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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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When foods containing fats and oils go bad, lipid oxidation is often to blame. Signs of lipid oxidation include nasty smells, disgusting flavors, and altered textures, resulting in foods that are generally unpalatable and often end up in landfills. Along with the economic and environmental tolls of food waste, rancid foods pose health concerns, as lipid oxidation often goes hand-in-hand with microbial contamination, and the ingestion of lipid oxidation products themselves has been linked to diseases like cancer. Therefore, food manufacturers are eager to reduce lipid oxidation as part of an overall strategy to improve the shelf lives of their products. However, several factors have made this task more difficult in recent years, necessitating innovations in food additives, product formulation, and packaging.

Laura Cassiday

Lipid oxidation, which leads to rancidity, is a major factor that limits the shelf life of foods containing fats and oils.

The replacement of saturated fats with polyunsaturated fats, as well as increasing consumer demand for natural ingredients, has challenged food manufacturers to develop new strategies to prevent lipid oxidation.

Natural antioxidants, product reformulation, and active packaging are three possible ways to meet this challenge.

There is no universal solution to preventing lipid oxidation; different strategies must be evaluated for each food application.
How good foods go bad

Lipid oxidation occurs when a free radical, such as OH\(^\cdot\), removes a hydrogen atom from the methylene carbon of an unsaturated fatty acid, producing an alkyl radical (Fig. 1).

This first stage of lipid oxidation, called initiation, is followed by propagation, during which the alkyl radical undergoes addition with atmospheric oxygen to form a peroxyl radical. The peroxyl radical abstracts a hydrogen from another unsaturated fatty acid, generating another alkyl radical and a lipid peroxide. The oxidation cycle continues, with the transfer of a radical from one fatty acid to another. In a process called β-scission, lipid peroxides decompose into alkoxyl radicals, forming a second free radical that can attack additional fatty acids and cause an exponential increase in oxidation rates. Lipid peroxides also decompose into secondary oxidation products, including volatile compounds that are responsible for rancid odors and flavors.

The kinetics of the lipid oxidation reaction are complex, with different rate constants for the initiation and propagation phases (Barden, L., and Decker, E. A., http://dx.doi.org/10.1080/10408398.2013.848833, 2013). Initially, the accumulation of lipid oxidation products is slow as free radicals form and preferentially oxidize antioxidants in the food. This period is called the lag phase (Fig. 2). Once available antioxidants are consumed and propagation and β-scission accelerate, the reaction becomes exponential.

The shelf life of a fat-containing food corresponds to the length of time a food may be stored before a consumer can detect volatile lipid oxidation products that affect flavor. Because some lipid oxidation products have very low sensory threshold values, people can often perceive rancidity during the lag phase—before oxidation products can be detected chemically. Thus, the length of the lag phase is the most important factor that determines shelf life (Barden, L., and E. A. Decker, http://dx.doi.org/10.1080/10408398.2013.848833, 2013).

Trends and challenges

During lipid oxidation, the initiating free radical attacks methylene carbons. Therefore, the higher the degree of unsaturation in a fatty acid chain, the more susceptible the fatty acid is to oxidation. The addition of a single double bond to a polyunsaturated fatty acid doubles its oxidation rate. This fact explains the high vulnerability to oxidation of some nutritionally important polyunsaturated fatty acids, for example, the omega-3 fatty acids eicosapentaenoic acid (EPA; 5 degrees of unsaturation) and docosahexaenoic acid (DHA; 6 degrees of unsaturation) (Kleiner, L., A&G, 2013).

Current dietary guidelines in the United States and elsewhere recommend replacing saturated fatty acids in foods with unsaturated fatty acids, which are generally perceived as more healthful (Dietary Guidelines for Americans, 2010). However, doing so results in a drastic reduction in shelf life of foods containing fats and oils, especially now that partial hydrogenation, a means of stabilizing unsaturated fatty acids, is deemed unacceptable because of health concerns.

Coinciding with the drive to eliminate saturated fats is an increasing consumer demand for natural products. As a result, many of the tried-and-true methods for preventing lipid oxidation, such as the synthetic antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene

FIG. 1. Mechanism of lipid oxidation. Credit: Wikimedia Commons

FIG. 2. Lipid oxidation curves for three different compounds (A–C). The lag phase corresponds to the time before the oxidation reaction becomes exponential. A and B have similar lag phases, but different rates of oxidation in the exponential phase. Sample C has a longer lag phase but then rapidly oxidizes, characteristic of samples containing tocopherols. (From Barden, L. and E.A. Decker, Lipid oxidation in low moisture food: a review, Critical Reviews in Food Science and Nutrition, November 26, 2013. Reprinted with permission of publisher, Taylor & Francis Ltd, http://www.tandfonline.com.)

