl ethers, and eight phthalates on a federal "chemicals of concern" list.

■■■

Renderer Darling International (Irving, Texas, USA) is acquiring the assets of animal by-products recycler Rothsay, a division of Maple Leaf Foods Inc., for approximately $621 million. Rothsay has a network of five rendering plants in Manitoba, Ontario, and Nova Scotia, and a biodiesel operation in Québec, Canada.

■■■

The contribution of canola to the Canadian economy has reached $19.3 billion per year, according to a report released by the Canola Council of Canada. This latest economic impact estimate was based on data from three crop years: 2009–2010, 2010–2011, and 2011–2012. The report details economic impact by sector, and found that crushing activities accounted for $1.1 billion and refining for $206 million of the $19.3 billion annual total.

■■■

Phosphate rock reserves are limited, nonrenewable, and controlled by a handful of countries, including China, Morocco, Tunisia, and the United States, leaving Europe completely dependent on imports. PhosFarm, a project funded by the European Union (EU), aims to reclaim organic phosphorus from readily available sources such as municipal wastewater, livestock manure, and digestate from biogas plants using immobilized enzymes. After separation of the solid fraction, the released phosphate dissolved in the liquid fraction can be precipitated as magnesium ammonium phosphate and calcium phosphate, which in turn are directly usable as high-value fertilizing salts. See http://tinyurl.com/PhosFarm for more information. ■

NEWS & NOTEWORTHY

US FDA delivers double whammy to veg oils industry

The US Food and Drug Administration (FDA) delivered its long-awaited proposed animal food and feed regulation on October 26, 2013. Just 10 days later, the agency announced it had made a preliminary determination that partially hydrogenated vegetable oils (PHO) are not Generally Recognized as Safe. The announcements put a strain on the edible oils and food industry, which had only 120 days to comment on the food safety regulation and a mere 60 days to comment on the potential ban on PHO in foods.

The animal food and feed regulation under the Food Safety Modernization Act is covered in a separate article in this issue of *Inform* (see page 16). Because the PHO announcement came as *Inform* went to press, the implications of this ban will be discussed in greater depth in the February issue.

EFSA releases preliminary report on occurrence of 3-MCPD in food

The European Food Safety Authority (EFSA) released a preliminary report on September 26, 2013, on the occurrence in Europe of 3-MCPD and 3-MCPD esters in foods. It drew immediate comment from industry groups in Europe and the United States. AOCS also submitted an official response to the report, noting the recent publication of four new validated AOCS methods for the characterization of MCPD-esters, 3-MCPD-esters, and glycidyl-esters (GE) in vegetable oils (see box, page 22).

Esters of 3-monochloropropane-1,2-diol (3-MCPD-E) are process contaminants formed during the refining of vegetable oils.

CONTINUED ON NEXT PAGE

AOCS methods for characterization of 3-MCPD-E and GE

Three new validated indirect methods are available on the AOCS website. This is in addition to the direct method for the characterization of GE published in 2012.

- AOCS Official Method Cd 29a-13 (see http://tinyurl.com/29aTrans)—2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by Acid Transesterification.
- AOCS Official Method Cd 29b-13 (see http://tinyurl.com/GC-MS-29b)—Determination of Bound Monochloropropanediol- (MCPD-) and Bound 2,3-epoxy-1-propanol (glycidol-) by Gas Chromatography/Mass Spectrometry (GC/MS).
- AOCS Official Method Cd 29c-13 (see http://tinyurl.com/29c-differential)—Fatty-acid-bound 3-chloropropane-1,2,diol (3-MCPD) and 2,3-epoxi-propane-1-ol (glycidol), Determination in Oils and Fats by GC/MS (Differential Measurement).
- AOCS Official Method Cd 28-10 (see http://tinyurl.com/GE-direct)—Glycidyl Fatty Acid Esters in Edible Oils.
- In early 2014, AOCS Press will publish the text *Processing Contaminants in Edible Oils: MCPD and Glycidyl Esters*. Edited by Shaun MacMahon of the US Food and Drug Administration, the book covers mitigation and removal strategies as well as analytical techniques and toxicology. Visit www.aocs.org/newbooks for the latest on this new addition to AOCS' resources on process contaminants.

(For more information, visit http://tinyurl.com/ProcessContam to access the AOCS online resources on process contaminants.) The presence of 3-MCPD-E in vegetable oils was first reported in 2006. At that point—in the absence of collaboratively studied methods—little was known about the occurrence, toxicokinetics, or toxicity of 3-MCPD-E. Many data gaps were identified then and still remain.

Studies of the unesterified compound—3-MCPD—have linked it with infertility in rats, suppression of immune function, and possible carcinogenicity. The European Union's (EU) former Scientific Committee on Food established a Tolerable Daily Intake (TDI) of 2 µg/kg body weight for 3-MCPD and, later, legislation in the EU mandated a maximum concentration level of 20 µg/kg of body weight for 3-MCPD in hydrolyzed vegetable proteins and soy sauce. (The TDI is an estimate of the amount of a substance in air, food, or drinking water that can be taken in daily over a lifetime without appreciable health risk.)

The German food safety agency, Bundesinstitut für Risikobewertung (BfR), issued a risk assessment on 3-MCPD-E in 2008. BfR based its risk assessment on toxicological data on free 3-MCPD, under the assumption that 100% of 3-MCPD is released from its esters during digestion. EFSA's Scientific Panel on Contaminants in the Food Chain (CONTAM) agreed with the BfR estimate, in the absence of toxicokinetic data.

EFSA REPORT

The September 2013 EFSA report is titled "Analysis of occurrence of 3-monochloropropane-1,2-diol (3-MCPD) in food in Europe in the years 2009–2011 and preliminary exposure assessment" and is available online at http://tinyurl.com/EFSA-3MCPD.

EFSA reviewed data from 14 EU member states on the occurrence of 3-MCPD in foods. The review found that although most of the population groups consumed less than half the TDI, toddlers and the elderly could be at risk of consuming more than the recommended maximum amount. Kartika Liotard—a member of the European Parliament (EP) for the Netherlands and a member of the EP's Committee on the Environment, Public Health and Food Safety—has called for further action on 3-MCPD-E (see http://tinyurl.com/Liotard-EP). Specifically, Liotard asked:

- "Will the Commission . . . lay down 3-MCPD content limits for margarine and similar products? If so, when? If not, what was the reasoning behind establishing limit values for soy sauce and not for margarine and similar products?
- "In setting limit values, will the Commission take special account of the eating habits of senior consumers, whose excessive 3-MCPD intake, according to EFSA findings, gives particular cause for concern?
- "The survey also shows that certain types of baby milk powder and follow-on formula contain high 3-MCPD concentrations. However, it gives daily intake figures for only two groups of babies. Does the Commission consider this sufficient to conclude that their intake does not exceed the safety limit? In view of the high 3-MCPD content in baby milk powder and follow-on formula revealed by the survey, will the Commission seek to impose maximum limits in respect of these products?"

Liotard's response to the report received significant press coverage in the Netherlands. As *Inform* went to press in late October 2013, the European Commission had not yet responded to her questions or a request by *Inform* for comment. *Inform* also asked the US Food and Drug Administration for comment; a spokesperson said, "FDA is aware of the report and is reviewing its findings."

INDUSTRY RESPONSE TO EFSA

FEDIOL—the trade group representing the EU vegetable oil and protein meal industry—responded to the EFSA report on October 3, 2013 (see http://tinyurl.com/FEDIOL).

"As acknowledged by EFSA, its report is preliminary," FEDIOL noted. "Further work is needed to reduce the uncertainty factors detailed by the report and to build a risk assessment upon it. It should also be noted that the data in the EFSA report have been collected between 2009 and 2011, at a time where analytical methods available for vegetable oil and fats were not validated yet.

"The availability of validated methods from 2012 has enabled our sector to explore technologies to reduce the occurrence levels of 3-MCPD-E and their viability," FEDIOL concluded.

IMACE, the European Margarine Association, said in a written statement: "The food industry, and among them margarine

"Will the Commission lay down 3-MCPD content limits for margarine and similar products?"

manufacturers, take[s] very seriously all new scientific insights on this class of compounds formed during the refining of vegetable oils and fats and not during the margarine production process. EFSA's preliminary exposure assessment is a first step toward the risk assessment to be carried out by competent authorities."

In the United States, the Institute for Shortening and Edible Oils (ISEO; Washington, DC) affirmed that its "members view food safety as their utmost concern; therefore, ISEO will continue to monitor scientific developments and to assist academic researchers, industry partners, and government agencies when possible to expand our knowledge of 3-MCPD esters. In the meantime, caution should be exercised in translating the EFSA occurrence findings to intakes in the United States and other areas around the globe. Consumption patterns and food formulations, including the types and amounts of fats in oils, may differ markedly from region to region."

The vegetable oil industry has also worked to develop mitigation strategies. The key unit operation in the refining process for the mitigation of 3-MCPD-E is bleaching, according to Wim De Greyt, R&D manager at Desmet Ballestra Group (Zaventem, Belgium), which supplies engineering services as well as plants and equipment for all fats and oils-related industries.

3-MCPD-E are not formed during the bleaching process, De Greyt explained in an email, but precursors such as acid and chlorine are brought into the oil during bleaching if HCl-activated bleaching earth is used. "We therefore recommend the use of non-HCl-activated or neutral bleaching earth," he said.

AOCS' RESPONSE AND METHOD VALIDATION

AOCS submitted a formal response to the EFSA report on October 4, 2013. In it, Technical Director and Chief Science Officer Richard Cantrill details the Society's efforts, from forming an Expert Panel on Process Contaminants in 2009 to publishing four validated methods for the analysis of 3-MCPD-E and GE in 2012 and 2013.

"The AOCS Expert Panel has monitored the development of indirect methods," Cantrill noted in the response, "and in 2012 it established protocols for the three most robust indirect methods. This decision was based on two needs: first, laboratories involved in food analysis needed a method that was cost effective and simple; and, second, they needed a comparison of popular methods to determine their precision and accuracy in a side-by-side comparison."

AOCS conducted a collaborative study with 20 participants from eight countries that validated three indirect methods for characterizing 3-MCPD-E, 2-MCPD-E, and GE in oils and fats. These methods were published in September 2013.

"AOCS has worked together with the global edible oil industry to validate methods so that occurrence and exposure data can be as robust as possible," said Cantrill. "Although primarily focused on direct analysis of contaminants, the Expert Panel decided to benchmark the three most commonly referenced indirect methods using a set of carefully manufactured authentic reference standards. Our experts were encouraged to find that the three methods not only performed equally well but also gave results close to the known content of the manufactured reference materials. AOCS is proud to have provided this valuable analytical contribution."

In related news, Cantrill recently announced that he took part in informal discussions about the EFSA report during the October 2013 Euro Fed Lipid meeting. Those discussions led to the decision for the AOCS Expert Panel on Process Contaminants to put out a call for extraction methods for fat-containing food matrices. AOCS will then conduct a collaborative study of those extraction methods, comparing the products of extractions using the recently validated AOCS methods (see box) for the characterization of MCPD- and glycidyl-esters in oils and fats. For more information, or to submit a method, contact Cantrill by email (rcantrill@aocs.org) or phone (+1 217-693-4830). ■

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www.aocsfoundation.org/auction

SUSTAINABILITY WATCH

A companywide campaign to curb emissions, reduce energy use, and decrease water consumption is showing early results at Archer Daniels Midland Co. (ADM; Decatur, Illinois, USA), according to the company's recently released Corporate Responsibility report.

Between 2010 and 2012, implementation of energy-saving projects generated an estimated 4.3% reduction in ADM's total energy consumption and a 2.6% reduction in global greenhouse gas emissions, both on a per-unit-of-production basis. This puts the company on-track to achieve its goal of a 15% reduction in both areas by 2020, ADM said.

The report also notes that from 2008 to 2012, the company reduced water use 11.8% at the six US corn-processing facilities responsible for 70% of its global water consumption. The company has committed to a 15% reduction per unit of production by 2018. The report is available at www.adm.com/responsibility.

■■■

Cargill announced in September 2013 that its palm oil plantation in West Kalimantan, Indonesia, PT Harapan Sawit Lestari (HSL), has received international recognition for its sustainable palm oil practices. HSL was awarded the certification for sustainable palm oil supply for biofuels according to the International Sustainability and Carbon Certification (ISCC) standards in the European Union (EU). This certification covers HSL's entire operations, including smallholder plantations managed by Cargill under the KKPA (Kredit Koperasi Primer Anggota) scheme as well as the mill and warehousing operations.

ISCC is a certification scheme that promotes responsible farming by allowing sustainable products to be differentiated from nonsustainable ones, including greenhouse gas emissions at different stages of the value chain. It is approved by the EU to cover the EU Renewable Energy Directive.

■■■

Big River Resources, LLC (West Burlington, Iowa, USA) and Prairie Gold, Inc., (Bloomington, Illinois, USA) are forming a joint venture to produce corn zein protein—a high-value co-product that can be produced from corn prior to the ethanol fermentation process. The new venture, Big River Prairie Gold, LLC, will construct and operate a zein production facility near Galva, Illinois, on the site of one of Big River's 110-million-gallon (420-million-liter) ethanol plants. Zein proteins (which are also found in sorghum) are soluble in organic solvents, such as ethanol but are not soluble in water. The proteins can be processed into a polymer that may be used as a plastic, fiber, film coating, confectioner's glaze, or gum. ■

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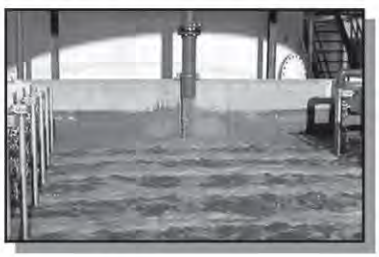

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BRIEFS

Tecnon OrbiChem initiated a new Bio-Materials & Intermediates Chemical Business Focus in September. It is intended to present detailed, comprehensive coverage of markets, prices, and developments for bio-based chemicals and bio-polymers alongside that for their petrochemical equivalents. Further information is available at www.orbichem.com/announcement-bio.aspx, as is access to a sample issue.

■■■

A new ASTM International standard issued in September 2013 covers butanol intended to be blended with gasoline at 1–12.5 volume percent for use as an automotive spark-ignition engine fuel. ASTM D7862-13 establishes performance requirements and test methods for butanol content, water content, acidity, inorganic chloride, solvent-washed gum, sulfur content, and total sulfate. The specification covers 1-butanol (currently being developed by Cobalt Technologies, Mountain View, California, USA), 2-butanol (being developed by Gevo, Inc., Englewood, Colorado, USA; and Butamax Advanced Biofuels LLC, Wilmington, Delaware, USA), and 2-methyl-1-propanol. It specifically excludes *tert*-butyl alcohol.

■■■

The US National Renewable Energy Laboratory released a report in October 2013 (http://tinyurl.com/E15-E10-NREL) finding that the available literature ". . . did not show meaningful differences between E15 [fuel comprising 15% ethanol and 85% petroleum-based gasoline] and E10 in any performance category." Furthermore, NREL found that the study by the Coordinating Research Council (CRC) that reported mechanical engine failure when operating on E15, "is not supported by the data." The CRC is a nonprofit organization supported by the American Petroleum Institute and a number of automobile manufacturers. ■

BIOFUELS+

The marigold on the left is growing in a pot made from bioplastics. The plant on the right is growing in a pot made from a petroleum-based plastic. Photo courtesy of the ISU Center for Crops Utilization Research, Iowa State University, Ames, USA.

Iowa State proposes Center for Bioplastics and Biocomposites

Iowa State University (ISU; Ames, USA) is seeking funding from the National Science Foundation to support a proposed Center for Bioplastics and Biocomposites based at ISU, in cooperation with the University of Massachusetts-Lowell, and the agriculture and plastics industries.

The basis for the program already exists in the ISU Center for Crops Utilization Research, which is working cooperatively with the ISU Biopolymers & Biocomposites Research team. One project already in progress is a study of the production and performance of different kinds of bioplastic pots used in horticulture. The accompanying photograph pictures a marigold (*Tagetes*) grown in a biorenewable pot made from a plastic comprising 50% soy polymer and 50% polylactic acid (left) and one grown in a pot made from petroleum-based plastic. The soil in each pot was the same, and the plants were grown under the same light and at the same temperature. Both were fertilized for two weeks, then no fertilizer for four weeks.

The plant in the soy-based pot needs to be fed much less frequently because the pot degrades over time, slowly releasing nitrogen, phosphorus, potassium, and other nutrients, according to James Schrader, an assistant scientist in horticulture at ISU. Researchers also found that, in transplanting plants into open ground, the pot could be crumbled and the pieces transferred into the hole to act as a fertilizer source for the rest of the growing season.

Elevance authorizes next stage of its second biorefinery

In October 2013, Elevance Renewable Sciences, Inc., headquartered in Woodridge, Illinois, USA, announced it had authorized the

CONTINUED ON NEXT PAGE

next significant stage of its second biorefinery in Natchez, Mississippi, USA. (The first is a joint venture with Wilmar International Ltd. in Gresik, Indonesia.)

Elevance's business is based on olefin metathesis technology. At the Natchez facility, the initial products will be derived from canola or soybean oil; the Gresik biorefinery currently operates on palm oil. Both plants are able to run on multiple renewable oil feedstocks, including jatropha or algal oils when they become commercially available.

When it is up to full scale, the Natchez facility will produce novel specialty chemicals, including multifunctional esters such as 9-decenoic methyl ester; a distribution of bio-based alpha and internal olefins including decene; and a premium mixture of oleochemicals. It will have a capacity of 280,000 metric tons per year.

Both Elevance facilities produce renewable chemicals that can function as building blocks, including renewable olefins (≥C10) and di-functional specialty molecules. These molecules combine the functional attributes of an olefin, typical of petrochemicals, and a monofunctional ester or acid, typical of bio-based oleochemicals, into a single molecule. These chemicals enable detergents to be more concentrated and clean better in cold water, improve solvency for better hard-surface cleaners; yield lubricant base oils with improved stability and fuel economy; and provide monomers for bio-based polymers and engineered plastics.

The Natchez facility has moved from batch to continuous operations and begun site preparation for biorefinery construction. Elevance expects full production at the facility will be in place by 2016.

Elevance announced in September the availability of octadecanedioic acid, which is being produced at its biorefinery in Gresik. As a diacid, or, if converted to diamine, this compound enables the creation of more than a dozen new base polymers that can result in more than a hundred new compounds or formulations.

Canada funds oilseed development

The government of Canada announced at the end of the third quarter of 2013 that it will support the development of *Camelina sativa* as an environmentally sustainable high-value oilseed crop. It will also fund the further development of mustard seed production. Each of these efforts will receive about $5-million initiative funds over the next five years.

Camelina. Linnaeus Plant Sciences (Saskatoon, Saskatchewan) and Soy 20/20 (Guelph, Ontario) plan to improve agronomic traits in camelina—herbicide and disease resistance, and yield enhancement—and to increase the value of camelina oil and co-products for industrial uses. Jack Gruschcow, president and chief executive officer of Linnaeus, said in a company statement that camelina has the potential to become the preferred oilseed platform crop for the production of high-value industrial fatty acids, presenting alternatives to current petrochemicals and reducing the refining expense and accompanying pollution of petrochemicals. Linnaeus and Soy 20/20 will help create a grower value chain by distributing agronomic data to potential growers, setting the groundwork for a new industry association and raising public awareness of the crop. Agriculture and Agri-Food Canada (AAFC) is supplying about two-thirds of the grant, with the rest coming from industry.

Mustard. Mustard 21 Canada, a nonprofit corporation initiated by the Saskatchewan Mustard Development Commission and the Canadian Mustard Association, will use its grant to fund research focusing on developing new and higher-yielding varieties of condiment mustard through traditional and molecular breeding applications as well as for industrial mustard for use in biofuels. AAFC will collaborate on many of these projects. In developing industrial oilseed varieties, there will be emphases on high oil and protein content, early maturing properties, and disease resistance.

BELOW: *Diagram of the Elevance facility at Natchez, Mississippi, USA. The Mississippi River is in the foreground. Courtesy: Elevance Renewable Sciences, Inc.*

Partnership to produce estolide industrial lubricants

Biosynthetic Technologies announced in October 2013 that it would partner with Albemarle Corp., an expert in catalyst chemistry scale-up, to begin operations of their demonstration plant within Albemarle's existing Baton Rouge (Louisiana, USA) facility.

Working with the US Department of Agriculture's National Center for Agricultural Utilization Research (Peoria, Illinois), Biosynthetic Technologies has developed a new, high-performance, bio-based drop-in synthetic alternative to petroleum used in motor oils and industrial lubricants. The starting materials are estolides, found in such materials as castor oil and oil from the *Lesquerella* plant. They are characterized by a secondary ester linkage of one fatty acyl molecule to the alkyl backbone of another fatty acid fragment. Estolides can be free acids, esters, or found within a triglyceride structure.

Allen Barbieri, chief executive officer of Biosynthetic Technologies, headquartered in Irvine, California, USA, said in a company statement, "Several of the world's largest motor oil companies have invested heavily to test and validate biosynthetic oil in their labs, in engine testing and in numerous field trials. This third-party validation has effectively demonstrated that biosynthetic oils achieve very high scores in key areas."

Motor oils formulated with this oil will be the first bio-based motor oils offered by major motor oil brands and certified to the latest industry standards of the American Petroleum Institute (API). There is also a high potential for estolides to be used in greases, gear oils, cooling fluids, metal-working fluids, hydraulic fluids, and marine lubricants. Testing has shown that motor oils and lubricants formulated with biosynthetic base oils can be recycled and re-refined just like petroleum.

Biosynthetic Technologies is moving forward with plans to develop a full-scale commercial facility, likely in California.

Indonesia mandates more biodiesel

As of January 2014, PT Shell Indonesia and PT Total Oil Indonesia must sell diesel fuel containing at least 10% FAME (fatty acid methyl ester) biodiesel at their Indonesian gas stations. Any retailer that fails to comply will face sanctions, such as business license revocation or suspension of operations. Up through 2013, Shell and Total mixed only 1% biodiesel into their diesel fuel, according to Rida Mulyana, the Indonesian Energy and Mineral Resources Ministry director general for renewable energy and energy conservation.

Rida promised that gas stations would be inspected from time to time to ensure they follow the new requirement.

In response, diesel retailers urged that the Indonesian government provide a complete explanation of the new regulation in an effort to head off resistance from consumers who feared the higher levels of biodiesel would affect the engines of their transportation vehicles and heavy equipment. But Juwono Andrianto, secretary-general of the Association of Indonesian Automotive Manufacturers, said the government should instead provide proof that biodiesel producers in the country could produce FAME at a high-enough quality not to hurt engine performance (http://tinyurl.com/JakartaPost-biodiesel)

The regulation also requires a minimum of 10% biodiesel in diesel fuel mixes intended for industrial and commercial purposes, and 20% for those used in power plants.

The decree is intended to reduce the importation of petrodiesel so as to counter the government's current-account deficit and to strengthen the rupiah, the national currency.

Castor bean oil slated as biodiesel feedstock

Evogene Ltd. (Rehovot, Israel), a plant genomics company specializing in enhancing crop productivity for the food, feed, and biofuel industries, announced at the end of the third quarter that its subsidiary, Evofuel Ltd., had complete three years of successful field trials in Brazil for the development of castor bean as an alternative feedstock for production of biodiesel and other renewable chemicals.

The field trials, conducted in cooperation with the agricultural producer SLC Agrícola S.A. (Porto Alegre, Rio Grande do Sul), demonstrated strong yield performance of Evofuel's proprietary castor seed varieties under rain-fed conditions in northeast Brazil. Evofuel envisions castor seed being a competitive feedstock for biodiesel as well as for traditional industries currently using castor oil, such as the lubricant and biopolymer industries.

Based on these field tests, advanced product development and pre-commercial trials are targeted to begin in 2014. Evofuel estimates its castor seeds will reach the commercialization stage in 2016.

The collaboration with SLC is aimed at developing castor as a rotation crop with soybeans, with the sowing of the castor seeds taking place after the harvesting of the soybeans. Growing castor plants during a period in which rainfall levels are typically not sufficient to cultivate other crops means castor production will not compete with the production of staple crops and will provide farmers with a high-value crop providing an additional revenue source. ■

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BRIEFS

Krill oil has passed flaxseed oil to become the second-best selling omega-3 supplement (with 14% of the market), according to Norwegian krill oil supplier Aker BioMarine and to Nutraingredients.com, while fish oil remains No. 1. Aker said it is doubling its research and development team as a result of the increasing popularity of krill oil.

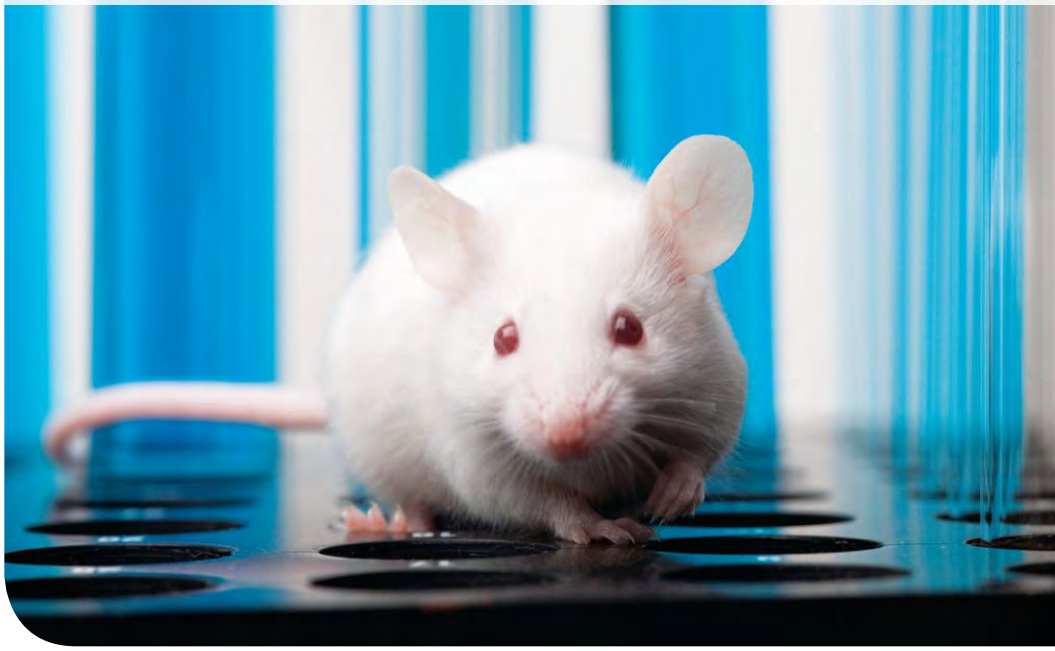
■■■

Researchers have identified a new catalytic antioxidant that could be an alternative to statins—the most prescribed cholesterol-lowering drugs in the world. Statins reduce cholesterol levels in the body by acting as competitive inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase—the enzyme that catalyzes cholesterol biosynthesis. The scientists showed that the catalytic antioxidant, 1-Fe, and its analogs, inhibit HMG-CoA reductase in a different way to statins. The research appeared in *Chemical Communications* (doi:10.1039/c3cc44740e, 2013) and was led by Adi Haber of the Israel Institute of Technology.

■■■

Research that reportedly is the first to estimate intake of individual polyunsaturated fatty acids (PUFA) by US children under the age of 5 has found what might be a troubling deficit in the diet of many youngsters. The cross-sectional study, published in *Maternal and Child Nutrition* (doi:10.1111/mcn.12077, 2013), used data on nearly 2,500 children aged 12 to 60 months from the US National Health and Nutrition Examination Survey. The research—which was led by Sarah Keim of Nationwide Children's Hospital (Columbus, Ohio, USA)—projected a ratio of omega-6 to omega-3 intake of about 10:1, which is considered by researchers in the area to be high. (The omega-6/omega-3 ratio remains a contentious subject among scientists; suggestions regarding an optimal ratio range anywhere from 1:1 to 5:1.) In addition, the estimated intake of docosahexaenoic acid in the study group was lower than what most infants generally consume and did not increase with age. ■

FOOD, HEALTH & NUTRITION



Lipids and cancer

Knocking out a single enzyme dramatically cripples the ability of aggressive cancer cells to spread and grow into tumors, according to a new study by researchers at the University of California, Berkeley in the *Proceedings of the National Academy of Sciences* (doi:10.1073/pnas.1310894110, 2013).

The paper, the authors say, sheds new light on the importance of lipids in the development of cancer.

Researchers have long known that cancer cells metabolize lipids differently than normal cells. Levels of ether lipids—a class of lipids that are more difficult to break down—are particularly elevated in highly malignant tumors, although the nature of that correlation has been unclear for decades. (Ether lipids are lipids in which one or more of the carbon atoms on glycerol is bonded to an alkyl chain via an ether linkage, as opposed to the usual ester linkage.)

"Cancer cells make and use a lot of fat and lipids, and that makes sense because cancer cells divide and proliferate at an accelerated rate, and to do that, they need lipids, which make up the membranes of the cell," said study principal investigator Daniel Nomura. "Lipids have a variety of uses for cellular structure, but what we're showing with our study is that lipids can also send signals that fuel cancer growth."

In the study, Nomura and his team tested the effects of reducing ether lipids on human skin cancer cells and primary breast tumors. They targeted an enzyme, alkylglycerone phosphate synthase, or AGPS, known to be critical to the formation of ether lipids.

The researchers first confirmed that AGPS expression increased when normal cells turned cancerous. They then found that inactivating AGPS substantially reduced the aggressiveness of the cancer cells.

"The cancer cells were less able to move and invade," said Nomura.

The researchers also compared the impact of disabling the AGPS enzyme in mice that had been injected with cancer cells.

"Among the mice that had the AGPS enzyme inactivated, the tumors were nonexistent," said Nomura. "The mice that did not have this enzyme disabled rapidly developed tumors."

The researchers determined that inhibiting AGPS expression depleted the cancer cells of ether lipids. They also found that AGPS altered levels of other types of lipids impor-

CONTINUED ON NEXT PAGE

tant to the ability of the cancer cells to survive and spread, including prostaglandins and acyl phospholipids.

"The effect on other lipids was unexpected and previously unknown," said study lead author Daniel Benjamin, doctoral student in the Nomura research group. "Other studies have investigated specific lipid signaling pathways, but what makes AGPS stand out as a treatment target is that the enzyme seems to simultaneously regulate multiple aspects of lipid metabolism important for tumor growth and malignancy."

Food as reward

The idea that certain foods (generally high-fat and sugary foods) are addictive has been the subject of a number of studies.

A report in *Science* (*341*:800–802, 2013) examines how high-fat foods activate a reward circuit in the brain involving dopamine, a neurotransmitter that regulates pleasure. Some work suggests that overconsumption of high-fat foods dampens this dopamine-induced reward sensation, leading to compensatory consumption of even more high-fat foods. The mechanisms by which dietary fat in the gut "talks" to the dopamine reward circuit are unclear, however.

A group led by Luis Tellez of the John B. Pierce Laboratory in New Haven, Connecticut, USA, has demonstrated that an intestinal lipid messenger known as oleoylethanolamine (OEA) may play a role—at least in mice. Mice on a high-fat diet had unusually low levels of intestinal OEA and exhibited deficient dopaminergic responses to gut stimulation with high-fat lipids. Infusion of OEA into these mice restored the dopaminergic response, and mice that had been accustomed to a high-fat diet began to eat more low-fat foods.

"Our results support the following conjectural model," the scientists write. "Gut lipid messengers such as OEA may function as homeostatic signals that dictate the amounts of dietary fat to be ingested (i.e., by either stimulating or suppressing intake toward a set point). . . . Reestablishing gut-lipid signaling may thus enhance the reward value of low-calorie foods, presumably by restoring gut-stimulated dopaminergic function. Whether this concept can be developed into a useful weight-loss strategy will require further research," they conclude.

Are guidelines on fish consumption during pregnancy wrong?

Researchers and health regulators agree: Exposure to very high levels of methylmercury by pregnant women can have undesirable effects on the developing fetal brain. Thus, advocates generally advise expectant mothers not to eat certain species of fish such as shark and swordfish.

However, research by scientists at the University of Bristol (UK) suggests that oily fish, whitefish, and shellfish actually account for only about 7% of the total variation in whole blood total mercury levels (TBM; untransformed and log-transformed) in the human body, indicating that guidelines concerning fish consumption during pregnancy may need to be revised. The study, which appeared in *Environmental Health Perspectives* (http://dx.doi.org/10.1289/ehp.1206115, 2013), analyzed 103 food and drink items consumed by 4,484 women during pregnancy and found that the 103 items together accounted for less than 17% of TBM in the participants.

The current findings come out of a study that is officially titled the Avon Longitudinal Study of Parents and Children (ALSPAC) and is unofficially known as the Children of the 90s study. The research has been running since 1991 and involves more than 13,000 women and almost 14,000 children. Research leader Jean Golding told *The Guardian* newspaper (see http://tinyurl.com/children90s) that a wide range of results from previous ALSPAC studies include the demonstration that peanut allergies are widespread and indications that children of mothers who put on the most weight in early pregnancy were more likely to be heavier themselves and to be more at risk of cardiovascular disease.

In the present study, the foodstuffs associated with the highest blood levels of total mercury (after white fish and oily fish) were herbal teas and alcohol, with wine consumption apparently leading to higher blood levels than beer. The researchers also reported that the women with the highest total mercury levels tended to be older, to have attended university, to be in professional or managerial jobs, to own their own homes, and to be expecting their first child. Overall, however, less than 1% of women had mercury levels higher than the maximum level recommended by the US National Research Council (0.7 μg/kg body weight/week).

The authors conclude that advice to pregnant women to limit seafood intake is unlikely to reduce mercury levels substantially.

Skin cancer and health

Do the benefits of exposure to the sun outweigh the risks? A study in the *International Journal of Epidemiology* (doi:10.1093/ije/dyt168, 2013) suggests it might.

"Sunlight is a known risk factor for skin cancer," notes the Vitamin D Council (VDC; www.vitamindcouncil.org) in a recent blog entry. "However, sunlight also helps you make vitamin D, creating a strong possibility that sunlight protects against other diseases and illnesses." Adding to an already complex risk-benefit analysis, the VDC says, is the fact that persons with a high level of exposure to the sun often get more outdoor physical activity. That said, if the benefits of sunlight outweigh the risks, then being diagnosed with skin cancer could possibly show a protective association against other diseases and illnesses, the blog suggests.

In the study, researchers at the Gentofte Hospital in Copenhagen examined the entire Danish population above the age of 40 from 1980 to 2006. In total, the study included 4.4 million individuals. The team, which was led by Peter Brøndum-Jacobsen, used national registries to find any diagnoses of nonmelanoma skin cancer, melanoma skin cancer, myocardial infarction, hip fracture, and deaths from any cause.

The researchers concluded, "In a nationwide study of 4.4 million individuals above [the] age [of] 40 years, having a diagnosis of skin cancer was associated with less myocardial infarction, less hip fracture in those below age 90 years, and less death from any cause" compared with general population controls. Although causal conclusions cannot be made from the data, the scientists called for further studies to examine the possible relationship between sun exposure and beneficial effects on health. ■

BRIEFS

In mid-September, the Dow Jones Sustainability Index (DJSI) announced that it considered the industrial enzyme manufacturer Novozymes A/S, headquartered in Bagsværd, Denmark, to be the most sustainable company within the Biotechnology industry sector. Novozymes was awarded an overall score of 86 out of 100. This is the 12th time that the DJSI has recognized Novozymes for its sustainability efforts.

The DJSI is an international sustainability ranking system that comprises the leading companies around the world. It is run by Standard & Poor's Dow Jones and RobecoSAM Sustainability Investing. Three overall dimensions are considered: economic, environmental, and social.

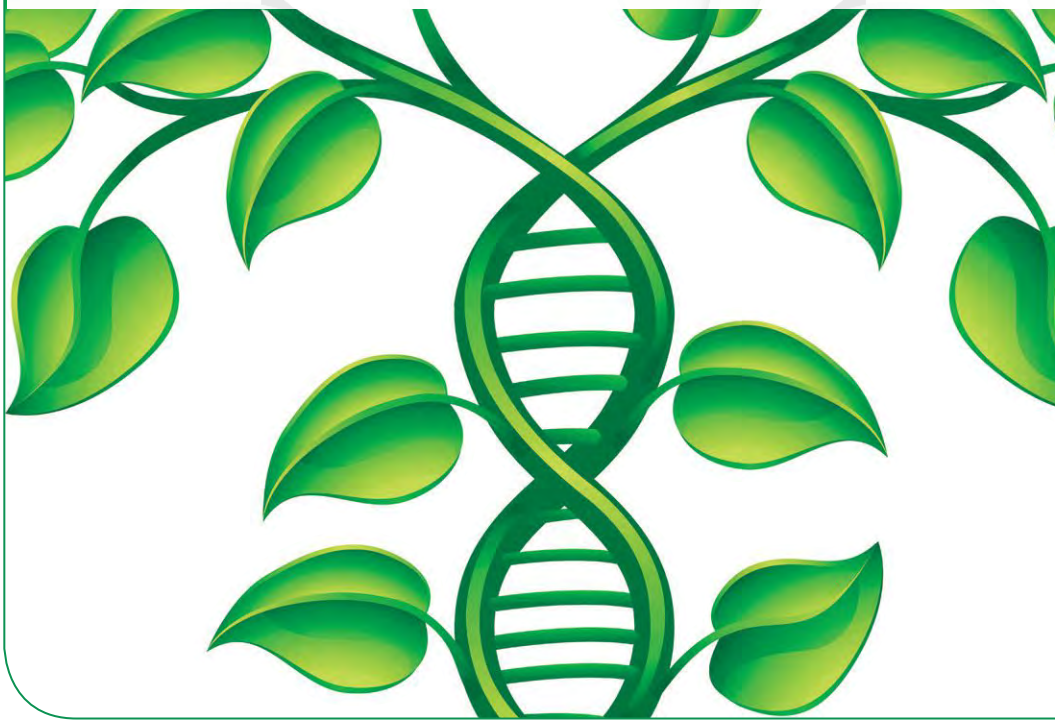
■■■

The Grocery Manufacturers Association (GMA) in September 2013 created a website (http://factsaboutgmos.org) designed to answer questions about genetically modified organisms (GMOs) and GM crops. Initially, the content included mainly links to third-party sources, such as *The New York Times*, Bloomberg.com, and *Scientific American*. The creation of this web site follows on the heels of the establishment by the Council for Biotechnology Information of its site, www.gmoanswers.com, in July 2013. Both sites represent industry efforts to change the popular conversation about the merits of GM crops and foods. According to the GMA, GM crops require fewer pesticides and less water and reduce the price of crops used for food, such as corn, soybeans, and sugar beets, by as much as 15–30% (http://tinyurl.com/GMA-benefits-GM).

■■■

Recent research (Microbial production of short-chain alkanes, *Nature*, doi:10.1038/nature12536, 2013) reported the development of genetically engineered *Escherichia coli* strains that are capable of producing short-chain alkanes, free fatty acids, fatty esters, and fatty alcohols. The short-chain alkanes are of especial interest since they largely reproduce the composition of gasoline. ■

BIOTECHNOLOGY



Scientists identify genes for increasing oil in leaves

Researchers with the US Department of Energy's Brookhaven National Laboratory (Upton, New York) have identified the key genes required for oil production and accumulation in plant leaves and other vegetative plant tissues. Enhancing expression of these genes resulted in greatly increased oil content in leaves—a finding that could have important implications for increasing the energy content of plant-based foods and renewable biofuel feedstocks.

"If we can transfer this strategy to crop plants being used to generate renewable energy or to feed livestock, it would significantly increase their energy content and nutritional values," said Brookhaven biochemist Changcheng Xu, who led the research.

In nature, oil storage is the job of seeds, where the energy-dense compounds provide nourishment for developing plant embryos. The idea behind Xu's studies was to find a way to "reprogram" plants to store oil in their more abundant forms of biomass.

The first step was to identify the genes responsible for oil production in vegetative plant tissues. Although oil is not stored in these tissues, almost all plant cells have the capacity to make oil. But until these studies, the pathway for oil biosynthesis in leaves was unknown.

Working with *Arabidopsis thaliana*, a plant that is genetically similar to canola, the researchers enhanced the factors that normally increase oil production in seeds, but found that these changes had no effect on oil production in leaves and in some instances caused growth and developmental problems in the plants. But when the scientists caused an overexpression of the gene for phospholipid:diacylglycerol acyltransferase (PDAT), there was a 60-fold increase in leaf oil production.

The researchers also observed that the excess oil did not mix with cellular membrane lipids but instead occurred as oil droplets within the leaf cells. They resembled the droplets found in seeds, only much larger. Large droplets are not good, according to Xu, because they are easily broken down by other cellular enzymes. In seeds, oil droplets are coated with a protein called oleosin, which

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"Further experiments with further manipulations identified genetic changes that led to oil productions and accumulations that were 170-fold higher than in control plants."

prevents the droplets from fusing together, keeping them smaller while also protecting the oil inside.

Next, the scientists overexpressed both PDAT and the gene for oleosin. Results showed a 130-fold increase in leaf oil production compared with control plants—and the oil accumulated in large clusters of tiny oleosin-coated oil droplets.

Further experiments with further manipulations identified genetic changes that led to oil productions and accumulations that were 170-fold higher than in control plants. At that point, oil accounted for nearly 10% of the leaf's dry weight.

"These studies were done in laboratory plants, so we still need to see if this strategy would work in bioenergy or feed crops," said Xu. "And there are challenges in finding ways to extract oil from leaves so it can be converted to biofuels. But our research provides a very promising path to improving the use of plants as a source of feed and feedstocks for producing renewable energy."

The research appeared in two journal articles: *The Plant Cell,* doi.org/10.1105/tpc.113.117358, 2013, and *The Plant Journal,* doi:10.1111/tpj.12343, 2013. See also http://tinyurl.com/oil-in-leaves.

Potential solution for green canola seed problem

Canola, also known as rapeseed, is a major crop in Canada. Its seeds can be pressed to yield canola oil, a popular cooking oil, as well as meal, which can be used in animal feed and as fertilizer.

The Canadian climate can affect canola oil quality, however. If there is a light, non-lethal frost (–2 to 0°C) near the time of seed maturity, the green color of the not-quite-mature seeds will be fixed in up to 20% of the seeds. The plant can continue to grow to maturity, but the presence of chlorophyll in mature seeds affects the oil quality, produces unpleasant flavors and odors, and reduces the shelf life of the oil.

Researchers from the University of Toronto (Canada), the University of Bordeaux (France), the Institut National de la Recherche Agronomique (Villenave d'Ornon, France), and the University of Calgary (Canada) recently reported results of their studies on possible ways to circumvent this problem (*Proc. Natl. Acad. Sci.*, doi:10.1073/pnas.1308114110, 2013). Using a strain of *Arabidopsis*, a well-studied plant that is a close genetic relative of canola, the team found a protein, called ABI3, that is important in removing seed chlorophyll and enabling the seeds to de-green.

Marcus Samuel, team leader, said in a statement released by the University of Calgary, "Given the similarity of *Arabidopsis* and canola, it would be easy to isolate the same genes from canola and use transgenic technologies (which introduce new genes into organisms) to create varieties that could withstand freezing conditions, yet produce mature brown-black seeds." He added, "We actually have demonstrated in our laboratory tests that the canola genes work the same way."

The University of Calgary team is working with Siniazo Biotech (Ottawa, Ontario, Canada), a biotechnology company, to file a provisional patent on this finding. It has also contacted companies that have developed transgenic canola to assess their interest in this idea (90% of the canola grown in Canada is transgenic or genetically modified for herbicide resistance). Further information is available at http://tinyurl.com/GreenCanola.

Can all plants learn to fix nitrogen?

Legumes such as soybeans and alfalfa form associations with soil bacteria called rhizobia. In these associations, the bacteria infect the roots, forming small nodules. The outcome of this infection is symbiotic—the bacteria receive nutrients from the plant roots, and the bacteria are then able to fix atmospheric nitrogen, which the plants can use as an "in-house" source of nutrition. Recently published research from the University of Missouri (MU), Columbia, USA has identified why not all crops can form this symbiosis.

"The problem is that corn, tomatoes and other crops . . . don't support an intimate interaction with the rhizobia, thus making farmers apply larger amounts of nitrogen than might otherwise be necessary," said Gary Stacey, leader of the research. "Scientists have known about this beneficial relationship since 1888, but it only exists in legume crops, like soybeans and alfalfa. We're working to transfer this trait to other plants like corn, wheat, or rice, which we believe is possible since these other plants recognize the bacteria. It's a good first step."

"There's this back and forth battle between a plant and a pathogen," said Yan Liang, a co-author of the study and post-doctoral fellow at MU. "Rhizobia eventually developed a chemical to inhibit the defense response in legumes and make those plants recognize it as a friend. Meanwhile, corn, tomatoes and other crops are still trying to defend themselves against this bacteria."

In the study, Stacey and Liang treated corn, soybeans, tomatoes and other plants to see how they responded when exposed to the chemical signal from the rhizobia bacteria. They found that the plants did receive the signal and, like legumes, inhibited the normal plant immune system. However, tomatoes, corn, and these other plants don't complete the extra step of forming nodules to allow the bacteria to thrive.

"The important finding was that these other plants didn't just ignore the rhizobia bacteria," Stacey said. "They recognized it, but just activated a different mechanism. Our next step is to determine how we can make the plants understand that this is a beneficial relationship and get them to activate a different mechanism that will produce the nodules that attract the bacteria instead of trying to fight them.

Former AOCS President Richard Wilson, who has also conducted research on nitrogen fixation, said in an email, "I feel confident this work shows a step toward strategies that may enable N_2 fixation in nonlegumes someday."

The research appeared in the journal *Science* (*341*:1384–1387, 2013; doi: 10.1126/science.1242736). See also http://tinyurl.com/N2fixation-NOD.

Terminator technology revival shut down

On October 16, 2013, a Constitutional Commission in Brazil's House of Representatives considered Bill PL 268/2007. The bill, sponsored by house member Eduardo Sciarra from the State of Panará, would allow the selling of bioreactor plant seeds containing Genetic Use Restriction Technologies (GURTs), also known as terminator genes. Terminator seeds are genetically engineered to be sterile in the second generation of seeds, and bioreactor seeds are modified to produce proteins or compounds intended for therapeutic or industrial uses.

The committee chose to withdraw Bill PL 268/2007 before considering it after facing pressure from national and international organizations. The president of the commission also stated that the bill would not be revived so long as he remained president. However, another bill that would allow all uses of GURTs, PL 5575/2009, is in another committee.

In 2000, the United Nations imposed a moratorium on terminator technology through the Biodiversity Convention, and no other country has commercialized the technology. Brazil has had its own eight-year ban on the technology.

Genetic manipulations showing promise in increasing bioethanol yields

Scientists interesting in developing second- and third-generation biofuels, that is, biofuels formed from feedstocks other than food such as corn and soybeans, are finding new ways to generate bioethanol more efficiently and in higher yields.

Changing a metabolic pathway. Chemical engineering researchers at the University of California Los Angeles (UCLA; USA) have created a new synthetic metabolic pathway for breaking down

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glucose that could lead to a 50% increase in the production of biofuels from sugars. The new pathway is intended to replace the natural metabolic pathway known as glycolysis. In that pathway, four of the six carbon atoms found in glucose are converted into two-carbon units called acetyl-CoA, which is a precursor to biofuels such as ethanol and butanol as well as other products. The two remaining glucose carbons are lost as CO_2. Loss of these two carbon atoms represents inefficiency in the process.

The UCLA research team's new synthetic glycolytic pathway, which they are calling non-oxidative glycolysis (NOG), converts all six glucose carbon atoms into three molecules of acetyl-CoA, with no loss as CO_2. NOG uses enzymes found in several distinct pathways in nature. The team first tested and confirmed that the new pathway worked *in vitro*. Then they genetically engineered the *Escherichia coli* bacterium to use the synthetic pathway and demonstrated complete carbon conservation.

Lead author Igor W. Bogorad, a graduate student in the laboratory of James C. Liao, said in a statement released by the university, "This is a fundamentally new cycle. We rerouted the most central metabolic pathway and found a way to increase the product of acetyl-CoA. Instead of losing carbon atoms to CO_2, you can now conserve them and improve your yields and produce even more product."

The researchers also point out that the NOG pathway can be used with many kinds of sugars. The original research report appeared in *Nature* (doi:10.1038/nature12575, 2013).

Engineering yeast to consume acetic acid. The yeast *Saccharomyces cerevisiae* is good at fermenting simple sugars, such as those found in corn kernels and sugar, into ethanol, which can be blended with gasoline for fuel. Yeasts do not generally do as well converting lignocellulose into ethanol. Researchers at the University of California-Berkeley (USA), the Lawrence Berkeley National Laboratory, and the University of Illinois (Urbana-Champaign, USA) started with the premise that the decomposition of hemicellulose, a component of lignocellulose, leads to xylose and acetic acid. Although yeasts such as *S. cerevisiae* can be engineered to ferment xylose, the accompanying by-product acetic acid is toxic to the organism.

The researchers identified a bacterium that could convert acetic acid into alcohol and the enzymes that catalyzed the process as well. Computer simulations of the possible effects of inserting these bacterial genes into the yeast's metabolic repertoire predicted a positive outcome, and subsequent experiments showed that the "new" yeast was making ethanol from acetate, a first for *S. cerevisiae*. Ethanol production increased about 10%, in line with computer calculations. The research appeared in *Nature Communications* (doi: 10.1038/ncomms3580, 2013).

General Mills CEO comments on GM ingredient labeling

During the September 2013 meeting of General Mills shareholders, Ken Powell, CEO of the food products company, said he supports a national system that labels foods as containing no genetically modified (GM) ingredients, instead of a system that labels those foods that do contain GM ingredients.

Powell's comments, appearing in the company's home-town newspaper, the Minneapolis (Minnesota, USA) *StarTribune*, reflect the worry among food industry executives that labels identifying GM ingredients will scare consumers. The outcome proposed by General Mills is unlikely to happen, though, since voters would first have to support the labeling of foods containing GM ingredients, according to the article. Powell said the company uses GM ingredients because they are safe, but opponents to GM ingredients claim the long-term effects of these foods are unknown and consumers have a right to know what is in their food.

Hawaii a new battleground for GM practices

Hawaii residents and environmental activists have pushed for new laws in The Aloha State restricting the ability of seed companies to develop and grow genetically engineered crops. The Hawaii government answered this call and has written a law that may change how international seed companies Monsanto, DuPont Pioneer, Dow Chemical Co., BASF, and Syngenta do business in the state.

The county of Hawaii—which is coterminous with the island of Hawaii—is considering Bill 113, which would prevent the growth of all genetically engineered crops, with the exception of papayas and other crops currently being cultivated. The bill would also prevent seed companies from operating in the county. The bill passed its first reading, but as of press time, the bill must pass its second reading and be signed by the mayor of Hawaii County before becoming a law.

According to an October 7 article in *The New York Times* newspaper, testing new GM crops in the state of Hawaii is easier and less expensive than in other areas; multiple crops can be harvested in a year due to the warm climate, accelerating the cross-breeding process. Almost any corn seed sold in the United States touches Hawaii somewhere during its development. Additionally, most of the main island's papayas are genetically engineered to resist a virus that nearly wiped out the crop in the 1990s. Seed company representatives have argued that the bill would force them off the island.

UK politician calls GM opponents "wicked"

Owen Paterson, Secretary of State for the United Kingdom's Department of Environment, Food, and Rural Affairs (DEFRA), called environmental groups "wicked" in October 2013 for opposing the development of "golden rice." This plant, a genetically engineered variety of sativa rice, biosynthesizes β-carotene, a precursor to vitamin A, in its seeds.

According to an article on FoodNavigator.com, golden rice in the diets of impoverished populations in African and Asian countries could help to prevent deaths and blindness from vitamin A deficiency.

Paterson, who supports GM research, said there is no scientific basis for opposition to GM crops. Groups that oppose GM research are "casting a dark shadow over attempts to feed the world," he said.

A DEFRA spokeswoman later said that Paterson's comments reflected his own views and not those of the government. ■

The US Environmental Protection Agency (EPA) has released the final Integrated Risk Information System (IRIS) summary for the solvent 1,4-dioxane, as well as a new toxicological review (see www.epa.gov/iris/subst/0326.htm). The agency has also published the interagency science discussion draft of the 1,4-dioxane IRIS assessment. Under EPA's *Guidelines for Carcinogen Risk Assessment*, 1,4-dioxane is "likely to be carcinogenic to humans." The compound is a by-product of the ethoxylation process and thus may be found in some detergents, cosmetics, and personal care products.