CONTINUED ON NEXT PAGE
(BHT), and tert-butylhydroquinone (TBHQ) have fallen out of favor. “People want cleaner labels; they want to be able to understand the ingredients in their foods,” says Leslie Kleiner, food science project coordinator at Roquette America, Inc. (Geneva, Illinois, USA). “With antioxidants like BHT or TBHQ, people don’t know what they are, so they don’t like them.” Augmenting consumer wariness are sensational news reports of studies linking large doses of synthetic antioxidants to health problems in animal studies.

Going natural

Adding antioxidants—synthetic or natural—to foods remains the most common approach for reducing lipid oxidation. Widely used classes of antioxidants include free radical scavengers, chelators, and singlet oxygen quenchers. Free radical scavengers donate a hydrogen atom to alkyl and peroxyl radicals, producing alcohols and peroxides. The resulting antioxidant radical is sufficiently stable to preclude further initiation and propagation reactions. Chelators bind transition metal ions such as iron and copper, keeping them from generating free radicals. Singlet oxygen ($^1\text{O}_2$) quenchers deplete the excess energy of singlet oxygen, which is the excited state of normal triplet oxygen generated by light or photosensitizers. Singlet oxygen is unstable and can react with unsaturated fatty acids to form hydroperoxides. Other classes of antioxidants work by absorbing UV light that triggers lipid photo-oxidation or by scavenging oxygen.

Natural alternatives are available for most types of synthetic antioxidants (Table 1). “Natural antioxidants and blends have been confirmed to be effective in food products containing fats and oils, but the challenge is that natural antioxidants are more expensive than synthetic counterparts and, in most cases, are less efficacious,” says Min Hu, senior scientist at DuPont Nutrition and Health (New Century, Kansas, USA), and editor of *Oxidative Stability and Shelf Life of Foods Containing Oils and Fats*, an AOCS Press book that will be available in January 2016. As a result, food manufacturers may need to use high concentrations of natural antioxidants—which can affect food flavor, color, or viscosity—or antioxidant blends.

Essential oils are liquid mixtures of volatile compounds obtained from aromatic plants, most commonly by steam distillation. Some essential oils derived from herbs such as oregano, thyme, and rosemary have antioxidant activity. Scientists have traced the radical-scavenging activity of essential oils primarily to phenolic compounds such as carvacrol and cymene and cyclohexadiene-like compounds such as γ-terpinene (Amorati, R., et al., http://dx.doi.org/10.1021/jf403496k, 2013). However, essential oils are mixtures of several components that can interact synergistically or antagonistically. In some essential oils such as cuneate Turkish savory (*Santureja cuneifolia*), pro-oxidant components counteract antioxidant ones, resulting in overall pro-oxidant behavior.

The method used to extract antioxidants from plants influences the extract’s composition and activity. Ignacio Vieitez Osorio at the Universidad de la República (Montevideo, Uruguay) has used an extraction technique called supercritical carbon dioxide extraction to characterize antioxidant activities of South American medicinal plants. The technique uses a fluid state of carbon dioxide (supercritical carbon dioxide) as an extraction solvent. According to Vieitez, traditional methods for extracting natural products typically use organic solvents that are flammable or toxic and high temperatures that can degrade products and form impurities. In contrast, carbon dioxide has a moderate critical temperature, is non-flammable, and is generally recognized as safe (GRAS) by the food industry.

Vieitez and his colleagues recently used supercritical carbon dioxide extraction to make extracts from four herbs: rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), and the South American herbs marcela (*Achyrocline satureioides*) and carqueja (*Baccharis trimera*). They then tested the extracts’ abilities to stabilize purified sunflower oil.