■■■

In October 2013, Swiss specialty chemicals manufacturer Clariant signed an agreement to divest its Detergents & Intermediates business to International Chemical Investors Group (ICIG) for CHF 58 million, or roughly $64 million. ICIG is a privately owned industrial holding company based in Luxembourg and Frankfurt, Germany. According to Clariant, all employees of the research and development, applications, sales, and marketing units will transfer to ICIG, along with production plants and sites.

■■■

Solvay, the Brussels-based chemical manufacturer, is acquiring Chemlogics, a maker of specialty chemicals for oil and gas exploration based in Paso Robles, California, USA. Solvay said it will add the company to its Novecare unit, which markets a variety of compounds used in chemically enhanced oil recovery.

■■■

Using a particular type of titanium dioxide—a common ingredient in cosmetics, food products, toothpaste, and sunscreen—could reduce the potential health risks associated with the compound, according to research published in *Chemical Research in Toxicology* (doi:10.1021/tx400285j, 2013). One of the two most commonly used crystalline forms of titanium oxide (rutile) easily washed off and had little effect in pig skin tests, while the other commonly used form (Anatase) was difficult to wash off and damaged the outermost layer of skin—even in low ultraviolet light. ■

HOME & PERSONAL CARE

New "game-changing" regulations in California may force reformulation

The US state of California has often taken the lead in legislating environmental policy and regulation that ends up forcing change elsewhere. New regulations that came into force on October 1, 2013, are no exception and may well force the reformulation of many consumer products worldwide.

Calling Assembly Bill 1879 (the California Green Chemistry Initiative) and Senate Bill 509, which passed in 2008, "a uniquely murky state legislative directive," attorney Lynn Bergeson further characterized the Safer Consumer Products (SCP) regulations as "precedent-setting" and "game-changing." Bergeson, a partner in the Washington, DC, USA, law firm of Bergeson & Campbell PC, gave her perspective on the regulations during a Chemical Watch webinar on October 16. Also speaking were Emily Tipaldo, manager, Regulatory and Technical Affairs, American Chemistry Council, and Karl Palmer, chief, Toxics in Products Branch, California Department of Toxic Substances Control (DTSC).

FIVE YEARS IN THE MAKING

Early attempts to craft SCP regulations that were acceptable to both industry and environmentalists failed; a second round of draft and proposed regulations began in 2011, culminating in a "revised text of proposed regulations" issued on August 23, 2013. These are the regulations that became final on October 1, 2013.

The SCP regulations "are largely a framework for future regulation," notes Ward Benshoof, a partner at Alston & Bird, and author of a summary of the regulations that appeared in *HAPPI* magazine (see http://tinyurl.com/SCP-HAPPI.) The regulations involve a four-stage process (see Table 1, page 39) with the following steps:

- Identifying candidate chemicals: DTSC must name "candidate chemicals" by November 1, 2013, from its initial list of chemicals posted in September 2013. This is the initial list of compounds in consumer products that DTSC believes require further research and potential regulation as "chemicals of concern."

  As of October 1, there were more than 1,100 chemicals on the initial list (www.dtsc.ca.gov/SCP/ChemList.cfm) from which DTSC will choose the candidate chemicals for the first round of regulation. This winnowed list will include chemicals that exhibit one or more hazard traits (i.e., are known carcinogens or mutagens) *and* are also on one or more of the 23 authoritative exposure indicator

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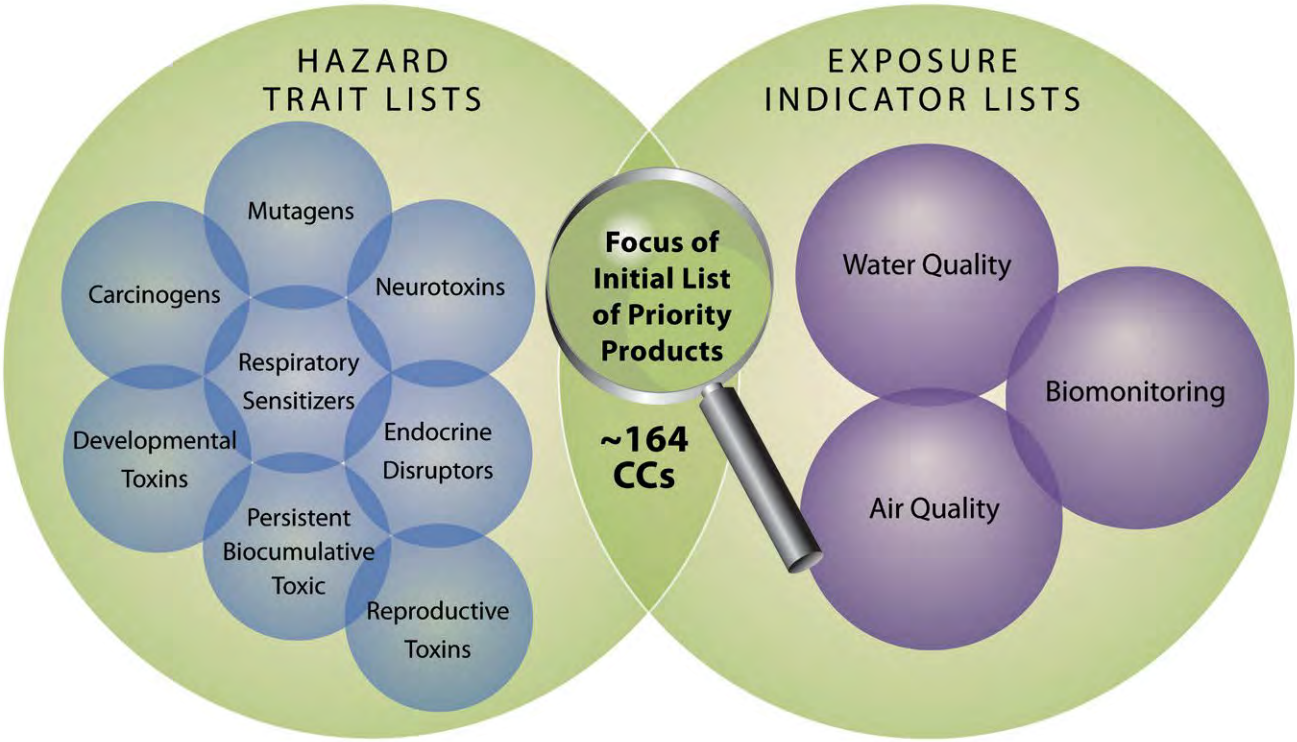

FIG. 1. *Initial list of candidate chemicals. Courtesy of Department of Toxic Substances Control.*

lists developed by other (mostly governmental) agencies. (See Fig. 1.) One class of chemicals notably absent from the initial list is that of so-called endocrine disruptors, or chemicals believed to affect the endocrine system in humans or animals. Endocrine disruptors were not listed because of a problem with the wording in the regulation. Once that wording is corrected, the compounds will be placed on the list, DTSC's Karl Palmer said.

- Identifying priority products: Within 180 days of the publication of the candidate chemicals list, DTSC must complete an evaluation of combinations of candidate chemicals that presumably pose the greatest threat to health or the environment. Based on that analysis, DTSC will name three to five consumer priority products (for example, nail polish that contains toluene) for evaluation and possible reformulation.
- Conducting alternative analyses: Manufacturers of priority products sold in California must inform DTSC of the presence of a Chemical of Concern in their products. Then they must provide an Alternative Analysis (AA) naming any alternative compounds that could replace the Chemical(s) of Concern or explaining why no alternatives exist. This process is a matter of great uncertainty for consumer products manufacturers; DTSC plans to provide guidance only after the priority products are named, probably in early 2014.
- Determining a Regulatory Response: After reviewing the AA, DTSC must then decide what to do. The possibilities range from doing nothing at all, to requiring warning labels, to banning the product from sale in California.

ADVICE TO MANUFACTURERS AND FORMULATORS

Chemical and consumer product manufacturers must above all else be realistic, cautions attorney Lynn Bergeson. "Do not rely on the almost-certain legal challenges we expect will be forthcoming," she said. "Litigation is probable. Success is unclear. Relying on a favorable outcome and doing nothing now is unwise. Prepare for the worst."

The requirement to conduct AA will fall on manufacturers and importers, she noted. However, businesses involved in the supply chain of consumer products sold in California could have obligations as well. To "get ahead of the curve," Bergeson offered the following suggestions. Companies should:

- Understand their firm's role with regard to each particular Priority Product (PP).
- Determine if an exemption applies.
- Know all the components of consumer products subject to the regulations. "It is critically important for consumer product manufacturers to know every component of their products down to the detection level (e.g., parts per million)".
- Review the candidate chemicals List. "Companies should begin now to review the Initial Candidate Chemicals List to determine if any of their consumer products contain one or more of these substances."
- Determine responsible entity roles and potential consolidation of efforts. There is limited time (60 days) provided for a responsible entity (manufacturer, importer, or retailer) to respond to the identification of a PP. Companies

TABLE 1. Implementation timeline[a,b]

Section	What	When
Candidate Chemical identification	Initial Candidate Chemical List	Date of regulations: October 1, 2013
Priority Product selection	First proposed Priority Products List	6 months after regulations' effective date
	First Priority Products List finalized; requires rulemaking	Up to 12 months
Alternative Assessment	Priority Product Notification due	2 months after final Priority Products List
	Preliminary AA Report due	6 months after listing on final Priority Product List
	DTSC reviews Preliminary AA report; NOC issued	2 months after receiving preliminary AA report
	Final AA Report due to DTSC	12 months after receiving NOC for the Preliminary AA Report
	DTSC reviews Final AA Report; NOC issued	2 months after receiving Final AA Report
Regulatory response	Regulatory response implementation	Specified in the regulatory response determination

[a]Adapted from a presentation by Karl Palmer, chief, Toxics in Product Branch, DTSC. Downloaded on October 17, 2013, from http://chemicalwatch.com/california-webinar-download.
[b]Abbreviations: AA (Alternative Assessment), DTSC (California's Department of Toxic Substances Control), NOC (Notice of Compliance).

need to have a strategy in place now, and they should not overlook the possibility of saving time and money through the use of consortia in conducting AA. "Memorialize these understandings in contracts, though." Consolidate efforts by sorting out relationships and responsibilities now. Consumer products companies should identify other entities similarly affected as well as companies in the supply chains that may have information or may be considered responsible entities under the regulations.

- Monitor the DTSC Portal. DTSC will post guidelines and other vital information at the DTSC Safer Consumer Products Web Portal at www.dtsc.ca.gov/SCP/index.cfm.

DTSC's Karl Palmer noted that the European Union's regulatory framework for chemicals—Registration, Evaluation and Authorisation of CHemicals, or REACH—could help companies meet requirements under the SCP regulations, but companies need to understand that the two regulations are different.

When asked how the agency will deal with data gaps, Palmer said DTSC is "going to use the weight of evidence as best as we can. It is going to be a process of collecting all the information available and evaluating [chemicals of concern] ultimately in the alternatives analysis."

The objective of the SCP regulations, noted ACC's Emily Tipaldo, is to promote innovation in consumer products to reduce or replace the presence of substances in those products considered to pose a risk of harm.

"But innovation needs responsible protection," she said. And precisely how industry and DTSC will protect confidential business information and trade secrets remains unknown. ■

Chemical Watch: new benefit for AOCS members

Did you know that AOCS has teamed up with the Chemical Watch (CW) global regulatory news and information service? As a result, we are now posting two free CW articles per month on the MyAOCS member page. Simply log in to your membership account at www.aocs.org/Account/login.cfm to access the reports. AOCS members also can receive 20% off the annual subscription rate by visiting http://chemicalwatch.com/aocs-offer.

The recording and PowerPoint presentations from the CW webinar on California's Consumer Product Safety regulations, however, are available free of charge to members and nonmembers alike at http://chemicalwatch.com/california-webinar.

We at AOCS are getting excited! After months of hard work, our new Association Management System (AMS) will be set to launch in early to mid-January.

Once the new AMS is integrated with our website, you will notice a slightly different login process. Rest assured we will have complete instructions on how to access your membership or store account on the website. Keep an eye out for email communications from AOCS with updates, but you will always find the most up-to-date information on our website.

We hope to have as seamless a transition as possible, but understand glitches may occur. You can be sure staff at AOCS will be standing by to help with any problems occurring after the transition.

The implementation of the new AMS will enable us to serve you better — we look forward to it, and hope you do too!

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PEOPLE/INSIDE AOCS

Green receives Lipid Research Award

Green

Allan Green of CSIRO Plant Industry (Canberra, Australia) is the recipient of the AOCS Australasian Section Lipid Research Award. The award recognizes persons who have made a significant contribution in basic or applied research and whose research either has had a significant effect on advances in the discipline and that hold substantial promise for its effect in the near future. Green has been at the forefront of research in plant lipid biosynthesis and genetic manipulation for over three decades. The award was presented to him at the biennial meeting of the Australian AOCS, held November 6–8, 2013, in Newcastle, New South Wales.

Mezzenga recognized

Mezzenga

The American Chemical Society (ACS) journals *Biomacromolecules*, *Macromolecules*, and *ACS Macro Letters* in partnership with the ACS Division of Polymer Chemistry announced the selection of AOCS member **Raffaele Mezzenga**, of ETH Zürich, Switzerland, as well as **David M. Lynn** of the University of Wisconsin-Madison, as winners of the inaugural Biomacromolecules/Macromolecules Young Investigator Award. They were honored during an award symposium at the ACS national meeting held in September 2013.

Mezzenga was recognized for his contributions to the fundamental understanding of self-assembly processes in polymers and biological colloidal systems. Lynn's award was for his contribution to the design and synthesis of polymers and materials of biotechnological and biomedical significance.

Mezzenga also received the 2011 AOCS Young Scientist Research Award.

Bioriginal celebrates 20th anniversary

AOCS Bronze Corporate member **Bioriginal**, headquartered in Saskatoon, Saskatchewan, Canada, celebrated its 20th anniversary in September 2013. In its early years the company worked directly with growers in the region to produce the company's first omega-3 fatty acid ingredient, borage oil. Over the last 20 years, Bioriginal has developed proprietary methods and innovative science-based omega-3 solutions with both plant and marine-based omega-3 fatty acids.

Widlak retires

Widlak

After 18 years with Archer Daniels Midland (ADM), **Neil Widlak** and his wife Trudy have retired to Anthem, Arizona, USA. Most recently, Widlak worked for ADM's Cocoa Division in Milwaukee, Wisconsin, as director of Product Services and Development, culminating a career in edible applications of fats, oils, and emulsifiers that included research and product development in packaged oils, frying fats, baking shortenings, coating fats, margarine, salad dressing, chocolate, and confectionery coatings. Besides ADM, Widlak's career included associations with Kraft Foods, Intermountain Canola, Lou Ana Foods, and Best Foods.

He may be contacted at nwidlak@att.net.

Widlak plans to continue his active role in AOCS, including attending the 105th AOCS Annual Meeting & Expo in San Antonio, Texas, USA, in May.

Cargill announces appointment

Cargill Inc. announced in mid-September 2013 that its board of directors had elected **David W. MacLennan** as the corporation's next chief executive officer effective December 1, 2013, succeeding **Gregory R. Page**, who will serve as executive chairman. MacLennan retains the title of president and continues as a director of the company. The transition is the result of the company's ongoing succession planning with the board.

MacLennan joined Cargill, which is headquartered in Minneapolis, Minnesota, USA in 1991 and has held various leadership positions within the financial, risk management, energy and animal protein businesses in the United States, London and, Geneva. He was elected Cargill's president and chief operating officer in 2011.

In his role as executive chairman, Page will continue to lead the board and will represent the company in a variety of interactions and public forums with customers, policymakers and others, and will be available as a resource to the company. Page was elected chief executive officer in 2007 and chairman later that same year. ■

IN MEMORIAM

William James Johnson

William ("Bill") J. Johnson died July 9, 2013, at his home in Memphis, Tennessee, USA, at the age of 90. His wife, Elsye, preceded him in death. He is survived by his children Laura and Teresa, two grandsons, four great-grandchildren; and two sisters.

Johnson was born in Los Angeles, California, USA, in 1923 and served in the US Army Air Corps during World War II, flying 46 combat missions as a bombardier.

He spent two years at Pasadena (California) City College and Southwestern College at Memphis Tennessee, where he majored in chemistry and physics.

He started with the Buckeye Cotton Oil Co. (Memphis, Tennessee) as a chemist in 1947, and remained with the company until his retirement. As part of his work, Johnson participated in the AOCS Smalley Check Sample Program and was recognized for his skills.

Satish C. Singhal

AOCS has received word that Satish C. Singhal of New Delhi, India, died on August 7, 2013. He joined AOCS in 1982 and became an emeritus member in 2008.

Singhal was quite active in the India Section of AOCS and served as its president continually from 1996 until his death. He received the AOCS Award of Merit in 2003.

Harold H. Perry

Harold H. Perry, co-founder of Plant Maintenance Service Corp. (Memphis, Tennessee, USA), died on September 11, 2013, at the age of 78.

Perry started his career in metal fabrication with the Southern Boiler and Tank Works (SBTW) in Memphis in 1953 and achieved the classification of Industrial Boiler Maker while he was with SBTW.

In 1967, he and his partners Robert L. Baker and Enoch E. ("Bubba") Radford formed the Plant Maintenance Service Corp. Over the years the company earned a high reputation for providing design, fabrication, and field services for chemical, agricultural, petrochemical, and pulp and paper industries. Its products included pressure vessels, tanks, columns, kilns, soybean oil extractors, heat exchangers, evaporators, crystallizers, extractors, and assorted process equipment. It also offered erection, repair, and installations services.

Under Perry, Plant Maintenance Service Corp. was presented 10 times with Wausau Insurance Companies' Loss Prevention Gold Safety Award.

Perry joined AOCS in 1984 and was a member of the Mid South Section as well as the Processing Division. He was also active in the International Oil Mill Superintendents' Association.

He is survived by his wife of 58 years, Mary Helen; his children Debora, Russell ("Rusty"), Becky, and Christy; six grandchildren, two great-grandchildren, and a sister.

AOCS MEETING WATCH

May 4–7, 2014. 105th AOCS Annual Meeting & Expo, Henry B. Gonzalez Convention Center, San Antonio, Texas, USA. http://annualmeeting.aocs.org

October 6–9, 2014. World Conference on Fabric and Home Care—Montreux 2014, Montreux Music & Convention Centre, Montreux, Switzerland. http://montreux.aocs.org

May 3–6, 2015. 106th AOCS Annual Meeting & Industry Showcase. Rosen Shingle Creek, Orlando, Florida, USA

For in-depth details on these and other upcoming meetings, visit http://aocs.org/meetings or contact the AOCS Meetings Department (email: meetings@aocs.org; phone: +1 217-693-4821; fax: +1 217-693-4865).

Also, be sure to visit AOCS' online listing of industry events and meetings at http://tinyurl.com/industry-calendar. Sponsoring organizations can submit information about their events to the web-based calendar by clicking a link and completing a web form. Submission is free. No third-party submissions, please. If you have any questions or comments, please contact Valorie Deichman at valoried@aocs.org.

PATENTS

Omega-3 diglyceride emulsions

Deckelbaum, R.J., and Y. Carpentier, The Trustees of Columbia University in the City of New York, US8410181, April 2, 2013

The present invention relates to omega-3 diglyceride emulsions characterized in that the lipid phase comprises at least about 40 wt% of diglycerides. Preferably about 70 wt% of the acyl groups of said diglycerides are eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) groups. The invention further relates to methods of treatment using the omega-3 diglyceride emulsions.

Method for producing biofuels, transforming triglycerides into at least two biofuel families: fatty acid monoesters and ethers and/or soluble glycerol acetals

Hillon, G., *et al.,* IFP Energies Nouvelles, US8419810, April 16, 2013

A process for producing biofuels by transforming triglycerides into at least two families of biofuels, monoesters of fatty acids and soluble ethers and/or acetals of glycerol, comprises: at least one transesterification step in which said triglyceride is reacted by heterogeneous catalysis with at least one primary monoalcohol selected from methanol and ethanol to produce at least one methyl and/or ethyl ester of the fatty acids of the starting triglycerides and glycerol, said products being free of by-products; and an etherification step in which the glycerol is reacted with at least one olefinic hydrocarbon containing 4–12 carbon atoms; and/or an acetalization step in which the glycerol is reacted with at least one compound selected from aldehydes, ketones, and acetals derived from aldehydes or ketones.

Stabilization of fatty oils and esters with alkyl phenol amine aldehyde condensates

O'Brien, T.J., and B.R. Munson, Baker Hughes Inc., US8430936, April 30, 2013

Biodiesel fuels, renewable diesel fuels, and feedstocks to these fuels (derived from plant seed oils or animal fats) are viewed as more environmentally friendly, renewable alternative fuels or supplemental fuels with petroleum-based diesel. Alkyl phenol amine aldehyde condensates improve the stability of biofuels by inhibiting the degradation processes. Alkyl phenylene diamines employed together with alkyl phenol amine aldehyde condensates in the biofuels give synergistically improved stability of the fuels.

Method to stabilize the crystallization of a blend of fat and oil containing more than 10% cocoa butter and having a low *trans* fat content

Descamps, P., and Y. Kegelaers, Puratos Naamloze Vennootschap, US8414955, April 9, 2013

The invention relates to method for preparing a chocolate composition comprising at least 10% cocoa butter, said method comprising the steps of: (i) Preparing a chocolate mixture comprising at least 10% cocoa butter by admixing a chocolate mass with a blend of an oil and/or fat having a Solid Fat Content higher than 2% when measured at 30°C with IUPAC method 2.150 a, and having a Trans Fatty Acid Content below 4%, preferably below 2%, (ii) Feeding this mixture in molten state to a scraped surface heat exchanger, wherein the temperature of the scraped surface is below +10°C, advantageously is below 0°C, (iii) Crystallization and stabilization of said melt with the aid of said scraped surface heat exchanger, (iv) Optionally, extrusion of the thus chilled mixture in block shape. This method allows the preparation of *trans*-free chocolate fillings advantageously without the need of specialty products. The invention further relates to the prepared chocolate fillings and their applications in the chocolate industry.

Shortenings and methods of making and using thereof

Narine, S.S., *et al.,* Bunge Oils, Inc.; The Governors of the University of Alberta c/o Tec Edmonton, US8431177, April 30, 2013

Described herein are shortenings with improved properties such as increased hardness, minimal *trans* fat, and reduced saturated fats. Methods for preparing the shortenings involve the use of one or more structural enhancers in a vegetable oil followed by processing and tempering the admixture. The shortenings can be used to produce food products with reduced saturated fats and increased hardness as well as minimal *trans* fats.

Method for preparation of polyunsaturated fatty acid containing phosphatidylserine

Chen, S., and H. Kwong, US8431369, April 30, 2013

A method for the preparation of the polyunsaturated fatty acids containing phosphatidylserine, the method comprising: combining L-serine with a fish liver phosphatidylcholine having

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a polyunsaturated fatty acid to form a mixture; reacting the mixture with phospholipase D to effect transphosphatidylation of L-serine and the phosphatidylcholine having polyunsaturated fatty acids to produce the polyunsaturated fatty acids-containing phosphatidylserine.

French fry production method with reduced crumb generation

Hamann, M.L., *et al.*, J.R. Simplot Co., US8435583, May 7, 2013

An improved production process for preparing parfried and frozen French fry potato products with reduced crumb generation particularly upon finish preparation as by finish frying. Potato products such as French fry strips are cut, blanched, and parfried, followed by a water treatment step as by applying a light spray or mist of water at ambient temperature and preferably at a rate selected to avoid any substantial overspray. The potato products are frozen for shipment and/or storage awaiting finish preparation. Upon finish preparation as by finish frying in hot oil, the potato products exhibit substantially reduced crumb generation. The improved process is particularly useful when parfrying and/or finish frying the potato product in a liquid or substantially non-hydrogenated oil, such as zero grams *trans* fat (ZGTF) oil, with dramatically reduced crumb generation upon finish fry preparation while retaining a substantially optimized balance of sensory characteristics.

Enzymatic modification of triglyceride fats

ten Brink, H.B., *et al.*, Conopco, Inc., US8431370, April 30, 2013

Process wherein the fatty acid residues on a glyceride moiety are randomized over the terminal and middle positions, wherein the process proceeds to a conversion degree on the terminal positions, Re, ranging from 0.3 to 0.95, and wherein a conversion degree on the middle position, Ra, ranges from 0.06 to 0.75, and wherein Ra is greater than 0.32Re – 0.08, the process comprises the exposure of a triglyceride fat to a catalyst comprising a lipase wherein the lipase is a *Thermomyces lanuginosa* lipase which has an activity of at least 250 IUN corresponding to 22 g/(g·h) at the onset of the process.

Patent information is compiled by Scott Bloomer, a registered US patent agent with Archer Daniels Midland Co., Decatur, Illinois, USA. Contact him at scott.bloomer@adm.com.

EXTRACTS & DISTILLATES

Nonchromatographic speciation of selenium in edible oils using dispersive liquid–liquid microextraction and electrothermal atomic absorption spectrometry

López-García, I., *et al.*, *J. Agric. Food Chem. 61*:9356–9361, 2013.

A methodology for the nonchromatographic separation of the main selenium species present in edible oils is presented. Dispersive liquid–liquid microextraction is used to extract inorganic selenium (*i*Se), seleno-L-cystine (SeCys2), seleno-L-methionine (SeMet), and selenocystamine (SeCM) into a slightly acidic aqueous medium. The selenium total (*t*Se) content is measured in the extracts by electrothermal atomic absorption spectrometry. By repeating the microextraction stage using an ionic liquid instead of water, the sum of SeCys2, SeMet, and SeCM is obtained and *i*Se is calculated by difference. The detection limit is 0.03 ng of Se per gram of oil. The fractionation of the edible oils by solid phase extraction followed by dispersive liquid–liquid extraction and atomic absorption measurement also permits speciation of *i*Se to be carried out. Data for *t*Se and *i*Se levels of 15 samples of different origin are given.

Influence of olive oil on the bioavailability of carotenoids

Lakshminarayana, R., and V. Baskaran, *Eur. J. Lipid Sci. Technol. 115*:1085–1093, 2013.

Bioavailability is defined as "the fraction of an ingested nutrient that is available for utilization in normal physiological functions or for storage." Available studies on the bioavailability of carotenoids are based on the measurement of their levels in serum or plasma. Dietary components were reported to affect the rate of carotenoids absorption. On digestion, carotenoids are incorporated into the lipid phase and then are emulsified into small lipid droplets. The nature and amount of lipids in the diet greatly affect the emulsification, secretion of bile salts, and formation of mixed micelles, all of which are currently important subjects to understand the bioavailability of carotenoids. Specific lipids, vegetable oils, and their fatty acid moieties have been shown to affect the formation of mixed micelles that positively influence the absorption of carotenoids. Gavages and dietary studies revealed that oleic acid micelles and olive oil (oleic acid, C18:1) enhance the intestinal accessibility of carotenoids more than linoleic acid micelles or vegetables oils rich in polyunsaturated fatty acids. The chemistry of fats or oils, which may act differently at various stages of absorption and metabolism of carotenoids, is discussed. This review shows that dietary unsaturated fat appears to be a suitable carrier for carotenoids when oxidative stress is a critical issue in nutrition-related degenerative disorders.

Viscosity modification of high-oleic sunflower and castor oils with acid oils-derived estolides for lubricant applications

García-Zapateiro, L.A., *et al.*, *Eur. J. Lipid Sci. Technol. 115*:1173–1182, 2013.

The use of estolides as additives to increase the viscosity of vegetable oils for lubricant applications was investigated. For this purpose, high-oleic sunflower (HOSO) and castor (CO) oils were blended with estolides derived from acid oils resulting from vegetable oil refining processing (HOSO and olive pomace acid oils). These blends were characterized from both viscous and tribological point of views. Significant increments in viscosity were generally found when added estolides were synthetized using sulfuric acid as catalyst, compared to *p*-toluensulfonic acid catalyst. Estolides prepared from these two by-products gave rise to similar viscosity modification in HOSO, yielding maximal viscosity increments of 322 and 362% at 10°C, respectively. Smaller viscosity modifications were obtained for CO/estolide blends, with maximal increments in kinematic viscosity of 84 and 152%, respectively. Generally, the addition of estolides to HOSO yielded a significant increase in the temperature dependence of viscosity. The addition of acid oils-derived estolides slightly improved CO thermal dependence of viscosity for lubricant applications. Although wear was significantly reduced by adding these estolides to any of these vegetable oils, the addition of acid oils-derived estolides to CO increased the friction coefficient at low rotational speeds, extending the boundary lubrication regime to higher Sommerfeld number values. On the contrary, a single Stribeck master curve was obtained for HOSO/estolide blends.

Bubble stripping in closed system to remove residual methanol from crude biodiesel

Yamane, T., *et al.*, *Eur. J. Lipid Sci. Technol. 115*:1183–1192, 2013.

A novel, simple, compact, efficient system based on a unit operation of bubble stripping was developed for intensive

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methanol (MeOH) removal from crude biodiesel (BDF), which was synthesized by the potassium hydroxide-catalyzed transesterification reaction of triacylglycerol (TAG) with MeOH. As TAG, both rapeseed oil and used cooking oil were tested. In the system, gas was supplied by a gas pump through a gas bubbler into the crude biodiesel, then the exit gas containing MeOH vapor passed through a condenser (0°C) and the condensed MeOH was collected in a vessel. MeOH-free gas from the condenser returned into the inlet of the gas pump, thus gas was circulated in a closed system. After the intensive MeOH removal by the bubble stripping, insoluble sediment always appeared in the biodiesel, which was removed by centrifugation. As a final step, trace amounts of potassium (K) and free glycerol were removed by adsorbent treatment. MeOH, K, free glycerol, fatty acid methyl ester, and water contents, acid, and iodine values and oxidation stability of the purified biodiesel cleared the specifications of EN 14214 Standard. The proposed dry process consisting of the three steps will be applicable to industrial production of high-quality biodiesel.

Unraveling the complexities of the HDL lipidome

Kontush, A., *et al.*, *J. Lipid Res. 54*:2950–2963, 2013.

Plasma high density lipoproteins (HDL) are small, dense, protein-rich particles compared with other lipoprotein classes; roughly half of total high density lipoprotein (HDL) mass is accounted for by lipid components. Phospholipids predominate in the HDL lipidome, accounting for 40–60% of total lipid, with lesser proportions of cholesteryl esters (30–40%), triglycerides (5–12%), and free cholesterol (5–10%). Lipidomic approaches have provided initial insights into the HDL lipidome with identification of over 200 individual molecular lipids species in normolipidemic HDL. Plasma HDL particles, however, reveal high levels of structural, compositional, and functional heterogeneity. Establishing direct relationships between HDL structure, composition, and atheroprotective functions bears the potential to identify clinically relevant HDL subpopulations. Furthermore, development of HDL-based therapies designed to target beneficial subspecies within the circulating HDL pool can be facilitated using this approach. HDL lipidomics can equally contribute to the identification of biomarkers of both normal and deficient HDL functionality, which may prove useful as biomarkers of cardiovascular risk. However, numerous technical issues remain to be addressed in order to make such developments possible. With all technical questions resolved, quantitative analysis of the molecular components of the HDL lipidome will contribute to expand our knowledge of cardiovascular and metabolic diseases.

More Extracts & Distillates can be found in this issue's supplement (digital and mobile editions only).

JAOCS
JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY

***Journal of the American Oil Chemists' Society* (November)**

- Authentication of edible vegetable oil and refined recycled cooking oil using a micro-UV spectrophotometer based on chemometrics, Liu, T., Y. Zhou, J. Lv, Z. Chen, B. Li, and Y. Shi
- Changing the SFC profile of lauric fat blends based on melting group triacylglycerol formulation, Nusantoro, B.P., N. De Clercq, K. Anthierens, and K. Dewettinck
- Experimental solid–liquid phase equilibria of a methyl ester/amide/nitrile ternary system by DSC, Mekki-Berrada, A., S. Bennici, J.-L. Dubois, and A. Auroux
- Effects of acidity, temperature and emulsifier concentration on the distribution of caffeic acid in stripped corn and olive oil-in-water emulsions, Costa, M., S. Losada-Barreiro, F. Paiva-Martins, and C. Bravo-Díaz
- Purification of free DHA by selective esterification of fatty acids from tuna oil catalyzed by *Rhizopus oryzae* lipase, Bhandari, K., S.P. Chaurasia, A.K. Dalai, and A. Gupta
- Characterization of enzymatically interesterified canola oil and fully-hydrogenated canola oil blends under supercritical CO_2, Jenab, E., and F. Temelli
- Plasma modified membrane for daily recovery of oil from repeated frying operation with frequent oil replenishment, Onal-Ulusoy, B., E. Tur, and M. Mutlu
- Classification of Turkish monocultivar (Ayvalık and Memecik cv.) virgin olive oils from north and south zones of Aegean Region based on their triacyglycerol profiles, Gökçebağ, M., H. Dıraman, and D. Özdemir
- Oxidative stability and sensory attributes of fermented milk product fortified with fish oil and marine phospholipids, Lu, F.S.H., B.R. Thomsen, G. Hyldig, D.M.B. Green-Petersen, N.S. Nielsen, C.P. Baron, and C. Jacobsen
- Characterization of the volatile, phenolic and antioxidant properties of monovarietal olive oil obtained from cv. Halhali, Kesen, S., H. Kelebek, and S. Selli
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- Margarine from organogels of plant wax and soybean oil, Hwang, H.-S., M. Singh, E.L. Bakota, J.K. Winkler-Moser, S. Kim, and S.X. Liu
- Variability of phytosterols in *Jatropha curcas* germplasm, Corzo-Valladares, P.A., Á. Fernández-Cuesta, J.M. Fernández-Martínez, and L. Velasco
- Antioxidation behavior of milkweed oil 4-hydroxy-3-methoxycinnamate esters in phospholipid bilayers, Evans K.O., and R.E. Harry-O'kuru
- A proposal for physicochemical standards and antioxidant activity of Portuguese propolis, Falcão, S.I., C. Freire, and M. Vilas-Boas
- Kinetics of isothermal crystallization of hydrogenated castor oil-in-water emulsions, Yang, D., A.N. Hrymak, and S. Kedzior

- Synthesis, molecular characterization and preliminary antioxidant activity evaluation of quercetin fatty esters, Mainini, F., A. Contini, D. Nava, P.A. Corsetto, A.M. Rizzo, E. Agradi, and E. Pini
- Regeneration of used frying oil, Turan, S., and A. Yalcuk

JOURNAL OF SURFACTANTS AND DETERGENTS

Journal of Surfactants and Detergents (November)

- Rheological behavior of selected water-nonionic-anionic surfactant quasi-ternary systems, Chruściel, A.
- The relationship between the structure and properties of amino acid surfactants based on glycine and serine, Qiao, W., and Y. Qiao
- Mixed micelles of trisiloxane based silicone and hydrocarbon surfactants systems in aqueous media: dilute aqueous solution phase diagrams, surface tension isotherms, dilute solution viscosities, critical micelle concentrations and application of regular solution theory, Sastry, N.V., A. D. Thummar, and S.H. Punjabi
- Synthesis and properties of mono or double long-chain alkanolamine surfactants, Zhang, Y., Y. Xu, S. Qiu, and L. Yang
- Stability of dye dispersions in the presence of some eco-friendly dispersing agents, Gharanjig, H., K. Gharanjig, and S. Tafaghodi
- Synthesis, characterization, biodegradation, and evaluation of the surface active properties of nonionic surfactants derived from *Jatropha* oil, Negm, N.A., A.S. El-Tabl, I.A. Aiad, K. Zakareya, and A.H. Moustafa
- Physicochemical investigation of biocompatible mixed surfactant reverse micelles: III. Aqueous NaCl solubilization, thermodynamic parameters of desolubilization process and conductometric studies, Kundu, K., and B.K Paul
- Mixed micellar and interfacial interactions of a triblock polymer ($EO_{37}PO_{56}EO_{37}$) with a series of monomeric and dimeric surfactants, Banipal, T.S., and A.K. Sood
- Mixtures of nonionic surfactants made from renewable resources with alkyl sulfates and sodium *n*-alkanecarboxylates: comparison of mixing behavior using Rubingh's treatment, Jackson, L.P., C. Townsend, and B.P. Grady
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- Synthesis, surface active properties of novel gemini surfactants with amide groups and rigid spacers, Hu, D., X. Guo, and L. Jia
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Lipids

Lipids (November)

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Professional Pathways

Professional Pathways is a regular Inform *column in which AOCS members discuss their professional experiences and share advice with young professionals who are establishing their own careers in oils and fats–related fields.*

Ray Shillito has more than 30 years of experience in research and development. He is an inventor on several patents and has worked in a variety of fields. Currently, Shillito works at Bayer CropScience in Morrisville, North Carolina, USA.

Why did you join AOCS?

I am not in the oils and fats industry *per se*; I work in agricultural biotechnology. I became involved in AOCS when I started working in the ISO and Codex standards area. AOCS is the administrator of a number of ISO standards activities. I also worked with AOCS when we contracted with them to make reference materials for biotechnology products.

Describe your career path.

I essentially followed my interests and opportunities that arose. When completing my degree in quantitative biochemistry at the University of Hull, England, I heard of the area of plant tissue culture. I went to study in the laboratory of Prof. H.E. Street, one of the pioneers in the field, at the University of Leicester. Toward the end of my Ph.D. he mentioned to me that plant tissue culture was being used for putting genes into plants. He suggested that I get in contact with Prof. R.A. Schilperoort at his laboratory at the University of Leiden (Netherlands). I obtained a NATO grant to do a postdoc there. It was right at the beginning of the development of modern biotechnology, using Agrobacterium to introduce genes into plant cells. I also learned Dutch, which was fun and helped when I later came to learn Swiss German in Basel. At the end of the postdoc period I had to find work to tide me over so I spent a few months as a postman. It was interesting to get to know the people of the Netherlands other than those in the academic field.

I was able to secure an EMBO [European Molecular Biology Organization] fellowship in Basel, Switzerland with Dr. Ingo Potrykus (now known for Golden Rice technology) in 1983. That position allowed me to use my tissue culture skills learned in Leicester and Leiden to introduce genes into plants without using Agrobacterium. I like to think that we were present at the birth of the modern era of plant biotechnology. I was able to collaborate with many people there; I have always been interested in collaborating with others. We filed several patents at that time. The experience I gained

helping to write patent applications has been useful ever since. Our successes led to recognition in the field for all of us, and thus to further opportunities.

After 5.5 years of research—very satisfying eventually, as we had several breakthroughs—I took a job with Ciba-Geigy (a predecessor of Syngenta) in the United States where agricultural biotechnology was taking off. I got the opportunity to work on difficult projects at the edge of what was possible and to lead a team of excellent people. Our work involved the introduction of genes into maize and led to more patents.

During the merger that led to Novartis, I was laid off, but through networking, was able to secure a job quickly (with no break) with a predecessor company of Bayer CropScience, to work on the development (product registration) side of the business. In hindsight this was a blessing, as it allowed me to broaden my experience and eventually to move into the more expert role that I serve. As well as my internal responsibilities, I now work within industry and scientific associations and NGOs and build professional relationships with people who work along the product chain from crop to products.

What do you love about your job?

I have been able to follow the technology—from putting genes into single cells in 1980, through the application to crop plants in the 1990s—and its adoption by farmers to improve food production on millions of acres.

In my present job I have the pleasure of working and building relationships with a wide range of interesting and talented people inside and outside of Bayer. I enjoy that I have to integrate and do many different things, that I interact with people, and that I am able to think and act strategically. I also get great satisfaction when long-planned initiatives (often with more than five-year horizons) finally pay off. I like to travel so that is a positive, as my job often requires travel to meetings or workshops. For all that modern communication tools can be used effectively, there is no substitute for face-to-face relationship building and discussions.

What is the biggest challenge you have encountered in your career and how did you address it?

My biggest challenge at a personal level was being laid off during the formation of Novartis while I had young children to support. I prepared to try consulting, and actively searched for another position. In the end I was able to obtain a new position before my job at Novartis finished, through following up a lead from a colleague.

How has your industry changed since you entered the field?

When I entered the field in 1978 there was no biotech industry. We fantasized about what could be achieved, including glowing plants as street lights, but as young postdocs we couldn't fully imagine then the size of the industry as it now is, or the controversy that has arisen over agricultural biotech. In the beginning it was a research interest, although we (and others) were already filing patents by the early 1980s.

The industry started to take off in the 1980s, mainly manifested as research activities, and there were a lot of startups. With the advent of real products in the mid-1990s it became more focused on products rather than on the research aspects per se. Thus, a research project had to have a real product in mind. The industry underwent a series of mergers and acquisitions that have led to the six major companies we know now.

Today biotech is maturing in the adopting countries, becoming a part of an integrated agricultural product landscape that has multiple tools for increasing productivity.

Unfortunately the antiscience resistance to the technology has limited its adoption, to the detriment of all, and particularly developing countries. There is a general attack on science by a small proportion of society who are having a disproportionate effect and degrading our ability to improve the lot for society as a whole.

Do you have any advice for those looking to enter your field?

Follow your interests. You are best at what you are most interested in. There is a demand for good scientists at all levels. Decide whether you wish to focus more on the science side or management track—the prerequisites and career opportunites are different. You also have to choose between industry and academia. Academia has become a very tough area due to decreasing availability of public funding, necessitating a continual search for funding. However, I understand that it provides a freedom to follow your interests that is not so available in the industrial track.

How do you see the industry changing in the next five years?

Becoming more integrated into an agricultural product line that includes all the inputs for farmers. The seed, farming, grain, and food processing areas are becoming more interdependent, and thus I expect them to collaborate and communicate with each other in more ways than today.

Describe memorable job experiences.

Our first transgenic colonies of cells produced from protoplasts in 1983. Planting the first Bt tobacco field trials in North Carolina in July 1986. Seeing the first cell divisions of maize protoplasts. Inventing a method of identifying cells expressing an introduced herbicide tolerance gene and proving the concept within a week or two. Spending Easter weekend rebuilding a computer so a greenhouse control system could be managed. Intense teamwork inside and outside the company helping to manage our products. Working many hours with country experts in sessions at Codex meetings over eight years to complete a Codex guidance document.

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Please describe a course, seminar, book, mentor, or speaker that has inspired you in ways that have helped you advance your career.

My first big influence was Professor Street; he would always ask the right probing questions. Ingo Protykus put a good team of young researchers together and gave us room to develop our ideas. I learned the value of teamwork from working with Barbara Hohn in Basel. I was helped greatly by Mary-Dell Chilton at Ciba Geigy (now Syngenta). Her genuine interest in the science of what we were doing, in people, and her honesty, were what really influenced me. Margaret Gadsby at Bayer impressed on me the value of precision, brevity, and clarity in writing and communication, among other things (although I cannot always achieve it).

I cannot mention everyone here, but I have had the pleasure of working with and learning from a wide range of people, both within the companies I worked for, in associations such as the SIVB [Society for In-Vitro Biology], and industry and science associations. I only hope I have given something back.

Do you have any advice for young professionals who are trying to develop an effective network of other professionals?

Go to scientific meetings, if possible with someone that can introduce you to others. Present posters and talks. Do not be afraid of approaching the "known" people in the field in person to discuss science, but do not be too aggressive. For example, do not try to link on social networks (such as LinkedIn) unless you actually know the person.

If you were starting your career again, what would you do differently?

Don't think I would do anything different. I basically followed my interests.

What are the opportunities for advancement in your career/field and how can someone qualify for such advancements?

There are a lot of opportunities, from basic research to product development, communications, and marketing. Do good work. Be honest and professional. Acknowledge the contributions of others, but do not underplay your own achievements. Being able to work as part of and/or manage a team is a prerequisite to advance in today's world. In some companies the scientists used to have a good opportunity to become the top managers, but this seems to be less and less the case today—senior managers seem to be drawn more often from business and marketing backgrounds.

How would you describe the culture in your field, and how has it developed?

I believe there is a sadness that much of the true potential has been obscured by the attack on the technology by pressure groups. The cost of launching a product is so high that many potentially beneficial products never reach the consumer, especially in the smaller crops. In addition it is a shame that there has been an erosion of the ability to work on ag biotech in Europe, where much of the early work was done. However, those working in the field had and still have a high level of energy and belief in the benefits available through ag biotech and plant research, in the development of products, and in their commercialization.

In your area/field and considering today's market, is it more important to be well rounded or a specialist?

I think the trend is toward specialization. I am not sure that this is a good thing as someone who is well rounded will have a better idea of the unintended or less obvious effects of a proposed action, and be able to make better-informed decisions. We need both specialists and "renaissance" people, and they need to respect each person's value.

What is your opinion toward the value of obtaining or possessing a graduate degree during a challenging economy?

In the long run it is valuable and almost required to reach the higher levels in both the science and the science management track. I have watched my own children struggle with this question. Getting an advanced degree today is difficult—it takes too long, and costs too much, and by the time you have it, you may also have a family, or have delayed doing so. I myself benefited from the much more focused UK system, where it took much less time to obtain a Ph.D., so I could start on my career by my early 20s.

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Recent advances in producing omega-3 fatty acids from single-cell oils

Roberto E. Armenta

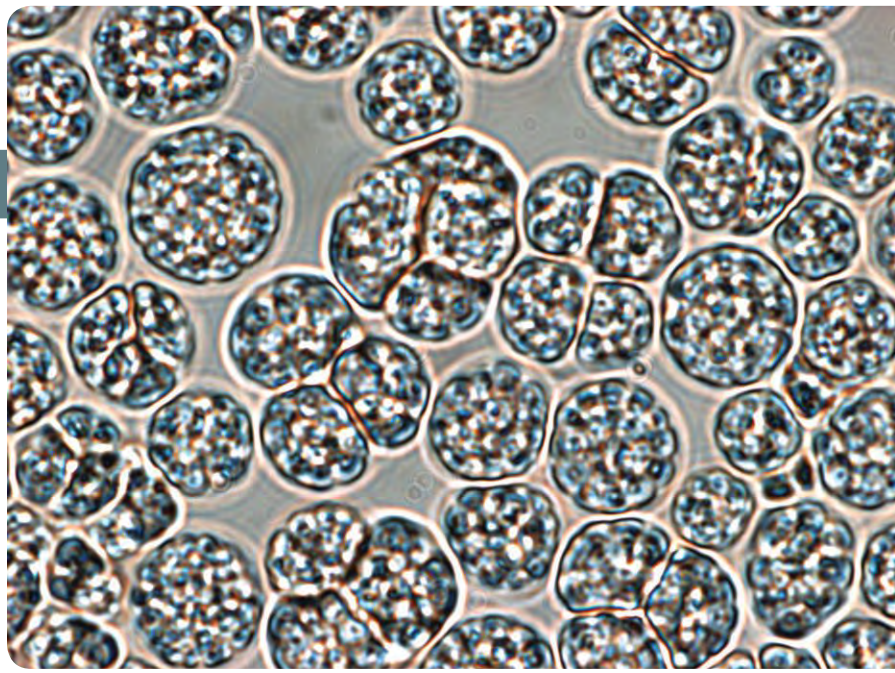

FIG. 1. Cells of a thraustochytrid, a producer of single-cell oil(SCO).

Polyunsaturated fatty acids (PUFA), specifically the omega-3 fatty acids (FA) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been linked to beneficial human health effects with respect to the heart and brain as well as several illnesses and diseases including asthma, rheumatoid arthritis, schizophrenia, depression, multiple sclerosis, and migraine headaches. Fish oil is presently the major source of omega-3 FA; however, recent high fish-oil market prices have increased interest in using single-cell oils (SCO), such as from algae, as an alternative to those from fish and land-based plant sources.

Microbes can be notable producers of SCO, and some of these oils may contain significant amounts of omega-3 and omega-6 FA such as EPA/DHA and arachidonic acid, respectively. Oil-producing microorganisms are found worldwide, both along coastlines and in the open ocean. Commercial microbial production of omega-3 FA is a relatively new field, and research in this area has grown rapidly over the last few years.

- **High fish-oil market prices have led to increased research on the use of single-cell oils as a source of omega-3 fatty acids.**
- **Technology breakthroughs will come from a combination of enhancements in fermentation, biology, and oil extraction.**
- **Production yields vary based on the microbe, the fermentative growing conditions, and the extractive procedures used to recover the oil.**

ORGANISMS THAT PRODUCE SCO

The term SCO was introduced in 1976 to define oils produced by single-celled microorganisms such as yeasts and molds. SCO not only are a commercial source of specialty lipids such as PUFA but have also been suggested as a promising source of biofuels. Current commercial SCO rich in omega-3 FA include oil with DHA from a *Schizochytrium* and EPA from the genetically modified yeast *Yarrowia lipolytica*. Thraustochytrids, which include the genus *Schizochytrium* and *Thraustochytrium*, are among the most promising microbes for producing omega-3 FA, with oil contents greater than 50% and more than 30% DHA within the

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FIG. 2. *Extracted SCO from a thraustochytrid.*

total oil produced. Thraustochytrids can produce both EPA and DHA; however, only one FA (often DHA) is produced in significant amounts—at least by nonrecombinant thraustochytrids. Thraustochytrid cells are depicted in Figure 1 and oil extracted from this organism is shown in Figure 2. The red-orange color of the oil is due to antioxidant carotenoids such as β-carotene and canthaxanthin.

Thraustochytrids are broadly called heterotrophic algae. However, their taxonomic classification is not yet settled. They have been associated with, and isolated from, decomposing algal and plant materials and from sediments and offshore water samples. Thraustochytrids are relatively unknown microbes, and new isolates have been reported around the world.

FERMENTATION

Several variables are considered when optimizing growth and FA accumulation by microbes in a fermentative process.

Carbon. The most commonly tested carbon feedstock is glucose, as the metabolic biochemical pathway that converts this sugar into FA is relatively efficient and is found in most microorganisms. The maximum theoretical lipid yield ($Y_{oil/glucose}$) for consumed glucose is 0.31, and 0.30 for glycerol ($Y_{oil/glycerol}$). This is paramount to consider when assessing microbial technologies that may suggest sugar–to-lipid conversion coefficient yields above the maximum theoretical limits.

Glycerol for producing SCO has become more generally available in the past five years because large amounts of raw glycerol are available as a by-product from the biodiesel industry. Nevertheless, soap and methanol contaminants in a glycerol by-product may inhibit microbial cell growth. The potential benefit of using glycerol by-product as a carbon feedstock is that its efficient use could reduce the cost of producing omega-3 FA.

Due to the large volumes of carbon used during fermentations, carbon is the most expensive fermentation ingredient when producing omega-3 FA. Thus, a current challenge regarding bioprocess optimization is finding and enhancing strains able to efficiently metabolize cheap and widely available sugars. After glucose, xylose, a five-carbon sugar, is the most abundant sugar monomer; it is found in hemicellulose. Typically, microbial metabolism of xylose is inefficient compared to hexoses such as glucose. For this reason, xylose is highly accumulated in industrial waste carbon feedstocks including agricultural and forestry wastes.

Nitrogen: This nutrient is needed for culture growth or biomass accumulation (first phase of growth, Fig. 3) as a component in amino acid and DNA synthesis, among other processes.

Once the nitrogen supply has run out, microbes use the remaining carbon to accumulate FA (second phase of growth, Fig. 4). Many

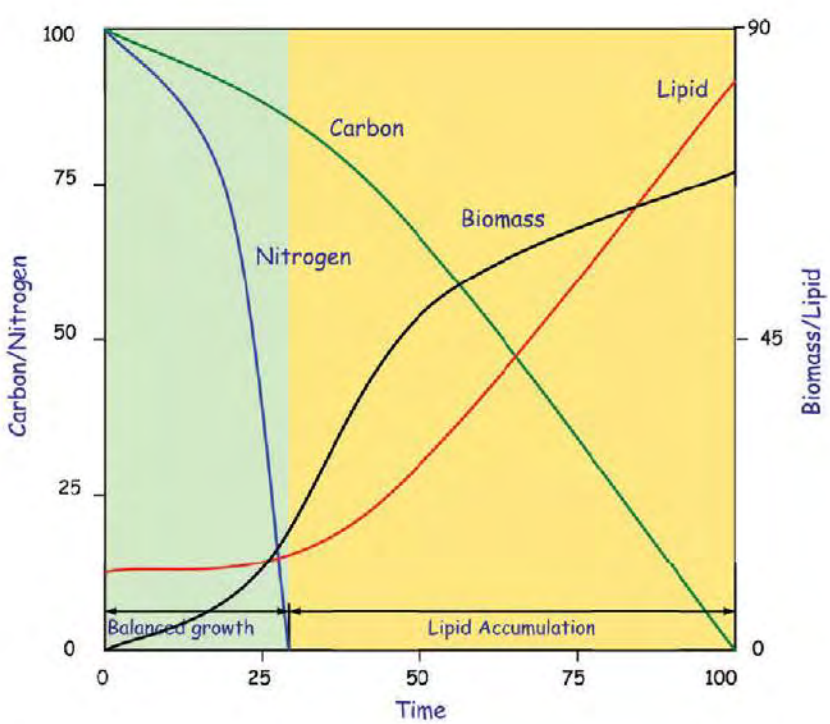

FIG. 3. *Growth cycle of SCO microbes*

sources of nitrogen are available for microbial growth; however, few published studies have targeted its usage optimization. Complex nitrogen sources, including yeast extract and peptones, are commonly used either separately or in combination. Also, nitrate, ammonium, and urea have been used.