### Table 1. Types and examples of antioxidants

<table>
<thead>
<tr>
<th>Type</th>
<th>Synthetic</th>
<th>Natural</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free-radical scavengers</td>
<td>BHA, BHT, TBHQ, propylene glycol</td>
<td>Tocopherols, essential oils (e.g., rosemary, oregano), plant extracts (e.g., green tea)</td>
</tr>
<tr>
<td>Chelators</td>
<td>EDTA, poly(acrylic acid)</td>
<td>Citric acid, lactoferrin</td>
</tr>
<tr>
<td>Singlet oxygen quenchers</td>
<td>Sodium nitrite</td>
<td>Tocopherols, polyphenols, carotenoids</td>
</tr>
<tr>
<td>UV absorbers</td>
<td>Benzotriazoles, benzophenones, pigments</td>
<td>Eucalyptus leaf extract</td>
</tr>
<tr>
<td>Oxygen scavengers</td>
<td>Metal-based powder</td>
<td>Ascorbic acid, enzymes, catechins</td>
</tr>
</tbody>
</table>
oil. Rosemary, marcela, and oregano extracts at concentrations of 500 ppm increased the oxidative stability of the oil by 8.7-, 3.1-, and 1.8-fold, respectively, whereas the carqueja extract had no significant effect. Interestingly, the rosemary extract caused a greater increase in the induction period at 100 °C (time needed to reach an increase in oxidation rate when the oil is heated) of sunflower oil than the synthetic antioxidants BHA and BHT—an increase similar to that of the powerful synthetic antioxidant TBHQ. "This result indicates that, despite being an unpurified 'crude' extract, rosemary extract may provide high stability to sunflower oil and appears to be a very attractive alternative to synthetic antioxidants in the stabilization of edible oils," says Vieitez.

However, Vieitez says that before these extracts can be used commercially to stabilize oils, their safety must be confirmed. "It would also be necessary to study the flavor of the oil preserved with these herbal extracts and to determine what impact this may have on consumer preferences," he says. In the future, Vieitez plans to optimize the supercritical extraction procedure and to determine the main active compounds in the plant extracts.

Matrix matters

According to Kleiner, there is no "one-size-fits-all" antioxidant; rather, researchers must determine the best antioxidant and dosage for foods on a case-by-case basis. Much of this variability has to do with dissimilarities in the physical and chemical properties of different food matrices.

For example, oil-in-water emulsions (salad dressings, mayonnaise, sauces, etc.) are much less oxidatively stable than bulk oils because they have a large surface area of lipid in contact with the aqueous phase, which facilitates interactions between lipids and water-soluble pro-oxidants such as metal ions (Waraho, T., et al., http://dx.doi.org/10.1016/j.tifs.2010.11.003, 2011). Nonpolar antioxidants tend to be more active than polar antioxidants in oil-in-water emulsions, whereas the reverse is generally observed in bulk oils. This so-called "polar paradox" can be explained by the different types of interfaces found in emulsions and bulk oils (Fig. 3, page 410). Antioxidants function best when they accumulate where lipid oxidation actually takes place. In oil-in-water emulsions, lipid oxidation occurs at the oil/water interface on the surface of droplets, whereas in bulk oil, lipid oxidation takes place primarily at the oil/air interface. Different types of antioxidants (nonpolar and polar, respectively) localize to these interfaces.

In oil-in-water emulsions, characteristics of the emulsion droplet such as charge, interfacial thickness, and interfacial permeability can influence oxidative stability. For example, anionic surfactants can promote rapid oxidation because they attract cationic metals such as Fe²⁺ to the droplet. In contrast, cationic surfactants decrease

CONTINUED ON NEXT PAGE
oxidation rates by repelling metal cations. Also, surfactants with large head groups can form a barrier that limits interactions between lipids and pro-oxidants in the aqueous phase. The emulsifier packing density impacts the diffusion of oxygen, free radicals, and pro-oxidants through the interfacial layer where they can interact with lipids. Because of these considerations, interfacial engineering to control the composition, thickness, and charge of the oil/water interface can be an effective strategy for inhibiting lipid oxidation in emulsions (Waraho, T., et al., http://dx.doi.org/10.1016/j.tifs.2010.11.003, 2011).

Minor components in bulk oils can interact with each other to influence lipid oxidation (Chen, B., et al., http://dx.doi.org/10.1080/10408398.2011.606379, 2011). For example, the small amount of water typically present in refined oils can act as a solvent for hydrophilic or amphiphilic pro- and anti-oxidants. In addition, water in bulk oil can enable association colloids (reverse micelles, microemulsions, etc. formed by minor oil components), which can act as lipid oxidation reaction sites. Trace amounts of photosensitizers like chlorophyll can produce singlet oxygen. Vegetable oils naturally contain tocopherol antioxidants, although about 30% of tocopherols are lost during the deodorization step of oil processing.

Low-moisture foods such as cookies, crackers, and breakfast cereals present their own challenges for preventing lipid oxidation (Barden, L., and Decker, E. A., http://dx.doi.org/10.1080/10408398.2013.848833, 2013). In these foods, water activity can be manipulated to help control oxidation. However, depending on the food, water might play protective or pro-oxidative roles. In baked goods, leavening, mechanical mixing, and kneading can cause air bubbles to form. If lipids associate with the bubbles, the interface could be a site of lipid oxidation. Also, flour contains components such as iron and riboflavin (a photosensitizer) that encourage lipid oxidation.