In SCO production, nitrogen is the second most expensive ingredient, after the carbon feedstock. The cost of nitrogen increases significantly when it comes from complex protein sources such as yeast extract, soy peptone, and glutamate. Thus, an alternative for cost reduction is to use inorganic nitrogen including NH_4OH and $(NH_4)_2SO_4$. However, replacing protein with mineral nitrogen would likely result in the need for additional trace nutrients including minerals and vitamins.

Rendered animal protein and municipal wastewater have been used as nutrient sources to produce microbial oil. Both can be a potential source of nitrogen, and wastewater can provide phosphorus as well. Research to define their optimal use for SCO production could be worthwhile. However, governmental regulations will play a significant role in defining whether, and how, such nitrogen feedstocks could be used to produce SCO aimed for human and animal markets.

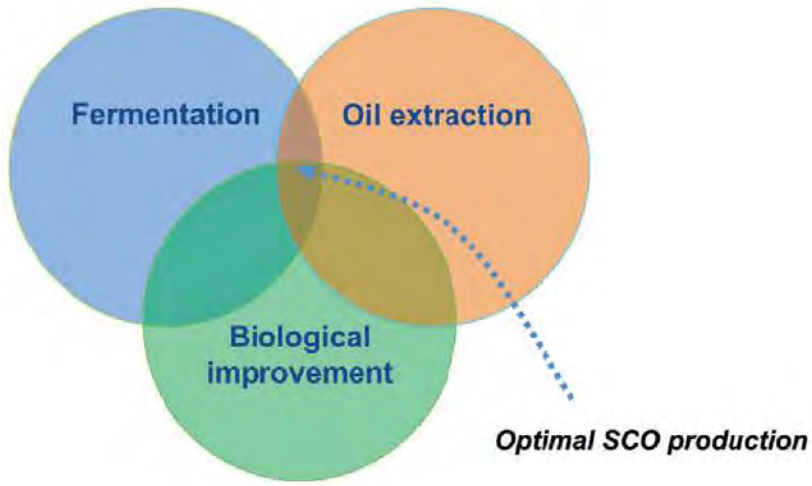

FIG. 4. *Process optimization for SCO production*

Dissolved oxygen (DO). During the initial growth phase of a fermentation process, the rate of oxygen consumption is high in a medium with abundant organic matter, as microbes use oxygen faster than it can be replaced. (A low DO is not a fermentation operating condition peculiar to a particular group of microbes.) During the second phase of fermentation, cellular oxygen demand drops and replication falls as nitrogen becomes limiting. At this point, microbes prioritize energy accumulation in the form of oil. Due to the many factors, individually and in combination, that can influence DO (e.g. fermentation media, temperature, agitation, pressure, etc.), DO is difficult to measure both at high and low percentages. Furthermore, the effect of DO on lipid desaturation of FA is highly debatable and remains to be investigated further.

pH. It is common to report the initial pH of fermentations; however, change in pH at the end of fermentation experiments is rarely indicated. When microbes are exposed to pH environments that are not optimal, they are forced to use energy to maintain the pH, thus microbial growth may be reduced as energy is diverted to pH maintenance.

Typically, pH is studied with the goal of defining optimal cell growth and oil productivity. However, one could study pH as a way to reduce sterilization needs in fermentation processes. Some oil-producing microbes are capable of growing at low pH, which could be a way to control contamination and, thus, reduce process energy inputs significantly.

Temperature. Overall, a higher temperature increases cell growth, while a cooler temperature promotes accumulation of FA. Nevertheless, the same temperature is generally maintained throughout microbial growth, and sometimes a dual temperature system is used where cultures are grown at a higher temperature for biomass growth (e.g., ≥25°C), and then at a cooler temperature (e.g., ≤20°C) for FA accumulation. The observed increase in DHA production at lower temperatures may be attributed to the microorganisms trying to maintain proper membrane lipid fluidity by the addition of more PUFA.

Salinity. Many oil-producing microbes are capable of growing in a variety of salinity levels. Many microorganisms require salt, in particular sodium ions, for growth, as it facilitates potassium ions' transfer across the cell membrane, which are also required for growth. In thraustochytrids, Na^+ is required owing to its role in cell osmotic regulation, not because of the direct Na^+ involvement in cell metabolism. However, there is evidence that thraustochytrid cells grow in the absence of Na^+, provided that cell osmotic regulation is adjusted by other means such as compatible solutes. Osmotic regulation in thraustochytrids is poorly understood.

INFORMATION

For an extended version of this article, please consult the following reference: Armenta, R.E., and M. Valentine, Single-cell oils as a source of omega-3 fatty acids: an overview of recent advances, *J. Am. Oil Chem. Soc. 90*:167–182 (2013).

OIL EXTRACTION

Oil extraction is the least investigated, or less reported, area of the whole bioprocess to produce SCO. Also, there is no universal method that will yield the best oil recovery for all microbes. In some microbes, cell walls are particularly thick and do an excellent job of maintaining oil within the cytoplasm; thus, a cell disruption method must be used to aid the recovery process. Examples

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of these mechanical techniques include sonication, bead milling, and the use of a French press.

Other techniques do not involve a separate cell disruption method but instead combine chemical extraction and FA esterification in one step with a mixture of solvents. This one-step process directly produces FA methyl esters (biofuel) and is typically used at the small scale to prepare samples for gas chromatography analysis of FA profiles. The concept behind solvent-based extractions is that solvents will extract compounds of similar polarity. Specifically, the solvent, or solvent mixture, must be able to efficiently enter the biomass, interact with the lipids, and form a solution whose components can later be easily separated to obtain the lipid fraction.

Supercritical fluid extraction (SFE) has also been used for oil extraction from microbial biomass. An SFE solvent has the ability to dissolve materials like a liquid and to diffuse through materials like a gas. SFE typically uses the solvent CO_2, and can extract a whole range of lipids depending on the type of microbial biomass and operating extraction conditions. When using SFE for oil extraction, microbial biomass must be dry as it does not work efficiently when samples contain high moisture.

To date, oil extraction with organic solvents (e.g., hexane) coupled with mechanical disruption remains the most efficient and economical technology to extract oil from microbes at an industrial scale. Other extraction technologies that minimize or avoid organic solvents in the extraction process are showing promising results at the laboratory and pilot levels, including pulse electric field, microwaves, sonication, and enzymes. However, these remain to be fully demonstrated and adapted at the industrial scale. The economics of these technologies must be determined as well.

Oil extraction can represent more than 40% of the cost of the finished microbial oil product due to high energy consumption required for this process. Hence, there is a need for developing cost-effective extraction technologies that can be scalable beyond the pilot plant level. Specifically, there is a need for technologies that both extract oil directly from wet cell biomass and avoid using, or at least use reduced amounts, of organic solvents, which would ease compliance with regulatory requirements for producing SCO intended for human consumption. This is a challenging task when the aim is to extract an SCO through a process that requires high extraction yields as well as effective protection against chemical oxidation of omega-3 FA.

BIOLOGICAL IMPROVEMENT

Genetically based strain improvement is the direct enhancement of product formation or cellular properties through modification of specific biochemical reactions using recombinant DNA techniques. This produces a genetically modified microorganism (GMO).

Classic mutagenesis is a type of genetic manipulation in which cells are exposed to a mutagen, then screened for potential enhancements, and cultivated as potentially enhanced species. Two widely used mutagens are ultraviolet radiation (UV) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). In one set of experiments, both UV and MNNG were applied on the microbe *Schizochytrium* sp. As a result there was an increase of more than 34% in oil production and a doubling of DHA yield. Several FA metabolic pathways are involved in the biosynthesis of PUFA. This adds significant complexity when regulation for a specific FA (e.g., EPA and DHA) is targeted.

Genome shuffling is another strain improvement technique that uses protoplast fusion from two different microbial cells. It may be used to enhance oleaginous microbes as it speeds up evolution by allowing multiple parental DNA to recombine.

Other ways of using metabolic engineering include providing an organism with an ability that was not previously present. This is accomplished through insertion of DNA that originated in a different species; the resultant microorganisms are termed transgenic. An example of this approach has been the genetic modification of *Saccharomyces cerevisiae* to produce EPA.

Genetic modification approaches have also been used to facilitate oil extraction. Initial research with both yeast and bacteria produced cells that secreted FA as they grow. Although oil yields were low, these results could be the base for future improvements that may ease extraction of SCO. A major challenge for making FA cellular excretion efficient would be to minimize consumption of released free FA by either the producing microbe or other microbes present in mixotrophic cultures.

As the body of biological knowledge increases, breakthroughs for SCO production will come from advances in metabolic engineering. Except for *E. coli, S. cerevisiae*, and *Chlorella,* full genomes for other oil-producing microbes are of limited availability. *Chlorella*, a relatively well known oil-producing microbe, is perhaps the most viable algae for genetic improvement aimed to customize FA profiles. *Chlorella* can grow in both autotrophic and heterotrophic conditions, and it is grown in dark fermenters to maximize oil productivity. Regardless of any genetic modification to which *Chlorella* and other microbes may be subjected, maximal theoretical lipid-to-sugar yields cannot be overcome.

CHALLENGES AND OPPORTUNITIES

Research on SCO as a source of omega-3 FA and other potential bio-products is relatively new and require further investigation. Advances that could result in cost savings at the industrial scale will likely come from a combination of enhancements in fermentation, oil extraction, and genetics (Fig. 4, page 53).

Of course, any processing changes would need to comply with regulatory requirements for commercial production of edible SCO; and benefits achieved by genetic engineering would also need to be weighed against the risks of negative consumer perception of GMO. Finally, there is a need to reduce the cost of oil extraction, where the challenge is to develop a technology that not only eliminates or reduces effects on the chemical stability of omega-3 FA but also is economically and ecologically sustainable.

Roberto E. Armenta has worked extensively with biological processes to produce both microbial high-value products (including carotenoids and nutritional oils) and commodities (such as biofuels). He directed the research and development effort at Ocean Nutrition Canada to produce nutritional lipids from microalgae. Currently at Mara Renewables (Dartmouth, Nova Scotia), he leads the most advanced R&D program in Canada to produce heterotrophic microalgal oil, including work on genetics, fermentation optimization, and downstream processing. He can be contacted at rarmenta@maracorp.ca.

How *trans* fats almost got saturated

This article was already in press when—on November 7, 2013—the US Food and Drug Administration announced its preliminary determination that partially hydrogenated oils are not Generally Recognized as Safe. See this issue's News & Noteworthy department for more on that story.

David Schleifer

Are *trans* fats saturated fats? The answer may seem obvious to an oil chemist. But in the late 1990s and early 2000s, the US Food and Drug Administration (FDA) had to decide whether *trans* fats should be distinguished from saturated fats on Nutrition Facts labels. How did the FDA come to face this question and how did industry respond?

In the late 1980s, many food manufacturers and restaurant chains replaced saturated fats with partially hydrogenated oils. They were praised for using these supposedly more "heart-healthy" oils by the Center for Science in the Public Interest (CSPI) and by Phil Sokolof's National Heart Savers Association. But in 1990, the *New England Journal of Medicine* published Mensink and Katan's study concluding that *trans* fatty acids both raised low density lipoprotein (LDL) choloesterol and lowered high density lipoprotein(HDL) cholesterol). Willett and colleagues published epidemiological research associating *trans* fats consumption with an increased risk of heart disease (Willett *et al.*, 1993). In 1994, US Department of Agriculture researchers published findings that more or less confirmed Mensink and Katan's conclusions (Judd *et al.*, 1994). Meanwhile, the US federal Nutrition Labeling and Education Act of 1990 (NLEA) was scheduled to take effect in 1994. Rather than enumerating *trans* fatty acids separately, the initial NLEA rules would include them as part of the "total fat" measurement on Nutrition Facts labels.

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- **The US Food and Drug Administration considered grouping *trans* fats together with saturated fats on Nutrition Facts labels.**
- **Food and edible oil industry representatives argued that labeling the two fats would create an incentive to reformulate.**
- **The two fats are now labeled separately, allowing manufacturers to show consumers the extent to which they have replaced *trans* fats.**

INFORMATION

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CSPI submitted a *trans* fat labeling petition to the FDA on February 14 (Valentine's Day) 1994. It proposed that the FDA "should amend the definition of saturated fat … to include *trans* fat" and even that the FDA should change the term "partially hydrogenated" to "partially saturated" (pp. ii–iii). CSPI wanted manufacturers to add up the grams of saturated fats and *trans* fats and to list the combined total as "saturated fat" on Nutrition Facts panels. CSPI also argued that foods labeled "saturated fat free" should contain less than 0.5 grams of saturated plus *trans* fats combined per serving. The organization's petition recognized that "*trans* fatty acids are not saturated by a chemical definition" (p. 4). But it maintained that the point of labeling was to assist consumers and that it would be easiest for them if these two types of "unhealthy" fats were combined and labeled as "saturated fats" (pp. ii–iii). CSPI actually amended its petition in 1998. It suggested that the FDA consider asking manufacturers to list the combined total of *trans* fat plus saturated fats as "saturated fat*." A footnote would indicate the precise quantity of *trans* fats.

AOCS resources on *trans*-fat replacement

AOCS has been working with industry and regulatory agencies for years, validating methods of analysis for *trans* fatty acids, while AOCS members have been researching and developing reformulation strategies. To access AOCS resources on the topic of *trans*-fat replacement, go to our new resource page at http://www.aocs.org/Resources/content.cfm?ItemNumber=19138&navItemNumber=19191.

CSPI's petition did not mention how labeling would affect industry. But for the FDA, informing consumers about *trans* fats was a way to encourage manufacturers to replace partially hydrogenated oils. When the FDA published its proposed labeling rule in 1999, it began by noting that only mandatory labeling would provide manufacturers with "sufficient incentive for reformulation" (Food Labeling: Trans Fatty Acids in Nutrition Labeling, Nutrient Content Claims, and Health Claims. *Federal Register 64627-62764*, 1999.).

How did the FDA propose rendering *trans* fats on labels in order to create this incentive? The agency essentially endorsed CSPI's 1998 proposal to identify the combined total of *trans* and saturated fats as "saturated fat," with asterisks referring to a footnote listing grams of *trans* fats. The agency designed the following sample:

The FDA also proposed that products bearing either a "*trans* fat free" or "saturated fat free" claim would have to have less than 0.5 grams of saturated fat plus *trans* fat combined per serving. But the FDA conceded doubts about categorizing what the regulators described as two chemically distinct substances as one. The agency invited responses to what it described several times as their "tentative" proposal.

Edible oil companies, oil suppliers, trade associations, food manufacturers, and other industry actors sent hundreds of letters to the FDA in which they largely agreed that labeling could create an incentive for manufacturers to replace *trans* fats. But industry almost unanimously argued that in order to create that incentive, labels should distinguish between *trans* fats and saturated fats.

For example, the Institute of Shortenings and Edible Oils (ISEO) wrote to the FDA arguing that the only "rational approach" to labeling was to list *trans* fats separately from saturated fats because of uncertainty about their relative physiological effects and because combining them would destroy "the incentives needed to encourage healthful product reformulation." Similarly, Cargill's letter argued that grouping the two fats together would create "very little incentive for companies making these types of foods to reformulate." The United Soybean Board, National Sunflower Association and US Canola Association and other trade associations wrote noting that breeders were already working on alternative technologies. But they maintained that trade associations and suppliers would only have reason to continue their efforts if manufacturers' labels showed consumers that products had been reformulated.

Willett's team wrote several letters to the FDA maintaining that saturated fats have benefits such as increasing HDL cholesterol, whereas *trans* fatty acids are completely unhealthy. They also argued that categorizing the two fats separately would create incentives for manufacturers to reformulate. And they pointed out that if the FDA limited saturated fat content for foods claiming to be "*trans* fat free" then not even olive oil would qualify. Chocolate, pork, dairy, and beef industry representatives wrote letters noting that if the FDA wanted to classify fats according to their effects on cardiovascular risk factors, then Nutrition Facts labels would have to disaggregate stearic acid from other types of saturated fats because of

its ostensibly neutral effects on LDL and beneficial effects on HDL cholesterol.

Ultimately, in its 2003 final rule, the FDA decided that manufacturers would have to quantify *trans* fats separately from saturated fats, and list each on its own line on Nutrition Facts labels. The FDA decided to disallow "*trans* fat free" claims but to permit manufacturers to state "zero grams *trans* fat" on the fronts of packages. The final rule contended that the resulting "increased consumer attention" would "provide an incentive to food manufacturers to reduce the amount of *trans* fat in their products" (p. 41467).

In fact, the effect of labeling has arguably been less to inform consumers about which products do contain *trans* fats and more to allow manufacturers to publicize the tremendous extent to which most products no longer contain *trans* fats at all. In 2009, the Grocery Manufacturers Association estimated that manufactures had reduced or replaced *trans* fats in over 10,000 products sold in the United States. A 2013 analysis found that *trans* fats had been reduced or replaced in 66% of a sample of 270 food products sold in US supermarkets (Otite *et al.*, 2013).

Adding *trans* fats to Nutrition Facts labels took 12 years from petition to implementation. Breeders, growers, oil suppliers, and manufacturers developed and commercialized complex *trans* fat replacement projects during that time. The FDA hewed to chemical structure in labeling but clearly wanted firms to replace *trans* fats based on their physiological effects. This could be a foretaste of the lengthy and painstaking processes of changing other aspects of food labeling—such as genetically modified organisms or added sugars—and of creating incentives for suppliers and manufacturers to pursue other, equally complex changes.

David Schleifer (@david_schleifer on Twitter) is a senior research associate at Public Agenda (New York, New York, USA), where he designs and executes qualitative and quantitative research on domestic policy and public opinion. He can be reached at david.schleifer@gmail.com.

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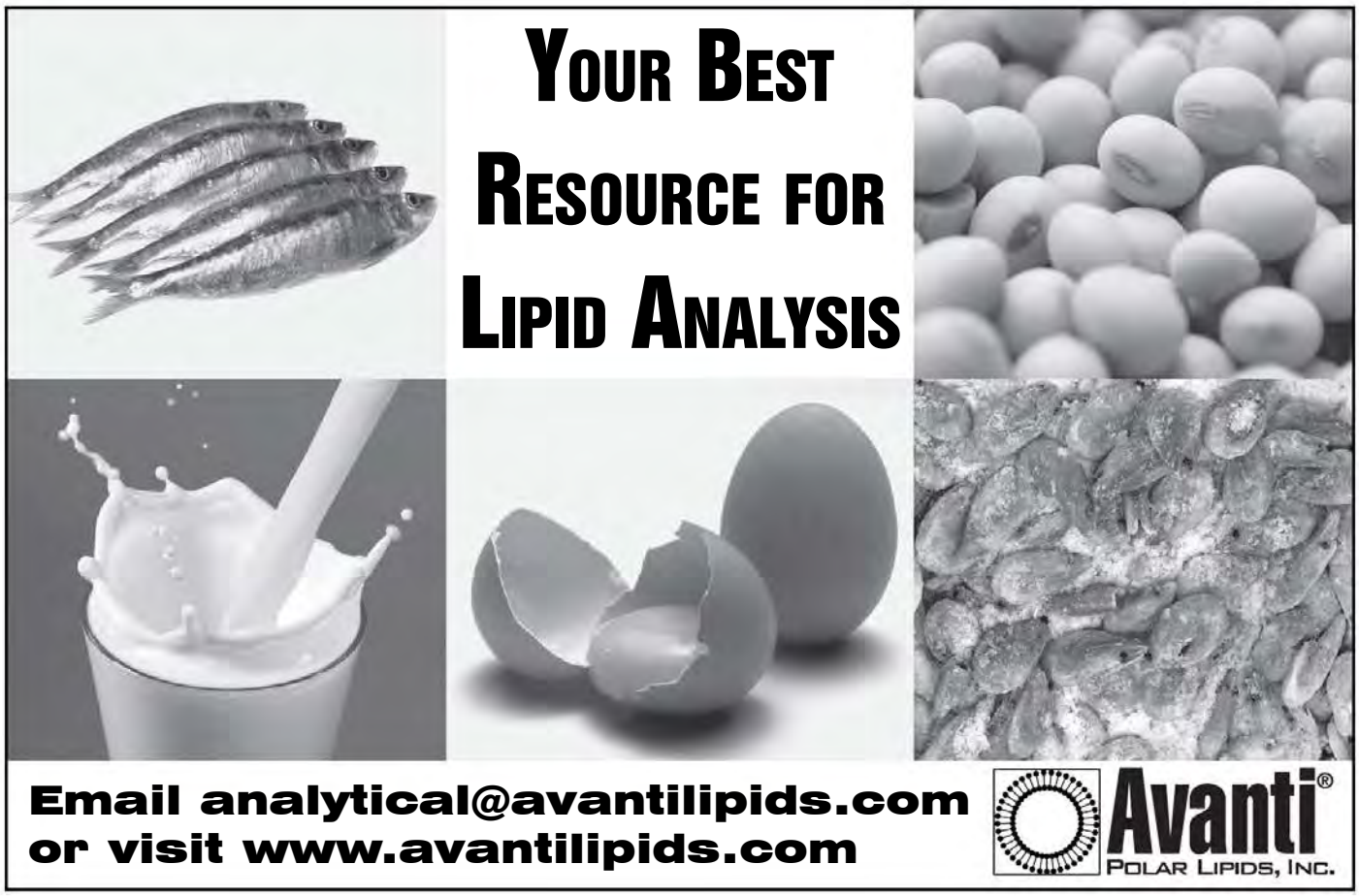

The contribution of individual fatty acids to the melting point pattern of bovine milk fat

Lars Wiking, Patrizia Buldo, and Mette K. Larsen

Milk fat contains a very high diversity of fatty acids (FA) and thereby triacylglycerides (TAG), which together result in a broad melting range from –40°C to 40°C. The quantity and type of FA in milk fat affect its melting properties, which are important for the mouth feel of butter. Milk fat melts in three fractions, low, medium and high, as shown in the differential scanning calorimetry (DSC) thermogram in Figure 1.

The FA composition of bovine milk fat is primarily affected by cow nutrition, and two main synthesis pathways in the cow are distinguished. Short- and medium-chain saturated FA (C4 to C14) and some palmitic acid (C16:0) arise by *de novo* synthesis, which occurs inside the mammary epithelial cell. The other pathway of milk FA involves plasma lipids. This pathway consists of FA that are derived from feed or from body fat mobilization, and it produces long-chain FA (≥C18 and some C16). Palmitic and stearic acids (C16:0 and C18:0) from the feed pass through the rumen unchanged, whereas the main unsaturated FA in feed—oleic (C18:1 *cis*-9), linoleic (C18:2 n-6), and α-linolenic acid (C18:3 n-3)—are biohydrogenated to a very high degree, resulting mainly in C18:0 together with smaller amounts of different monounsaturated C18 FA. In the mammary gland, some of the medium- and long-chain FA from the rumen are desaturated by stearoyl-CoA desaturase. The outcome of these syntheses is a composition with up to 400 different FA, and only 12 of these are present in amounts higher than 1%.

- **Predicting the melting points pattern of milk fat is difficult, as the contribution of individual fatty acids to these patterns is complex.**
- **However, multivariate data analysis can be used to get an overview of relations between fatty acid composition and melting behavior of milk fat.**
- **Our studies show that fatty acids present in the highest quantities generally determine the melting point of milk fat.**

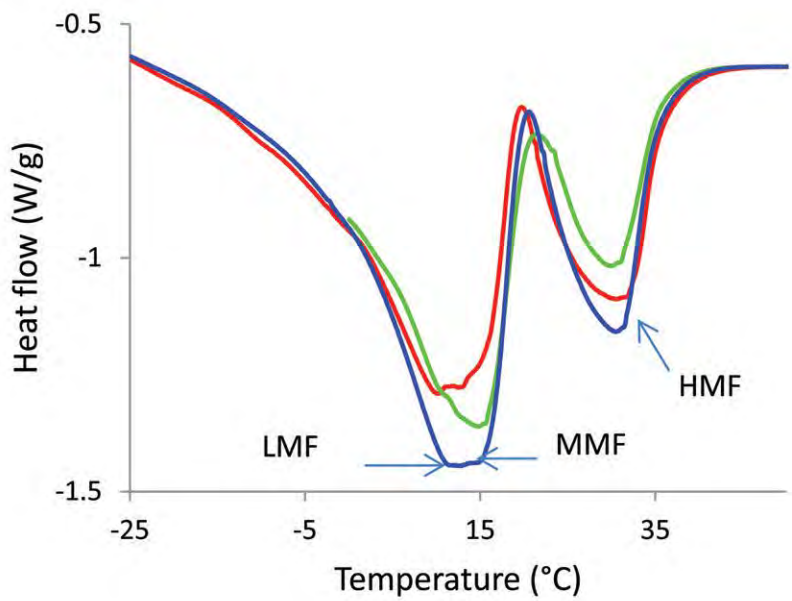

FIG. 1. *Differential scanning calorimetry melting curves of milk fat from three dairies in Denmark. The arrows shows the low- (LMF), middle- (MMF), and high melting fractions (HMF).*

One of our research focuses is to understand the relationship between the raw material composition and the product quality. FA composition of milk can be altered by manipulating the feed of the dairy cow, and science has primarily focused on the human nutrition quality of the produced milk fat. However, the properties of milk fat intended for further processing into butter and cheese are also an important issue. The melting point of fats is generally related to the TAG composition, but because milk has a very complex FA and thus TAG composition it is more complicated to link the two. We have started to use multivariate data analysis to get an overview of relations between FA composition and melting behavior of milk fat.

In a principal component analysis (PCA), a multivariate data set is summarized and visualized as a compressed representation of the observations in a lower dimensional space (most frequently a plane). Thus, similarities and differences among the data are described, and patterns of the data are identified. Regarding DSC scans, a lot of parameters can be identified such as melting on- and off-set and peak minima, but the entire thermogram also can be used as independent X variables to be analyzed by PCA to separate samples. By using partial least squares (PLS) analysis for multivariate calibration, quantitative relations between variables can be established.

PCA was used on data from a study including 33 dairy cows from four farms, where the cream from individual cows was analyzed by DSC for FA composition and for melting behavior. Results of this PCA are shown in Figure 2 (page 60). Samples from Farm C were grouped and separated from those of the other farms. The loadings explain the pattern in FA composition

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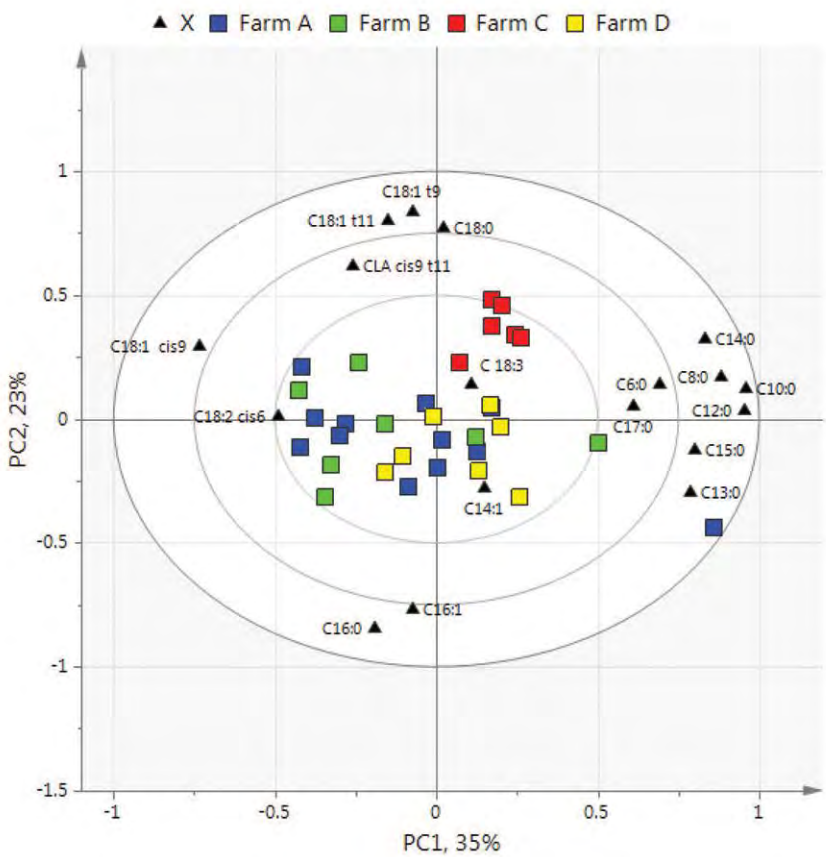

FIG. 2. *Score and loading plot of principal component (PC) analysis model for fatty acid composition of bovine milk from four Danish farms. Abbreviations: t, trans; CLA, conjugated linoleic acid.*

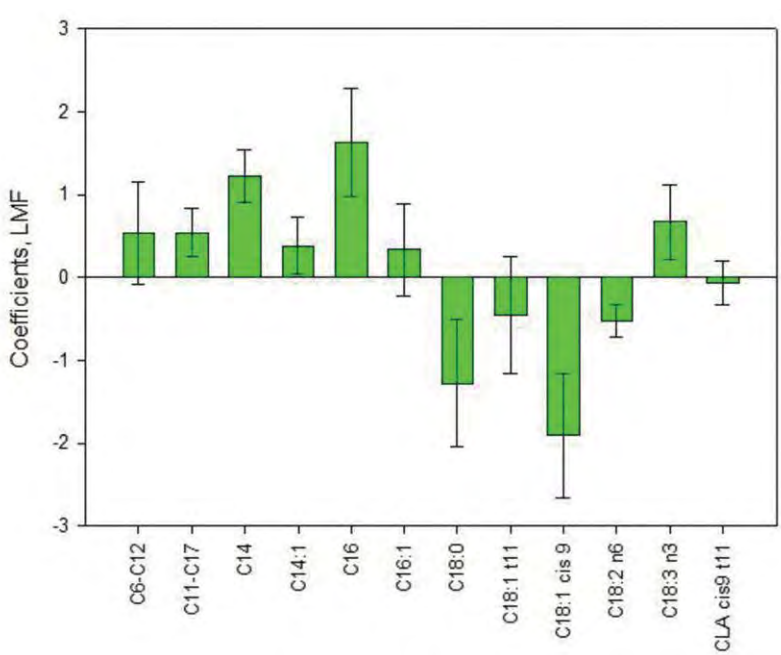

FIG. 3. *Scaled coefficient plot of partial least squares model for prediction of the melting point of the low-melting fraction (LMF) based on the fatty acid composition of milk fat.*

that causes the separation of Farm C: C18:0, C18:1 *trans*-9 and C18: *trans*-11 point in the same directions as Farm C, whereas C16:0 and C16:1 point in the opposite direction. Farm C administered the highest level of forage of the four farms, and this explains the higher proportions of C18 FA in the milk fat at the expense of C16. In another study we separated milk fat from different dairies and followed seasonal changes of FA composition by use of PCA. We also analyzed the TAG composition but found that it did not give a better separation in a PCA analysis than samples based on FA composition.

PLS analysis based on milk fat samples from three Danish dairies collected during a whole year—where FA were used as independent variables and melting points as dependent variables—gave a valid model for the melting point of the low-melting fraction; 66% of the variation could be explained. However, the explanations for the medium- and high-melting fractions were only 14% and 5%, respectively. For the melting point of the low-melting fraction, coefficients of the individual FA are shown in Figure 3. The main FA responsible for increasing the melting point of the low-melting fraction were C16:0 and C14:0, whereas the main FA decreasing the melting point of the low-melting fraction were C18:1 *cis*-9 and C18:0. Across our studies, there is a contradiction whether C18:0 or C18:1 *trans*--9/*trans*-11 and C18:2 *cis*-9,*trans*-11 (CLA) contribute negatively to the model. In general, the results demonstrate that the three or four FA present in the highest concentration have the highest effect on the melting point. It was not possible to predict the melting point of the medium- and high-melting fraction from the fatty acid composition which can be due to lower variations in these meting points compared to the low-melting fraction. Itmay as well be difficult to affect this fraction by altering the feed consumed by the dairy cow as the milk fat is kept fluid at cow body temperature as a function of the desaturase system.

Recent developments in algorithms of infrared (IR) spectrometry for analyzing milk composition have made possible fast determinations (at line or online) of the major FA. In combination with the prediction models we are working to develop, it could in the future be possible to optimize conditions in the butter manufacturing processes based on IR analysis of the milk.

Lars Wiking and Mette K. Larsen are associate professors in the Department of Food Science, Aarhus University, Denmark. They can be contacted at Lars.Wiking@agrsci.dk and Mette.Larsen@agrsci.dk, respectively.

Patrizia Buldo is a post-doctoral researcher in the Department of Food Science, University of Copenhagen, Denmark. She can be reached at PBU@food.ku.dk.

Omega-3 long-chain fatty acids and their use in traumatic brain injury and concussions

Michael Lewis

Traumatic brain injury (TBI) is a major cause of death and disability worldwide, especially in children and young adults. Of the 44 million youths in the United States who play organized sports, at least a half million are estimated to suffer a sports-related head injury each year, while the Centers for Disease Control and Prevention (CDC) estimates that, on an annual basis, sports, car accidents, and falls (the young and old are particularly at risk) combined cause between 1.7 and 3.4 million Americans to suffer a head injury. Survivors of TBI and concussions are often left with significant cognitive, behavioral, and communicative disabilities, and some patients develop long-term medical complications. According to the US National Institutes of Health (NIH), TBI has left more than 5 million Americans with a permanent need for help in performing daily activities, costing more than $76 billion annually.

According to two of the most up-to-date clinical websites (NIH and CDC), in addition to seeking appropriate medical care, the two most important things to do after a concussion are (i) to eliminate the possibility of additional blows to the head and (ii) to rest to allow the brain to heal as quickly as possible. When the brain is injured, its consumption of glucose and other

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- **Traumatic brain injury (TBI) is a major cause of death and disability worldwide.**
- **Long-term treatment primarily involves rest, and recovery times vary significantly.**
- **The following article considers whether supplementation with omega-3 long-chain fatty acids, which play a major role in brain development and health, could make it easier for the brain to heal itself.**

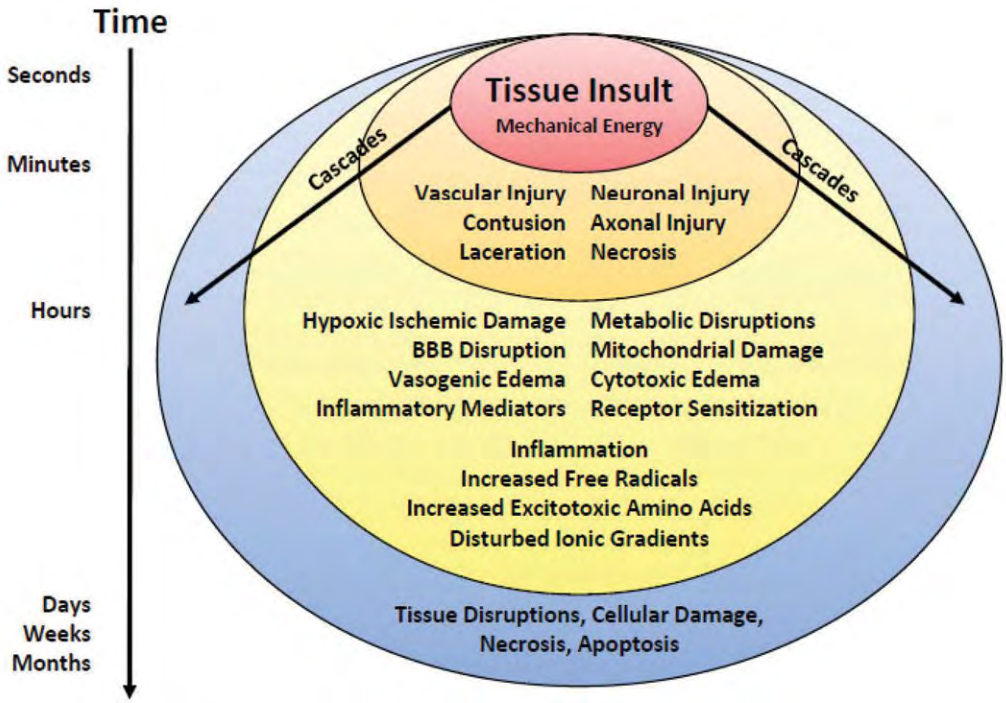

FIG. 1. *Secondary phase of brain injury after the primary insult. BBB, blood brain barrier.*

substrates is greatly increased. Physical rest, as well as rest from thinking and other cognitive activities such as reading, studying, using a computer, and playing video games, is critical for the brain to heal and return to its baseline metabolism. The length of time it takes for this to occur can vary widely, from hours to days to weeks to months.

BASICS OF TRAUMATIC BRAIN INJURY

The brain may sustain a primary injury when mechanical energy is transferred to the brain, whether through a motor vehicle accident, falling and hitting the floor, a head-to-head collision in football, a gunshot to the head, or exposure to a blast wave from an explosion. While the devastating effects of a severe TBI are immediately apparent, the effects of a concussion, sometimes called a mild TBI, may not be detectable right away—even with an MRI scan. In either situation, cascades of biochemical events cause secondary injuries over minutes, hours, days—even weeks after the initial injury. This secondary response leads to increased damage (see Fig. 1).

Major advances in the acute care of severe TBI have occurred over the past decade, particularly neurosurgical interventions born out of necessity on the battlefields of Iraq and Afghanistan by US military surgeons. However, despite considerable research efforts, current treatment of brain injury beyond acute surgical intervention is largely confined to supportive measures. These advances are not trivial. They have resulted in countless patients living through a severe TBI, when otherwise they would not have survived. But in both severe TBI and in concussions, the secondary injury phase of brain injury can be a prolonged pathogenic process characterized by neuroinflammation, excitatory amino acids, free radicals, and ion imbalance. There are no approved therapies to address these underlying cascades and limit the damage that continues to occur following the primary injury.

So what does medicine have to offer a patient after a severe TBI or a concussion that remains symptomatic? As the mother of one victim said, "He [her son] lived, he survived, and now what?" Herein lies the problem: *There is nothing.* Modern medicine has nothing to offer. Patients and their families are told only time will heal the brain and are offered little else except pharmaceutical intervention for each individual symptom. If the patient is having pain, they are prescribed a pain medicine (or two, or three, or worse, narcotic pain medications). Trouble sleeping? Prescription sleep medications. Anxiety? Benzodiazepines. Depression? SSRI [selective serotonin reuptake inhibitor] antidepressant medications. None of these interventions addresses the underlying damage to the brain.

As a physician in the US Army, I spent more than three years developing programs for the military on the use of omega-3 long-chain fatty acids (omega-3s) to treat TBI. My idea was to make it easier for the brain to repair itself by providing it with more of the raw materials it needs. Since omega-3s—particularly docosahexaenoic acid (DHA)—are the "bricks" of the brain's cell walls, my strategy was to speed the brain's recovery by delivering optimal amounts of omega-3s to saturate the brain, giving it the best opportunity to heal itself. In my clinical experience advising hundreds of patients over the past few years, not only do omega-3s help the brain heal, but patients often experience a rapid improvement of post-concussive

Omega-3s and brain injury

We know omega-3 long-chain fatty acids (omega-3s) are important throughout life. They are essential for the development of the fetal human brain, as well as through infancy and childhood. (The January 2013 issue of *Inform* featured a cover story about the omega-3 fatty acid docosahexaenoic acid—DHA—in the brain.) In fact, the male brain continues to grow until around age 25.

But while omega-3s are known to support cardiovascular and heart health as well as neurological health, the typical diet in most Western countries is woefully deficient in omega-3 intakes, particularly when compared to intakes of their competitive cousin, omega-6 long-chain fatty acids (omega-6s).

Omega-6s are commonly found in seeds, nuts, and the oils extracted from them. A common source, soybean oil, is widely used in snack foods, cookies, crackers, sweets, and fast food consumed in the United States. The consumption of soybean oil has increased by 1200% since around 1970 (Blasbalg *et al*., 2011). It is so ubiquitous in processed foods and fast foods that an astounding 20% of the calories in the American diet is estimated to come from this single source.

Omega-6s increase inflammation, blood clotting, and cell proliferation, whereas omega-3s modulate those functions. Both families of omega fatty acids must be in balance with each other to maintain optimal health. However, due to significant changes in the US food chain and dietary habits, that balance has been skewed in American society from one or two omega-6s for every omega-3 to a ratio of 25:1 or higher (Blasbalg *et al*., 2011; Lewis *et al*., 2011). Consequently, we generally have too many omega-6s and a deficit of omega-3s, which limits the brain's ability to repair itself with the anti-inflammatory building blocks it needs. This omega-3 deficiency puts us at increased risk of a difficult recovery from a concussion, particularly soldiers in theaters of war and youths who participate in collision sports such as American football, soccer, hockey, rugby, and others.

symptoms such as headaches, lethargy, insomnia, and cognitive difficulties.

According to Hee-Yong Kim of the NIH, omega-3s have three important functions during a brain injury. They are neuroprotective (keep brain cells alive); they are anti-inflammatory; and they promote neurite development and synaptogenesis (increase the length and number of branches from a brain cell, creating more connections between brain cells). A few animal studies clearly demonstrate the ability of omega-3s to preserve neurologic tissue in stroke (Belayev *et al*., 2009) and spinal cord injury models (King *et al*., 2006). Only one series of studies, conducted by researchers has shown their usefulness directly in TBI (Bailes and Mills, 2010). Unfortunately, there is not much to be found in the scientific literature beyond animal studies. Only two case reports have been published about the use of omega-3s in severe brain injury in people.

In January 2006, the Sago Coal Mine Disaster occurred in West Virginia. When faced with the lone survivor, Randy McCloy, physicians at West Virginia University's hospital had to employ extreme methods to save his life. McCloy had suffered severe carbon monoxide and methane gas poisoning resulting in kidney and liver failure, a heart attack, and significant brain damage from a lack of oxygen. Kidney dialysis and hyperbaric oxygen therapy were used aggressively. However, with nothing available to help the brain recover, the attending neurosurgeon began administering large doses of fish oil through McCloy's feeding tube. His thought was to use the same substance that is known to build the human brain *in utero* to see whether it could perhaps help the brain recover. It did and, and the results were published as a case report in 2008 (Roberts *et al*., 2008).

In March 2010, a Virginia teenager sustained a severe TBI in a motor vehicle accident. The attending neurosurgeon's impression was that the injury was likely lethal. Citing the Sago Mine Disaster case, Michael Lewis, at that time a physician in the US Army, recommended to the neurosurgeon and the patient's family that the use of high doses of fish oil containing omega-3s might be useful in promoting recovery. The son survived and even attended his high school graduation three months later (Lewis, Ghassemi, and Hibbeln, 2013).

While these two case reports are the only published experiences using omega-3s with severe brain injury (carbon monoxide poisoning in one; a severe TBI in the other), they have been well documented and featured on multiple news programs, such as CNN's *Sanjay Gupta, MD* show. As a result of this media exposure, hundreds of people have reached out to groups such as Brain Health Education and Research Institute asking for advice. In some instances, parents and doctors have taken action, such as in the case of an 8-year old girl who made a surprising recovery following a near drowning. She was administered omega-3 fish oil after being in a coma for over two months and within two weeks came out of her comatose state. This case was documented on a local ABC television affiliate in upstate New York. Physicians managing other cases of severe TBI and sports concussions continue to access protocols made available online by the Brain Health Education and Research Institute (www.brainhealtheducation.org).

WHAT CAN BE DONE FOR TBI NOW?

Conventional medicine can take survivors of severe TBI and symptomatic concussions only so far. Concussion patients may continue to experience symptoms for months, even years, without any effective therapies. The situation is even worse for severe TBI patients who often end up heavily medicated at home

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or in a nursing home, overwhelming family resources. According to Goldstein (2012), the father of a TBI survivor, unconventional therapies are not merely a reasonable option, they are a necessity. Several very safe therapies (nutrition such as omega-3s, hyperbaric oxygen, craniosacral manipulation, and others) can have enormous impact. These do not cure brain injury, but they optimize the brain's opportunity to heal itself and give a patient the best chance to regain as much function as possible.

The potential role for omega-3s seems obvious but has yet to be systematically studied outside the research laboratory (Lewis & Bailes, 2011). Unfortunately, sometimes even the best-trained, most-skilled, and well-intentioned professionals within the medical establishment suffer tunnel vision, sticking to their familiar, well-trodden paths rather than be pioneers blazing new trails to help patients and families. We don't yet have a randomized, placebo-controlled, clinical trial of omega-3s for either severe TBI or concussions. But we do know that omega-3s are the nutritional foundation of the brain and the neuronal cell wall. We know from scientific research that omega-3s are neuroprotective, modulate inflammation in the brain, and even promote synaptogenesis. And we know from growing amounts of clinical experience, that omega-3s can be immensely useful to decrease or eliminate many of the symptoms that plague patients following brain injury. With this knowledge, why not use the best available knowledge today to help brain injury patients?

Michael Lewis is a graduate of the US Military Academy at West Point and Tulane University School of Medicine (New Orleans, USA). He completed his post-graduate training at Walter Reed Army Medical Center, Johns Hopkins University, and Walter Reed Army Institute of Research. Lewis is board certified and a Fellow of the American Academy of Preventive Medicine. He retired at the rank of Colonel following over 31 years of US Army service. He is currently in private practice in the Washington, DC, area, and is the founder and president of the Brain Health Education and Research Institute. He can be contacted at dr.michael.lewis@gmail.com.

[FAST FACT]


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Dozens of 380-million-year-old intact biolipids have been found in the fossil of a crustacean from the Devonian Reef in the Kimberley region of northwestern Australia. The lipids, which are 250 million years older than any found previously, include original sterols from algae in the oxygenated part of the ocean, cholesterol from the crustacean itself, at least 70 different steroids, and a range of compounds formed by sulfate-reducing bacteriathat degraded the organic matter contained in the crustacean. Other compounds found in the fossil included aromatic steroidal hydrocarbons often found in petroleum. More information is available at http://phys.org/news/2013-11-reef-fossil-age-limit.html or in the original paper (Melendez, I., K. Grice, and L. Schwark, *Sci. Rep.*, doi:10.1038/srep02768, 2013).

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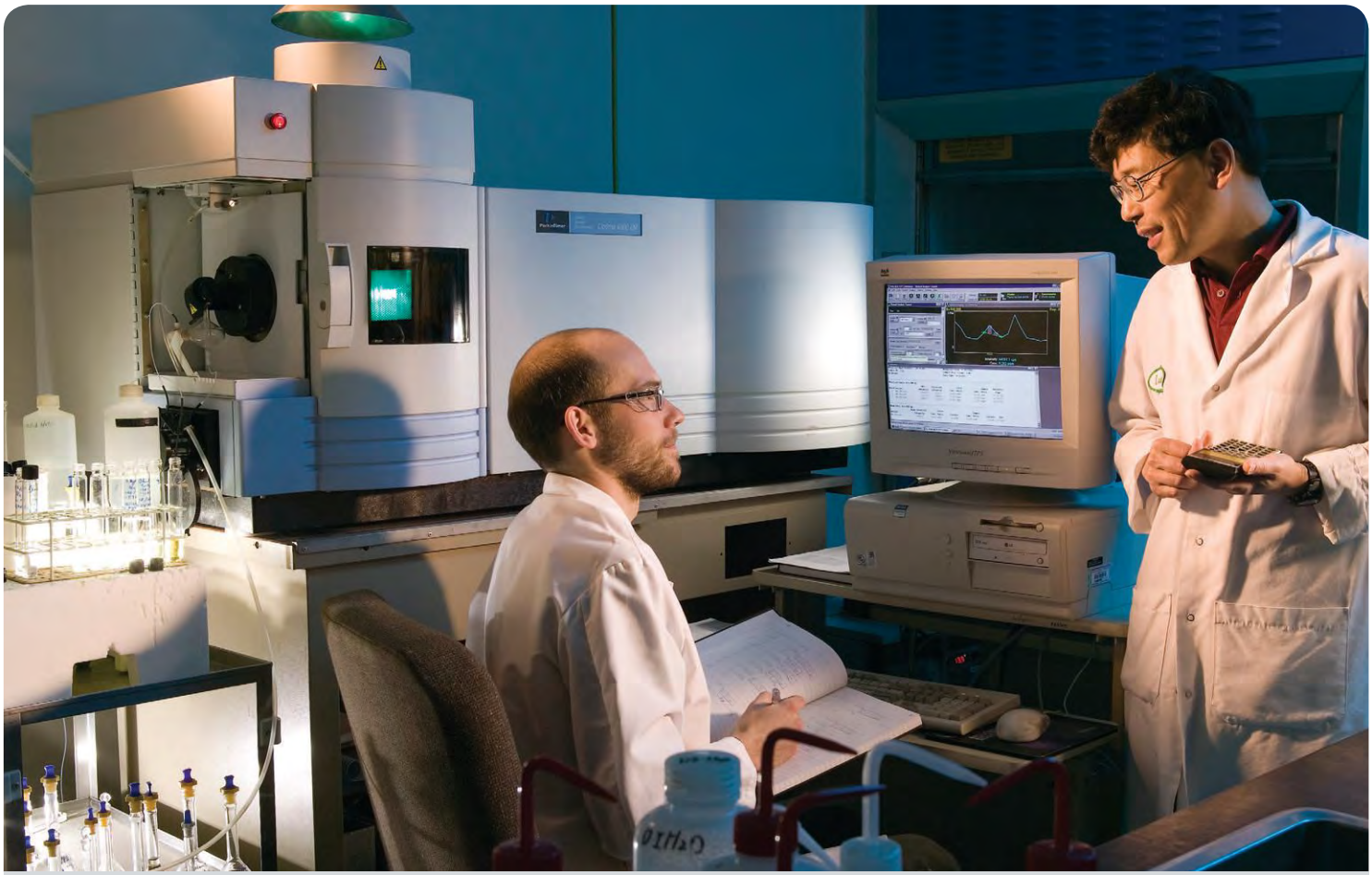

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INFORM

International News on Fats, Oils, and Related Materials

SUPPLEMENT

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How electronic media shape consumer attitudes about food safety

The skinny on high-oleic soybean oil

Unprecedented number of trade regulations taking effect in 2014

Matt Goodman

There are a number of major trade regulation changes in 2014, unprecedented in both number and magnitude. Many of these new regulations center around food import quality and safety, and if companies are not compliant they risk costly fines and penalties at or after an international border crossing.

These shifts affect every company that imports into, or exports from, Canada or the United States, with some regulations requiring new licensing and others mandating significant software updates. The most noteworthy are:

- *Food Safety Modernization Act (United States)*. This regulation requires companies to change registration processes for domestic and foreign facilities, as well as comply with added certifications for food safety. Food importers will need to contend with added food safety certifications because there will be more rigid requirements to identify their foreign suppliers.
- *Canadian Food Inspection Agency (CFIA) Inspection Modernization Program (Canada)*. The CFIA is developing a more comprehensive inspection approach across all imported and domestic food commodities – for both interprovincial and international trade. All regulated companies will need to obtain an Imported Food Sector Licence, and the CFIA will conduct more inspections and enforcement for non-compliance. In addition, the types of regulated commodities are expanding. While currently only importers of meat, fish, dairy and eggs are regulated, the new program regulates all other food commodities, including coffee, baked goods, spices, infant formula, snack foods, meal replacements and others.
- *Single-window initiative (United States, Mexico, and Canada).* This initiative applies to any goods coming into or leaving the United States. It requires shippers to interact with approximately 40 different partner government agencies through a single web-based interface system. Companies must transition from a paper-based process to a digital one to meet the initiative's goal of increasing real-time tracking and visibility of shipments. It began rolling out in 2014, and when it is completely implemented, shippers will know the status of their shipments from the partner government agencies more quickly and benefit from decreased wait time and exam costs.
- *e-Manifest (Canada)*. All shipments into Canada must be declared electronically before arrival. This requires new software and IT upgrades to ensure compatibility with the new system; otherwise, companies risk an additional duty fee at the border.

Although all of these updates will make the customs process smoother and more up to date, it is unprecedented to have this many major trade regulation changes implemented at the same time. To manage this level of change, investing more time now is necessary for businesses to meet the new requirements. Managing trade compliance is undeniably an investment, but it is offset by reducing the risk of costly audits and fines.

Matt Goodman is vice president of global trade management with Livingston International (Toronto, Ontario, Canada), which focuses on customs brokerage and trade compliance. He can be reached at info@livingstonintl.com.

EXTRACTS & DISTILLATES

Elevating bioavailability of curcumin via encapsulation with a novel formulation of artificial oil bodies

Chang, M.-T., *et al.*, *J. Agric. Food Chem. 61*:9666–9671, 2013.

Utilization of curcumin has been limited due to its poor oral bioavailability. Oral bioavailability of hydrophobic compounds might be elevated via encapsulation in artificial seed oil bodies. This study aimed to improve oral bioavailability of curcumin via this encapsulation. Unfortunately, curcumin was indissoluble in various seed oils. A mixed dissolvent formula was used to dissolve curcumin, and the admixture was successfully encapsulated in artificial oil bodies stabilized by recombinant sesame caleosin. The artificial oil bodies of relatively small sizes (150 nm) were stably solidified in the forms of powder and tablet. Oral bioavailability of curcumin with or without encapsulation in artificial oil bodies was assessed in Sprague–Dawley male rats. The results showed that encapsulation of curcumin significantly elevated its bioavailability and provided the highest maximum whole blood concentration (C_{max}), 37 ± 28 ng/mL, in the experimental animals 45 ± 17 min (t_{max}) after oral administration. Relative bioavailability calculated on the basis of the area under the plasma concentration–time curve (AUC) was increased by 47.7 times when curcumin was encapsulated in the artificial oil bodies. This novel formulation of artificial oil bodies seems to possess great potential to encapsulate hydrophobic drugs for oral administration.

The integral and extrinsic bioactive proteins in the aqueous extracted soybean oil bodies

Zhao, L., *et al.*, *J. Agric. Food Chem. 61*:9727–9733, 2013.

Soybean oil bodies (OBs), naturally pre-emulsified soybean oil, have been examined by many researchers owing to their great potential utilizations in food, cosmetics, pharmaceutical, and other applications requiring stable oil-in-water emulsions. This study was the first time to confirm that lectin, Gly m Bd 28K (Bd 28K, one soybean allergenic protein), Kunitz trypsin inhibitor, and Bowman–Birk inhibitor were not contained in the extracted soybean OBs even by neutral pH aqueous extraction. It was clarified that the well-known Gly m Bd 30K (Bd 30K), another soybean allergenic protein, was strongly bound to soybean OBs through a disulfide bond with 24 kDa oleosin. One steroleosin isoform (41 kDa) and two caleosin isoforms (27 kDa, 29 kDa), the integral bioactive proteins, were confirmed for the first time in soybean OBs, and a considerable amount of calcium, necessary for the biological activities of caleosin, was strongly bound to OBs. Unexpectedly, it was found that 24 kDa and 18 kDa oleosins could be hydrolyzed by an unknown soybean endoprotease in the extracted soybean OBs, which might give some hints for improving the enzyme-assisted aqueous extraction processing of soybean free oil.

Rapid determination of total conjugated linoleic acid content in select Canadian cheeses by ^{1}H NMR spectroscopy

Prema, D., *et al.*, *J. Agric. Food Chem. 61*:9915–9921, 2013.

The application of ^{1}H nuclear magnetic resonance (NMR) spectroscopy to the measurement of conjugated linoleic acid (CLA) content in the lipid fraction of dairy products is both a novel and inviting alternative to traditional methods such as gas chromatography (GC), which can require time-consuming sample derivatization. In this work, a newly developed, rapid, and reliable lipid extraction protocol was combined with simple, nondestructive ^{1}H NMR spectroscopic analysis to measure the total CLA content in CLA standards and in various Canadian cheeses from conventional, organic, and grass-fed dairy sources. The total CLA concentrations (mg/g cheese) obtained using these new extraction and analysis methods were consistent with amounts found using the modified Folch extraction and GC analysis (correlation coefficient of 0.948). Results showed that cheeses from exclusively grass-fed dairy cows were significantly higher in total CLA content than either conventional or organic cheese.

Differential effect of 14 free fatty acids in the expression of inflammation markers on human arterial coronary cells

Soto-Vaca, A., *et al.*, *J. Agric. Food Chem. 61*:10074–10079, 2013.

Cardiovascular disease is the leading cause of death in the United States, and circulating free fatty acids (FFA) are known risk factors associated with cardiovascular inflammation. The influence of 14 dietary FFA (including saturated, mono- and polyunsaturated, and *trans*) on the expression of inflammatory markers in human coronary arterial smooth muscle (HCASM) and endothelial (HCEC) cells using a cell culture model was investigated. HCASM and HCEC cell cultures were incubated with 200 μM of each FFA for 8 or 24 h, respectively, at 37°C in a 5% CO_2 humidified incubator. Inflammatory markers were assessed by ELISA or Western blot in the supernatant or cell lysates, respectively. Results showed significant differences in the expression of inflammatory markers among the fatty acid treatments and the control, with myristic and palmitic acids

CONTINUED ON NEXT PAGE

being identified as the most and linoleic acid as the least pro-inflammatory. This suggests that FFA may induce low-grade inflammation in human coronary arterial cells and provides more information on mode of action.

Bioactive lipids in the butter production chain from Parmigiano Reggiano cheese area

Verardo, V., *et al., J. Sci. Food Agric. 93*:3625–3633, 2013.

Bovine milk contains hundreds of diverse components, including proteins, peptides, amino acids, lipids, lactose, vitamins, and minerals. Specifically, the lipid composition is influenced by different variables such as breed, feed, and technological process. In this study the fatty acid and phospholipid compositions of different samples of butter and its by-products from the Parmigiano Reggiano cheese area, produced by industrial and traditional churning processes, were determined. The fatty acid composition of samples manufactured by the traditional method showed higher levels of monounsaturated and polyunsaturated fatty acids compared with industrial samples. In particular, the contents of n-3 fatty acids and conjugated linoleic acids were higher in samples produced by the traditional method than in samples produced industrially. Sample phospholipid composition also varied between the two technological processes. Phosphatidylethanolamine was the major phospholipid in cream, butter, and buttermilk samples obtained by the industrial process as well as in cream and buttermilk samples from the traditional process, while phosphatidylcholine was the major phospholipid in traditionally produced butter. This result may be explained by the different churning processes causing different types of membrane disruption. Generally, samples produced traditionally had higher contents of total phospholipids; in particular, butter produced by the traditional method had a total phospholipid content 33% higher than that of industrially produced butter. The samples studied represent the two types of products present in the Parmigiano Reggiano cheese area, where the industrial churning process is widespread compared with the traditional processing of Reggiana cow's milk. This is because Reggiana cow's milk production is lower than that of other breeds, and the traditional churning process is time consuming and economically disadvantageous. However, its products have been demonstrated to contain more bioactive lipids compared with products obtained from other breeds and by the industrial process.

Stability of omega-3 LC-PUFA-rich photoautotrophic microalgal oils compared to commercially available omega-3 LC-PUFA oils

Ryckebosch, E., *et al., J. Agric. Food Chem. 61*:10145–10155, 2013.

Microalgae are the primary producers of omega-3 LC-PUFA [long-chain polyunsaturated fatty acids], which are known for their health benefits. Their oil may thus be a potential alternative for fish oil. However, oxidative stability and hydrolytic stability of omega-3 LC-PUFA oils are important parameters. The purpose of this work was therefore to evaluate these parameters in oils from photoautotrophic microalgae (*Isochrysis*, *Phaeodactylum*, *Nannochloropsis gaditana*, and *Nannochloropsis* sp.) obtained with hexane/isopropanol (HI) and hexane (H) and compare them with commercial omega-3 LC-PUFA oils. When the results of both the primary and secondary oxidation parameters were put together, it was clear that fish, tuna, and heterotrophic microalgae oil are the least oxidatively stable oils, whereas krill oil and the microalgae oils performed better. The microalgal HI oils were shown to be more oxidatively stable than the microalgal H oils. The hydrolytic stability was shown not to be a problem during the storage of any of the oils.

Wax ester profiling of seed oil by nano-electrospray ionization tandem mass spectrometry

Iven, T., *et al.*, *Plant Methods 9*:24, 2013.

Wax esters are highly hydrophobic neutral lipids that are major constituents of the cutin and suberin layer. Moreover, they have favorable properties as a commodity for industrial applications. Through transgenic expression of wax ester biosynthetic genes in oilseed crops, it is possible to achieve high-level accumulation of defined wax ester compositions within the seed oil to provide a sustainable source for such high-value lipids. The fatty alcohol moiety of the wax esters is formed from plant-endogenous acyl-CoAs by the action of fatty acyl reductases (FAR). In a second step the fatty alcohol is condensed with acyl-CoA by a wax synthase (WS) to form a wax ester. To evaluate the specificity of wax ester biosynthesis, analytical methods are needed that provide detailed wax ester profiles from complex lipid extracts. We present a direct infusion electrospray ionization-tandem mass spectroscopy (MS) method that allows the semiquantitative determination of wax ester compositions from complex lipid mixtures covering 784 even-chain molecular species. The definition of calibration prototype groups that combine wax esters according to their fragmentation behavior enables fast quantitative analysis by applying multiple reaction monitoring. This provides a tool to analyze wax layer composition or determine whether seeds accumulate a desired wax ester profile. Besides the profiling method, we provide general information on wax ester analysis by the systematic definition of wax ester prototypes according to their collision-induced dissociation spectra. We applied the developed method for wax ester profiling of the well-characterized jojoba seed oil and compared the profile with wax ester-accumulating *Arabidopsis thaliana* expressing the wax ester biosynthetic genes *MaFAR* and *ScWS*. We developed a fast profiling method for wax ester analysis on the molecular species level. This method is suitable to screen large numbers of transgenic plants as well as other wax ester samples such as cuticular lipid extracts to gain an overview on the molecular species composition. We confirm previous results from atmospheric pressure chemical ionization-MS and gas chromography-MS analysis, which showed that

fragmentation patterns are highly dependent on the double bond distribution between the fatty alcohol and the fatty acid part of the wax ester.

The impact of cholesterol, DHA, and sphingolipids on Alzheimer's disease

Grimm, M.O.W., *et al.*, *Biomed. Res. Int.* doi.org/10.1155/2013/814390, 2013.

Alzheimer's disease (AD) is a devastating neurodegenerative disorder currently affecting over 35 million people worldwide. Pathological hallmarks of AD are massive amyloidosis, extracellular senile plaques, and intracellular neurofibrillary tangles accompanied by an excessive loss of synapses. Major constituents of senile plaques are 40–42 amino acid-long peptides termed β-amyloid (A β). A β is produced by sequential proteolytic processing of the amyloid precursor protein (APP). APP processing and A β production have been one of the central scopes in AD research in the past. In the last years, lipids and lipid-related issues are more frequently discussed to contribute to the AD pathogenesis. This review summarizes lipid alterations found in AD postmortem brains, AD transgenic mouse models, and the current understanding of how lipids influence the molecular mechanisms leading to AD and A β generation, focusing especially on cholesterol, docosahexaenoic acid, and sphingolipids/glycosphingolipids.

Lipid biology of breast cancer

Baumann, J., *et al.*, *Biochim. Biophys. Acta 1831*:1509–1517, 2013.

Alterations in lipid metabolism have been reported in many types of cancer. Lipids have been implicated in the regulation of proliferation, differentiation, apoptosis, inflammation, autophagy, motility, and membrane homeostasis. It is required that their biosynthesis is tightly regulated to ensure homeostasis and to prevent unnecessary energy expenditure. This review focuses on the emerging understanding of the role of lipids and lipogenic pathway regulation in breast cancer, including parallels drawn from the study of metabolic disease models, and suggestions on how these findings can potentially be exploited to promote gains in HER2/neu-positive breast cancer research.

Fish and marine omega-3 polyunsatured fatty acid consumption and incidence of type 2 diabetes: a systematic review and meta-analysis

Zhang, M., *et al.*, *Int. J. Endocrinol.*, 501015, 2013.

The purpose of this article is to examine the association between fish and marine long-chain omega-3 polyunsaturated fatty acid (LC n-3 PUFA) consumption and incidence of type 2 diabetes (T2D) in prospective cohort studies. Methods: Meta-analytic procedures were used to estimate the relative risk (RR) using random effects or fixed effects generic inverse variance model. Publication bias and study heterogeneity were assessed using Egger's test and I^2 statistic. We found no significant association between the intake of fish/seafood (pooled RR: 1.04., $P = 0.63$, 95% confidence interval [CI]: 0.9 to 1.2, 549, 955 participants) or marine LC n-3 PUFA (pooled RR: 1.08, $P = 0.39$, 95% CI: 0.90 to 1.30, 346, 710 participants) and T2D risk. Significant study heterogeneity was observed in fish/seafood and marine LC n-3 PUFA studies ($P < 0.00001$). Subgroup analysis revealed no obvious sources for high heterogeneity. We also found a significant protective effect of oily fish intake on T2D risk (pooled RR = 0.89, $P = 0.005$, 95% CI: 0.82 to 0.96). Dose-response analysis suggested that every 80 g per day intake of oily fish may reduce 20% risk of T2D. We found no significant effect of fish/seafood or marine LC n-3 PUFA intake on risk of T2D but a significant effect of oily fish intake on risk of T2D.

The maize leaf lipidome shows multilevel genetic control and high predictive value for agronomic traits

Riedelsheimer, C., *et al.*, *Scientific Rep.* 3:2479, 2013.

Although the plant lipidome show an enormous level of structural and functional diversity, our knowledge about its genetic control and its connection to whole-plant phenotypes is very limited. Here, we profiled 563 lipid species with UPLC-FT-MS [ultra pressure liquid chromatography-Fourier transform-mass spectroscopy] in 289 field-grown inbred lines genotyped with 56,110 SNPs [single nucleotide polymorphisms]. Genome-wide association study identified 174 associations for 76 lipids explaining up to 31.4% of the genetic variance (P-value 8.4×10^{-18}). Candidate genes were found for lipid synthesis, breakdown, transfer, and protection against peroxidation. The detected SNP-lipid associations could be grouped into associations with (i) individual lipids, (ii) lipids from one biochemical class, and (iii) lipids from several classes, suggesting a multilevel genetic control architecture. We further found a strong connection between the lipidome and agronomic traits in field-evaluated hybrid progeny. A cross-validated prediction model yielded correlations of up to 0.78 suggesting that the lipidome accurately predicts agronomic traits relevant in hybrid maize breeding.

Lipids of mitochondria

Horvath, S.E., and G. Daum, *Prog. Lipid Res. 52*:590–614, 2013.

A unique organelle for studying membrane biochemistry is the mitochondrion, whose functionality depends on a coordinated supply of proteins and lipids. Mitochondria are capable of synthesizing several lipids autonomously such as phosphatidylglycerol, cardiolipin, and, in part, phosphatidylethanolamine, phosphatidic acid and CDP [cytidine diphosphate]-diacylglycerol. Other mitochondrial membrane

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lipids such as phosphatidylcholine, phosphatidylserine, phosphatidylinositol, sterols, and sphingolipids have to be imported. The mitochondrial lipid composition, the biosynthesis, and the import of mitochondrial lipids as well as the regulation of these processes will be main issues of this review article. Furthermore, interactions of lipids and mitochondrial proteins, which are highly important for various mitochondrial processes, will be discussed. Malfunction or loss of enzymes involved in mitochondrial phospholipid biosynthesis leads to dysfunction of cell respiration, affects the assembly and stability of the mitochondrial protein import machinery, and causes abnormal mitochondrial morphology or even lethality. Molecular aspects of these processes as well as diseases related to defects in the formation of mitochondrial membranes will be described.

Recent trends in the use of lipidic nanoparticles as pharmaceutical carriers for cancer therapy and diagnostics

Mussi, S.V., and V.P. Torchilin, *J. Mater. Chem. B 1*: 5201–5209, 2013.

Lipidic nanoparticles have recently gained attention in cancer research. In this review we are focused on the solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC). They have significant advantages including low toxicity of the lipids and the controlled release of the drugs incorporated into the matrix. The recent trends described here contain functions added to nanoparticles to improve the therapeutic efficacy, such as long-circulation, co-loading of drugs, the combination with RNA/DNA, pH stimulus-sensitive drug release, incorporation of agents for imaging, and the attachment of ligands for active targeting. By putting it all together, it may be possible to obtain an ideal multifunctional nanocarrier for cancer therapy. Among the many efforts made so far to obtain one, SLN/NLC should have a place in this search for a combined therapeutic and diagnostic system with dramatically enhanced efficacy in cancer therapy.

The fat side of prostate cancer

Zadra, G., *et al.*, *Biochim. Biophys. Acta 1831*:1518–1532, 2013.

Prostate cancer (PCa) metabolism appears to be unique in comparison with other types of solid cancers. Normal prostate cells mainly rely on glucose oxidation to provide precursors for the synthesis and secretion of citrate, resulting in an incomplete Krebs cycle and minimal oxidative phosphorylation for energy production. In contrast, during transformation, PCa cells no longer secrete citrate and they reactivate the Krebs cycle as energy source. Moreover, primary PCas do not show increased aerobic glycolysis and therefore they are not efficiently detectable with F-18-FDG-PET [F-18 fluorodeoxyglucose positron emission tomography]. However, increased *de novo* lipid synthesis, strictly intertwined with deregulation in classical oncogenes and oncosuppressors, is an early event of the disease. Upregulation and increased activity of lipogenic enzymes (including fatty add synthase and choline kinase) occurs throughout PCa carcinogenesis and correlates with worse prognosis and poor survival. Thus, lipid precursors such as acetate and choline have been successfully used as alternative tracers for PET imaging. Lipid synthesis intermediates and fatty acid catabolism also emerged as important players in PCa maintenance. Finally, epidemiologic studies suggested that systemic metabolic disorders including obesity, metabolic syndrome, and diabetes as well as hypercaloric and fat-rich diets might increase the risk of PCa. However, how metabolic disorders contribute to PCa development and whether dietary lipids and *de novo* lipids synthesized intra-tumor are differentially metabolized still remains unclear. In this review, we examine the switch in lipid metabolism supporting the development and progression of PCa, and we discuss how we can exploit its lipogenic nature for therapeutic and diagnostic purposes.

Quantification of triacylglycerol molecular species in cocoa butter using high-performance liquid chromatography equipped with nano quantity analyte detector

Beppu, F., *et al.*, *J. Oleo Sci. 62*:789–794, 2013.

Triacylglycerol (TAG) molecular species were quantified through high-performance liquid chromatography (HPLC) equipped with a nano quantity analyte detector (NQAD). TAG standard compounds, i.e., 1,3-dipalmitoyl-2-oleoylglycerol (β-POP), 1-palmitoyl-2-oleoyl-3-stearoyl-*rac*-glycerol (β-POS), and 1,3-distearoyl-2-oleoylglycerol (β-SOS), and natural cocoa butter were used for analyses. NQAD gave the first order equation passing through the origin for all TAG standard compounds. TAG molecular species in cocoa butter were quantified using the calibration curves, and the obtained values were almost the same as the reported ones of conventional cocoa butter. Furthermore, a recovery test was also carried out, and the values were almost 100. Therefore, HPLC-NQAD can be successfully used for the quantification of TAG molecular species in natural fats and oils.

Resolution behavior of *cis*- and *trans*-octadecenoic acid isomers by AOCS official method using SP-2560 column

Yoshinaga, K., *et al.*, *J. Oleo Sci. 62*:781–788, 2013.

The gas chromatography–flame ionization detector equipped with a higher polarity column (i.e., SP-2560) has often been used for the quantification of *trans*-fatty acids in food. In particular, AOCS Ce 1h-05, the official method of the American Oil Chemists' Society (AOCS), is a highly effective method to separate the isomers of *trans*-fatty acids. In this study, the resolution behavior and the response factors of *cis*- and *trans*-octadecenoic acid methyl ester (C18:1-ME) isomers separated by the AOCS Ce 1h-05 method were investigated, and the contents of

STATISTICAL ANALYSIS FROM MINTEC

James Hutchings at Mintec

World soybean production is expected to rise by 6% year-on-year to reach a record 283.5 million metric tons (MMT) in 2013/14. US production will rise by 7% to 88.7 MMT with exports expected to rise by 10% year-on-year to 39.5 MMT, helped by an expected increase in demand from China, which is set to import a near-record 5.5 MMT of soybeans from the United States. Late-summer warmth in the United States helped make up for earlier delays in crop development caused by the dry August, and soybean yields have been revised upward.

Rapeseed (canola) oil's premium over soybean oil has shrunk significantly since May 2013. The world rapeseed market is also expected to see a record production level this season of 67.9 MMT, up 8%. This is largely due to a 16% rise in production from top exporter Canada, where near-perfect growing conditions have led to record yields and so, despite a drop in harvested area, rapeseed production will rise to a record 16.1 MMT. Canadian exports and stock levels are both expected to rise.

The steady drop in soybean oil prices seen in 2013 resulted in their falling below palm oil by November 2013 for the first time since mid-2012. Palm oil prices had been steady for most of 2013 but saw a rise in mid November due to concerns over the impact of the typhoon in the Philippines. While the typhoon has not affected the major palm oil-producing countries of Indonesia and Malaysia, it may reduce supplies of coconut oil in the short- to medium-term, and this could lead to increased demand for palm or palm oil products as substitutes. World palm oil production has continued to grow steadily to reach a record 58.3 MMT this season.

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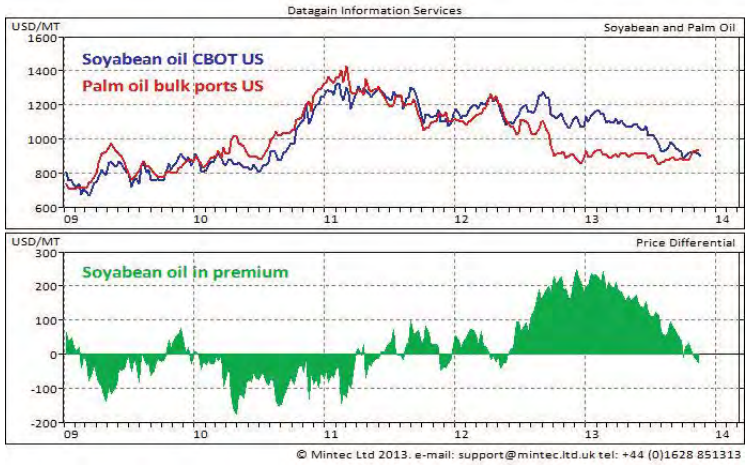

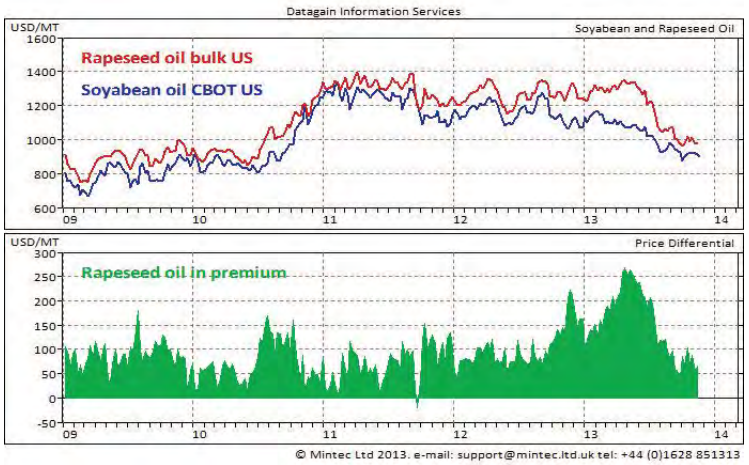

Abbreviations: USD, US dollars; MT, metric ton; CBOT, Chicago Board of Trade.

each *cis*- and *trans*-C18:1-ME isomer in partially hydrogenated vegetable oil (PHVO) and milk fat were quantified by using the calibration curves obtained for the respective isomers. The relative response factors for the *trans*- and *cis*-C18:1-ME isomers against the internal standard heneicosanoic acid methyl ester (C21:0-ME) were 1.031 ± 0.040 (mean ± standard deviation) and 0.990 ± 0.032, respectively. The relative response factors of *trans*-isomers tend to be higher than those of *cis*-C18:1-ME isomers. The peaks of *cis*-4-C18:1-ME, *cis*-5-C18:1-ME, *cis*-6-C18:1-ME, *cis*-7-C18:1-ME, *cis*-8-C18:1-ME, and *cis*-9-C18:1-ME isomers overlapped with those of *trans*-C18:1-ME isomers. Both PHVO and milk fat contained many types of *cis*- and *trans*-C18:1 isomers, and the total contents of the *trans*-C18:1 isomer in PHVO and milk fat were 28.01 g and 3.62 g per 100 g oil, respectively. When the *trans*-C18:1-ME isomer was separated from the *cis*-C18:1-ME by using a silver-ion cartridge column before the analyses, the total contents of the *trans*-C18:1 isomer in PHVO and milk fat were 23.03 g and 2.78 g per 100 g oil, respectively. The difference in the *trans*-C18:1 isomer content between the two methods was ascribed to the partial overlapping of *cis*-isomer peaks with the peaks of *trans*-C18:1-ME isomers in the chromatogram. ■

Speeding up the transition to greener SURFACTANTS

- Consumers increasingly base purchasing decisions on perceived health and environmental benefits. This is particularly true in cosmetics and personal care, where the number of products carrying green claims is on the rise.
- Identifying green alternatives for the conventional surfactants that hold the components of personal care products together in stable formulations is especially challenging, as there are few ways to determine which green surfactant will work best in any given formulation.
- The following article describes a quick, efficient, and accurate high-throughput screening method based on a well-known theory from the oil industry that cosmetic scientists could use to select appropriate green surfactant alternatives.

Edgar Acosta, Ian Callaghan, Steven Abbott, and Sander Van Loon

One key class of ingredients in cosmetics and personal care products is surfactants. Used to create emulsions and microemulsions, surfactants help the water and "oil" components to hold together in stable formulations. There's clearly a desire to replace conventional surfactants with naturally derived, sustainably sourced or biodegradable alternatives, but for the cosmetic scientist the challenge of reducing this switch to practice is a tough one.

There are very few ways for determining which surfactant is the best green replacement, especially when working with microemulsions. Trial and error takes time, eating into a company's competitive edge, so it is clear a framework for rational formulation design would be beneficial. Outlined here is a real alternative to the limited hydrophilic-lipophilic balance (HLB) approach.

HLD-NAC theory

A highly promising approach for identifying key parameters is the hydrophilic lipophilic difference (HLD)—net average curvature (NAC) methodology of Edgar Acosta of the University of Toronto, which is built on the HLD concept developed by Aubry, Salager, Sabatini and others. HLD theory is well established in the oil industry but little known for cosmetics.

The HLD uses a simple equation based on salt concentration, oil "number," and the surfactant characteristic curvature (Cc), which identifies the region in surfactant space where the surfactant is most useful. A simple equation determines how close a mixture is to the

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▲ ***FIG. 1.*** *Chemspeed Formax (VLCI, Amsterdam, Netherlands).*

HIGH THROUGHPUT

Specialized high-throughput equipment, such as the Chemspeed Formax, can be used to greatly increase processing power. Robotic determination of Cc values can be carried out four times faster than by traditional longhand phase scans, significantly decreasing the required human power and greatly improving accuracy. This is the first time high-throughput screening and HLD-NAC have been brought together.

"optimal formulation," where the smallest amount of surfactant gives the largest amount of (micro) emulsification.

If the Cc of the surfactant is far from this optimum, the emulsion is inefficient/poor. If the Cc is slightly lower than optimum, an efficient type I oil-in-water (o/w) emulsion is produced, and if it is slightly higher an efficient type II water-in-oil (w/o) emulsion is formed.

Importantly, the Cc of a mixture of surfactants is the weighted average of the two. Optimal surfactant mixtures with the desired Cc can be created from combinations of surfactants.

Surfactant mixtures are common in cosmetic formulation; and HLD theory, uniquely, makes it possible to find optimal mixtures efficiently.

Doing this process for multiple surfactants is tedious, time consuming and resource intensive. We therefore developed a quick, efficient, and accurate method to determine Cc values of multiple surfactants using a high-throughput screening method.

Cc VALUES OF GREEN SURFACTANTS

A number of the key surfactant suppliers generously provided a range of green surfactants. They were chosen to cover a large range from hydrophilic

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TABLE 1. Green surfactant characteristic curvature (Cc) values[a]

Name	Cc
C13C15 oxo alcohol and approx. 7 moles of EO	–0.7
Yucca saponins	–2.4
Sodium cocoyl sarcosinate	–2.8
Sodium lauroyl sarcosinate	4.2
Isotridecyl alcohol 8 mole ethoxylate	–0.1
Polysorbate 20 (POE sorbitan dodecanoate)	–7.9
Polysorbate 80 (POE sorbitan 9-octadecanoate)	–3.7
Sorbitan monolaurate	3.5
Soy lecithin	4
Sodium dihexyl sulfosuccinate	–0.9
Octyldecyl glucoside (Guerbet-type)	–2.4
Laureth-4	1.5
Protodioscin (beta-diglucorhamnoside)	–1
Sucrose palmitate	–0.8
Sucrose distearate	4
Fatty alcohol C12–C18 with approx. 14 moles EO	–2.9
PEG-20 glyceryl stearate	–2.4
Octyldodecanol (C20-Guerbet alcohol)	4
Laureth-7-citrate (citric acid ester of lauryl E7)	–0.75
Sodium stearoyl glutamate	–5.0
Fatty alcohol C8 with approx. 4 moles EO	0.25
Fatty alcohol C12–C14 with approx. 9.5 moles EO	-2.4

[a]Abbreviations: EO, ethylene oxide; POE, polyoxyethylene; PEG, polyethylene glycol.

THE 30-DAY FORMULATION CHALLENGE PROJECT

This article grew out of a 30-day challenge project supported by Intelligent Formulation Ltd., which aimed at stimulating innovation in the formulation space. Gavin Donoghue and Sarah Gregory from Syntopix Group plc identified a need for rational microemulsion formulation design. The challenge brought together two surfactant experts, Edgar Acosta at the University of Toronto (expert in HLD-NAC theory) and Ian Callaghan, an independent consultant. Steven Abbott of the University of Leeds (United Kingdom) and a consultant to Syntopix provided the Optimal Surfactant software and acted as project leader. Sander Van Loon led the VLCI team running the high-throughput scans. The team thanks David Calvert of Intelligent Formulation for overall coordination of the project. Intelligent Formulation Ltd. and Syntopix Group plc no longer exist.

DETERMINING Cc VALUES

Cc values are known only for a few dozen surfactants. Catalogs of green surfactants contain hundreds of possible choices but provide no guiding Cc values.

Measuring Cc is not very difficult. Take, for example, eight tubes containing measured amounts of water, surfactant, salt, and eight different oils spanning the appropriate HLD range. Phase transition from w/o to o/w via a type III bi-continuous oil and water emulsion determines the optimal point from which a Cc value can be calculated.

to hydrophobic, ionic and nonionic, ethoxylated and non-ethoxylated, sugar-based, natural extracts, and so on. Of the 22 surfactants tested, Cc values were determined for 13 (see Table 1, page S11).

For the remaining nine, definite values could not be defined within the bounds of this project, but minimum or maximum ranges were determined. In general it's desirable to formulate with surfactants with Cc values from –2.5 to +2.5. Within this range surfactants match well to typical oils and formulation conditions. Outside of this range surfactants are more difficult to match and therefore not a first choice as a principal ingredient.

THE FUTURE

All Cc values from the project have been placed in the public domain. In particular, the Optimal Surfactant software (www.stevenabbott.co.uk/HLDNAC.html) has these values included in its database, allowing users to find their own surfactant formulations. The team has also provided an Instant Guide to HLD-NAC. It is hoped this project will encourage surfactant suppliers to measure and provide Cc values to aid their customers in formulation design.

BUILDING FORMULATIONS BASED ON Cc VALUES: THE BENEFITS

Using the measured Cc values makes it much easier to get reasonable first approximations to good formulations. Although the HLD-NAC equations are simple, help from software (and the database of Cc values inside it) makes it even easier to use and to understand how to adjust formulations to improve them further. This system provides:

- Improved prediction of surfactant performance in a given formulation
- Reduced formulation development time, cost, and resource
- Easier selection of appropriate green surfactant alternatives
- Elimination of surfactant adjustment with alcohols; the fine tuning of formulations with mixtures of surfactants is possible by calculation of combined Cc value.

As a result of this project Syntopix Group plc identified two new green surfactants for incorporation into novel oral care microemulsions.

Edgar Acosta is a professor at the University of Toronto, Canada. Ian Callaghan is an independent consultant, Steven Abbott is a professor at the University of Leeds (United Kingdom), and Sander Van Loon is the chief executive officer of Van Loon Chemical Innovations (VLCI), http://www.vlci.biz/vlci/, which is based in Amsterdam, the Netherlands. For more information, contact Sander Van Loon at s.vanloon@vlci.biz.

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It's not all about the science

Aurora A. Saulo

"GMO health risks,"[1] "The sweet lowdown: Exposing the unhealthy truth about sugar,"[2] "Is high-fructose corn syrup bad for you?"[3] and "Chemical cuisine. Learn about food additives"[4]—these are the catchy titles of just some of the many articles easily obtained on the Web about the "bad" things found in our food. Many of these articles cite results of scientific studies, often not referenced and sometimes obtained from reputable scientific publications. Organizations publishing these articles, alerts and other information on food safety and health proclaim that they aim to protect consumer interests and appear to practice responsible reporting, even if only one side of the story is told. Some organizations may even have the word "science" in their names. It seems that some in the media believe in the importance of basing their story on science to project credibility. In a television interview about her book *Obsessed: America's Food Addiction—and My Own,* news show host Mika Brzezinski[5] asserted that "... we were drawn to the same things and we got that same brain reaction that science backs up. It's an actual reward when you eat certain foods, which points to addiction." Her statement, that she used science to arrive at her observations, was made to support her hypothesis that certain foods are addictive. This is a form of uncontrollable consumer activism on food safety and health using the media for dissemination.

For example, MSG (monosodium glutamate) is one of the most extensively researched food ingredients in the world, but it is also one of the most misunderstood. In a 1968

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opinion letter to the *New England Journal of Medicine*, Robert Ho Man Kwok coined the term "Chinese restaurant syndrome" to describe the symptoms sometimes felt by individuals sensitive to MSG, such as palpitating of the heart, flushing of the face and swelling of the area around the lips. "MSG symptom complex" has long replaced the term, and worldwide scientific organizations, including the Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives and the U.S. Food and Drug Administration, have reaffirmed its safe use for the general population at levels normally consumed as a flavor enhancer.[6, 7] The negative association, however, remains to this day.

In 2004, high-fructose corn syrup (HFCS) in beverages became the focus of the media when a commentary stated that HFCS was a possible cause of obesity.[8] Many more studies followed with findings establishing the same linkage, which resulted in local governments, starting in 2007, banning products containing HFCS. The continuous barrage of negative reports on HFCS was supported by other factors, including personal and moral stands against corn-processing companies viewed negatively by consumers as biotechnology-using companies.

When consumers were asked their reasons for avoiding MSG, however, they were uncertain why but still believed that MSG was bad for them.[9] And just as with MSG, when consumers with the most negative perceptions of HFCS were interviewed in focus groups, they were unsure why they perceived HFCS as harmful and how it was different from other sweeteners.[10] But consumers believed these reports that some foods and food ingredients were indeed harmful to their health and demanded that food manufacturers produce foods without those ingredients.

HOW CONSUMERS DO THEIR RESEARCH ON FOOD SAFETY AND HEALTH

So how do consumers with little or no scientific or technical background decide which information to consider or subscribe to in today's technological era? Today's consumers post online their information, experiences, insights and opinions on any topic for sharing with their social network. A proliferation of Web 2.0 applications specifically allows user-user interactions, resulting in discussions that then generate one or several collective positions on the topic to which consumers may subscribe. Knowingly or unknowingly, consumers use their family, friends and the different forms of analog and digital media as their major means of researching various information, including health and safety.[11]

USING ELECTRONIC MEDIA

According to their study, Eysenbach and Köhler[12] reported that consumers judged the credibility of a website by its source, the professionalism of its design, how scientific the Web pages look, ease of navigation and the language used. Internet users did not qualify source credibility by reading the "About Us" section when they sought health information. Consumers believed negative reports more than positive ones, because they could use negative information to make decisions that would help them avoid losses.[13] Furthermore, positive information seemed self-serving. Since the media have both audiences and shareholders to satisfy, the media tended to focus on negative reports, which have a greater impact.

When consumers have not formed attitudes toward an issue, Kumkale et al.[14] stated that consumers used source credibility mainly to form attitudes and not to change them. Consumers also depended only on the first few links given by search engines and completed their research in slightly less than 6 minutes. The information they retrieved was then shared among friends, family and followers, who further shared with others in their own respective social networks. When transmitted to others, the reports sometimes stayed the same, were repeated out of context or sometimes embellished with sensational details, resulting in urban legends, modern folklore, Internet hoaxes or myths. This rapid communication fuels consumer activism. Although consumers prefer the use of Web 2.0 applications (like Facebook), there is a concern for the quality of the information coming from this practice or discussions of groups of lay users.[15]

When consumers have formed attitudes toward an issue, they generally look for information that agrees with their beliefs.[16] This is especially true with their perception of biotechnology-enhanced foods. Those beliefs remained stable even in the presence of persuasive arguments. Giving factual or scientific statements to consumers isn't enough to allay their concerns.[17] Consumers consider "The U.S. has the safest foods in the world" a weak explanation of the safety of advanced technologies that they fear or do not understand well.[18] Consumers perceive the opposite beliefs conveying positive information as inaccurate and more biased.

USING TRADITIONAL MEDIA

Traditional media remain sources of information for the consumer. For example, Blue[19] stated that many obtain their knowledge of farming from the media, marketing efforts and advertisements and stories, but not from farmers. Many consumers don't know what is involved in growing the food they buy in supermarkets. They don't know the real differences in organic and conventional farming, pests that harm agriculture and the methods employed by farmers to get rid of them, the heat treatment (pasteurization) of milk to make it safe, or generally, the hazards that are always present in our food.

The media deTer mines The agenda

Through the information obtained from the media, consumers form their own beliefs and values, influencing

their political, social, economical and behavioral tendencies. Thus, consumers may demand produce with no pesticides, or raw milk and no bioengineered foods. The media helped consumers form these beliefs. An extension of these beliefs is the expectation that all foods we eat must be absolutely safe.

SAFETY CHARACTERISTICS

The media also helped decide for consumers what safety characteristics are important in the food they eat. For example, between 1993 and 1998 and 2006 and 2010, the media increased its coverage of food safety, thus deciding that food safety was important. In this case, increased media coverage of food hazards raised awareness of the hazards during the same periods of the coverage and increased vigilance in food-handling practices in the home.[20]

It was a different situation in 2008, when tomatoes in fresh salsa were initially blamed for a multistate salmonellosis outbreak that was later confirmed as due to uncooked jalapeño peppers. This premature media reporting of tomatoes as the source of contamination resulted in financial losses of approximately $450 million to both the tomato and pepper industries.[21] The same was true for the bovine spongiform encephalopathy (BSE) coverage in 1996 when, according to Texas beef farmers, Oprah Winfrey's statement that she would stop eating hamburger caused a steep decline in beef prices, although the U.S. did not have a single case of BSE.[22] The information released by the media was based on emotions, and consumers were emotional in their reactions.

DEMAND, PRICE, SERVICES

Extensive product coverage affects consumer perceptions and can influence demand and price for the product and services related to it. Particularly vulnerable to such marketing methods are adolescents[23, 24] and 3- to 5-year-olds who cannot differentiate between commercials and reality, and whose identities are defined by their consumer habits.[25] These young consumers watch advertisements and visit websites of food and beverage brands that offer games and product discounts, feature shows attractive to them and urge these young viewers to ask their parents to buy those products.[26] Such media coverage is intentionally dramatic and sensational to grab the attention of the young viewers. The media effectively influenced the attitudes of these young consumers.

SNACKS—THE CAUSE AND CURE OF OBESITY

However, the media also supports two strong and opposing attitudes. In its policy statement published by the media, The American Academy of Pediatrics Council of Communication and Media[27] defined "snacking behavior" as an undesirable eating pattern that contributes to obesity. The academy defined snacks as foods eaten mindlessly, such as during TV viewing, and that it claims have the same deleterious impact as fast foods have on health. The academy concluded that the "media clearly play an important role in the current epidemic of childhood and adolescent obesity" through increased screen time of "unhealthy eating practices" that are then emulated by these young consumers and supported its policy statement with a voluminous amount of scientific studies

On the other hand, a forward-thinking marketing group has been presenting healthy snacking as a possible solution to obesity.[28] The Hartman Group contends that snacking as traditionally defined has changed in the past 20 years. Americans are snacking more than they are eating meals. They have a changed eating behavior and snack foods are no longer just sweet and salty. Although still less so than meals, snack foods have evolved to include fresh foods of higher quality. Instead of demonizing snacks, The Hartman Group encourages embracing "our cultural love of snacking" and promoting snacking as small portions of "a memorable social experience" that may help curb obesity. The media is being used to modify consumer snacking behavior from "unhealthy" to "healthy." These are examples of the use of the media that influences the eating behavior of the American public in very different ways.

THE CONSUMER AND FOOD MANUFACTURERS

American consumers are some of the most affluent consumers in the world. There are many good-tasting and healthful foods that are economically available to them. Most food products resulted from the innovative application of food science methods and techniques consumers now publicly disown. Americans are some of the very few in the world who have the luxury to pick and choose foods according to the personal criteria they used to form their attitudes. Information obtained from the media, such as the absolute safety of foods, formed some of those attitudes. The criteria they use may not be always based on sound science, but they are what consumers believe and seek in the market. Since food manufacturers must meet consumer demands, they develop and prepare such products—"GMO-free," "no artificial colors," "no MSG," "fresh, organic, not processed" and "all natural, no additives." The demand for foods with clean labels, that is, devoid of "chemicals," continues even when those ingredients may enhance safety, prolong shelf life or increase palatability.

WHERE DO WE GO FROM HERE?

Consumers form their attitudes on health and safety based on information given to them by the media. This information shapes their beliefs and values. Their emotions, educational background and social network further influence attitudes beyond media input. These factors reinforce each other. As a result, much consumer information is

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not based on sound science but is rather an amalgamation of what consumers read, think, see, believe and feel. And of course, the attitudes they form are a lot about who they are to begin with.

Sometimes the science is good, and we scientists are satisfied with the resulting consumer attitudes. Sometimes the science is bad and the objective is wrong, but when written or presented by a popularizer, the topic becomes fascinating to the masses and is carefully read. Most of the time, the science is good but is not read.

Where do we go from here? Are we headed toward a world without science? Where celebrities and politicians shape our minds with messages of the terrors of genetic manipulation resulting in weird foods? Where processed foods and food additives are evil and cause obesity, addiction and learning disabilities? A world without science but with messages that make sense and are relevant to consumers?

Or will we have a world without emotions? Where dull, white-garbed scientists end up forming our attitudes? Where their messages are true, factual and science-based but make no sense and can't be understood? Where scientists want us to care about their messages without showing that they care about us first?

Or is it possible to have a happy compromise? Where food manufacturers always have our health and safety in mind? Where we can be wary of technologies we can't understand but still believe in the ultimate benefits of science? Where we have scientists who use words we can understand and ways that are relevant? After all, it's not all about the science.

***Aurora A. Saulo**, is professor of and the extension specialist in food technology of the College of Tropical Agriculture and Human Resources of the University of Hawaii at Manoa.*

This article was reprinted with permission from the October/November 2013 issue of Food Safety *magazine [http://www.foodsafetymagazine.com/magazine-archive1/octobernovember-2013/ite28099s-not-all-about-the-science/].*

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High-oleic soybean oil: Will recent FDA determination improve ITS CHANCES?

Michael Logli

The US soybean oil industry is looking to regain lost market share with bioengineered trans fat-replacement high-oleic soybean oil (HOSO). As the recent preliminary determination by the US Food and Drug Administration (FDA) that partially hydrogenated oils (PHO) are not Generally Recognized as Safe (GRAS) sends food manufacturers scrambling to remove the last vestiges of PHO from their products, will they replace them with HOSO?

Since the FDA-mandated *trans* fat labeling became effective in 2006, commodity soybean oil has lost 15% of its market share in the United States to other oils such as high-oleic canola and sunflowerseed, according to Qualisoy, a soybean oil industry initiative headquartered in St. Louis, Missouri, USA. Qualisoy's mission is to help the US soybean industry develop, commercialize, and promote soybeans with enhanced quality traits.

But according to Susan Knowlton, senior research manager for DuPont, many first-generation *trans*-fat alternatives did not deliver flavor or stability, required changes to recipes or manufacturing processes, or increased the saturated fat content in finished products (http://tinyurl.com/ly62zeq). HOSO may be the solution to these issues.

High-oleic soybeans are genetically modified (GM) to produce more monounsaturated (oleic) fatty acids than saturated or polyunsaturated (largely linoleic) fatty acids. HOSO contains about 75% oleic acid, 10–16% linoleic acid, 6–11% saturated fats, less than 3% α-linolenic acid (Table 1, page S18), and qualifies as having 0 g *trans* fat (less than 0.5 g/serving) for labeling purposes.

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- **The soybean oil industry has lost market share in recent years owing to labeling regulations and adverse public perception of dietary trans and saturated fats.**
- **High-oleic soybean oil (HOSO) is derived from a genetically modified plant that yields oil with greater oxidative stability than commodity soybean oil.**
- **Will the preliminary determination by the US Food and Drug Administration (FDA) that partially hydrogenated oils (PHO) are not Generally Recognized as Safe (GRAS) give HOSO an edge?**

TABLE 1. Fatty acid profile of soybean seeds

	Commodity soybean oil[a] (%)	DuPont Plenish[b] (%)	Monsanto Vistive Gold[c] (%)	Low-linolenic soybean oil[a]
Total saturated fat	12.5–19.6	10–11	6	16–17.8
Myristic (14:0)	0–0.2	0	0	0.1
Palmitic (16:0)	9.5–13.3	6–7	2.5	10.9–11
Stearic (18:0)	3.0–6.1	4	3.5	4–5.7
Total monounsaturated fat	17.7–29.3	75–78	75	25.2–27.7
Oleic (18:1)	21.9–26.6	75–78	75	25–27.5
Total polyunsaturated fat	59.2–64.7	10	19	54.5–59
Linoleic (18:2)	46.2–57.1	<10	16	51.5–55
α-Linolenic (18:3)	5.5–11	<3	3	3–4

[a]Firestone, D,, *Physical and Chemical Characteristics of Oils, Fats, and Waxes, Third Edition*, AOCS Press, Urbana, Illinois, USA, 2013.
[b]DuPont Pioneer press kit.
[c]Monsanto press kit.

HOSO has several advantages over commodity soybean oil. HOSO is perceived to be more healthful than commodity soybean oil because of its lowered saturated- and *trans*-fat content. HOSO also has a more neutral taste and offers higher heat stability, said Don Banks, AOCS member, president of Edible Oil Technology (http://tinyurl.com/kknauvz), and consultant for the United Soybean Board (USB; Chesterfield, Missouri, USA). The FDA's recent preliminary determination regarding PHO may also improve HOSO's chances.

"Since high-oleic soybean oil requires no hydrogenation to be functional in many foods, we view this as an opportunity to displace some PHO in affected foods," said Robert Reeves, director of public affairs for Qualisoy.

The USB, which administers funds from the US soybean checkoff program for soybean-related research and marketing, has pledged $60 million over the next five years to support HOSO production. USB predicts that the United States will need to produce 9 billion pounds (4 million metric tons) of HOSO by 2023 to meet global demand.

"We project that high-oleic soy could be 25% of the US crop eventually," said USB's Director of Nutrition and Public Relations Steve Poole in an article on BakingBusiness.com (http://tinyurl.com/StevePoole).

Two seed companies, DuPont Pioneer and Monsanto, have developed high-oleic soybeans for commercialization. DuPont (Wilmington, Delaware, USA) entered the market first with its Plenish® high-oleic soybeans. Using Plenish seeds, Archer Daniels Midland will sell HOSO under the name "Frymax Soy Supreme" through Stratas Foods, its joint venture with ACH Foods, a division of Associated British Foods. Bunge will also produce and sell HOSO using Plenish seeds. The oils developed by both companies will be used in frying applications and will be released for commercial use in early 2014. Two other companies—Cargill and Perdue AgriBusiness—also plan to introduce HOSO from Plenish seeds later in 2014.

Monsanto (St. Louis, Missouri, USA) has developed its own high-oleic soybeans under the Vistive® Gold brand. The seeds contain half the saturated fat of Plenish (6% vs. Plenish's 10–11%) and have a higher level of polyunsaturated fats (19% vs. Plenish's 10%).

"These soybeans deliver a yield per acre that is competitive with proven Monsanto varieties . . . and outperform Pioneer commodity soybeans," said Sarah Vacek, Monsanto's Vistive Gold product manager. "We think this will help processors contract for the acres of Vistive Gold."

Plenish and Vistive Gold soybeans have been deregulated in the United States through the Animal and Plant Health Inspection Service of the US Department of Agriculture. Plenish has also been deregulated in China. Monsanto is waiting for China's response to its application for deregulation before releasing Vistive Gold commercially. Monsanto and DuPont Pioneer have submitted applications to the European Commission for permits to import their GM high-oleic soybeans to the European Union. Both companies are waiting for the European Food Safety Authority to complete safety assessments of the products. Neither company is changing its marketing or production strategies in light of the FDA determination on PHO.

However, certain factors may prevent HOSO from taking hold. HOSO is produced from GM soybeans, and anti-GM activists continue to pressure food manufacturers. Com-

petition from other non-GM *trans*-fat replacements—high-oleic canola, sunflowerseed, and palm oils—may also make building a HOSO market difficult. Robert Reeves, director of public affairs for Qualisoy, said HOSO may be cost-competitive with high-oleic canola oil at first, but if acreage and production increase, it likely will become the cheaper *trans*-fat replacement.

HOSO also lacks an international identity standard to facilitate global trade. The Codex Committee on Fats and Oils (CCFO)—a committee created by the international food standards organization Codex Alimentarius—chose at its last meeting in 2013 not to revise the Codex Standard for Named Vegetable Oils (CODEX STAN 210-1999) to include HOSO. According to the *Olive Oil Times*, CCFO felt that market information and production numbers were insufficient to demonstrate that a large enough HOSO market exists. The committee created an electronic working group to revise the proposal in time for the next meeting in 2015 (http://tinyurl.com/codexHOSO). The lack of an international trade standard, as well as the absence of deregulation in China and the EU, could delay international acceptance of HOSO.

CCFO is also reviewing proposals for adding two other high-oleic oils to the Standard for Named Vegetable Oils—high-oleic, high-stearic sunflower, proposed by Argentina, and a variety of high-oleic palm, proposed by Colombia.

With high expectations and a number of hurdles to overcome, HOSO finds itself in a situation similar to that of low-linolenic (LL) soybean oil—one of the first *trans*-fat replacement oils. LL soybean oil contained roughly 3% α-linolenic acid—although some ultra-low varieties had as little as 1%—and 54–59% linoleic acid. Lower α-linolenic acid levels increased the oil's stability and made it less likely to oxidize. However, additional competition in the *trans*-fat-replacement market—from non-GM high-oleic canola and sunflowerseed oils—hurt profits. Additionally, producing LL soybean oil created logistical problems for smaller companies, such as the now-defunct Asoyia, Inc., based in Iowa City, Iowa, USA. Asoyia, which supplied Cargill with ultra-LL soybeans, closed in 2009.

"In a raw material situation, it costs more to transport and distribute grain than it does to grow it. Further, if you want to use the traditional handling and processing network for a specialty bean, there is a critical mass that you have to get beyond before the efficiencies are good enough for the specialty market to have any staying power at all. That fact weighs in the favor of larger companies," said Charles Hurburgh, professor of agricultural and biosystems engineering at Iowa State University (Ames), who worked with Asoyia (see *Inform 21*:213–215, 2010).

Since HOSO has the same levels of α-linolenic acid as LL soybeans, Monsanto and DuPont Pioneer no longer sell LL soybeans, according to spokespersons for both companies.

The jury is still out on whether HOSO will help the soybean oil industry regain its lost market share. But HOSO has advantages that LL soybean oil did not have, including the perception that HOSO has a more healthful fatty acid profile as well as having a neutral flavor that should be attractive to food formulators, increased oxidative stability (as compared with commodity soybean oil that has not been partially hydrogenated), and the possibility that it will, in the end, be less expensive than high-oleic canola and sunflowerseed oils. These benefits may lead to HOSO carving a place for itself in the *trans*-fat replacement market.

Michael Logli is an Inform *science writer and can be reached at michael.logli@aocs.org.*