**Formulation considerations**

In some cases, foods containing fats and oils can be reformulated to improve shelf life. Manufacturers may be able to avoid or minimize certain ingredients known to promote lipid oxidation, such as transition metals (iron) and photosensitizers (riboflavin, chlorophyll). Solid foods can be coated with proteins or polysaccharides to create an oxygen-impermeable barrier at the food surface. Encapsulating oils likewise reduces interactions with pro-oxidants in the air or food matrix.

Adjusting processing conditions can also help control lipid oxidation. If possible, high temperatures during processing should be avoided because oxidative reactions occur more rapidly. Also, minimizing oxygen exposure, for example, by reducing mixing or kneading times, can reduce lipid oxidation. During packaging, many snack products are flushed with nitrogen or other inert gases to reduce oxygen content.
with nitrogen gas to remove oxygen. This approach has had variable success in reducing lipid oxidation because oxygen remains in air pockets of foods and packaging materials.

Active packaging

Packaging materials have long provided a barrier to external conditions, but now researchers are designing new materials that play a more active role in extending shelf life. By incorporating antioxidants into packaging, manufacturers may be able to reduce the load of food additives in their products.

In the past, manufacturers have coated packaging polymers like low-density polyethylene and poly(lactic acid) with the synthetic antioxidants BHA and BHT to develop antioxidant packaging films (Tian, F., et al., http://dx.doi.org/10.1039/c3fo30360h, 2013). Although these materials are effective and stable, they are considered “label-unfriendly” due to their synthetic origin. As a result, researchers are now investigating the use of different types of natural antioxidants, such as tocopherols, enzymes, essential oils, and plant extracts, in active packaging.

Antioxidants can be introduced to packaging in several different ways. Sachets made of oxygen-permeable material contain oxygen scavengers such as iron, ascorbic acid, or catechol. These antioxidants react with oxygen, and the resulting reactive oxygen species are trapped within the sachet. This approach has reduced the oxygen level to less than 0.01% in foods such as bakery products, cheese, and chips. Alternatively, antioxidants can be coated onto packaging or dispersed throughout the polymeric matrix. In both cases, antioxidants migrate from the packaging materials into the food, which requires their labeling as food additives.

Another approach is to covalently immobilize antioxidants to the packaging material so that the antioxidants cannot migrate from package to the food. First, the packaging polymer must be pretreated, or functionalized, to introduce reactive sites. Then, researchers can covalently attach antioxidants or other functional molecules to the active sites.

Julie Goddard at the University of Massachusetts (Amherst, USA) has linked metal chelators to the packaging materials polyethylene, polypropylene, and polyethylene terephthalate to remove trace amounts of iron from products. “We’ve shown that these materials can extend the lag phase in lipid oxidation by a factor of four to five times,” Goddard says. “In one variant, the chelating active packaging materials even outperformed EDTA in solution.” Goddard’s team is currently working on ways to cost-effectively produce the materials at an industrial scale.

New antioxidants needed

Although researchers are developing approaches to meet the challenges posed by a changing food industry, the field desperately needs new materials with which to work. According to Eric Decker at the University of Massachusetts (Amherst, USA), only a handful of new antioxidants—synthetic or natural—have been introduced to the market over the past 30 years. “Although there are examples of antioxidants all through nature, there just haven’t been good systematic approaches to find antioxidants that actually work in foods,” says Decker. Sometimes the antioxidants aren’t soluble in foods or don’t localize to the site of lipid oxidation. Other times, crude extracts contain both pro- and anti-oxidants, limiting their effectiveness.

“I think the biggest problem the food industry has is there’s not a good fundamental understanding of lipid oxidation, so they can’t take a systematic approach,” says Decker. “Food companies will just take every antioxidant they can off the shelf and randomly mix them in different combinations in the hope that something works.” Therefore, the key to finding new antioxidants and other approaches that extend shelf life may be more basic research on the process of lipid oxidation, he says.

Laura Cassiday is an associate editor of Inform at AOCS. She can be contacted at laura.cassiday@aocs.org.
Natural solutions for improved stability of omega-3 oils and omega-3-enriched foods

Xin Tian and Jill McKeague

The health benefit of omega-3 fatty acids was first discovered in the 1970s, when Bang et al. found that the Greenland Inuits, who had high consumption of fish and marine animals, had a significantly lower incidence of cardiovascular mortality. Later, it was found that consuming omega-3 could also enhance brain development in infants, improve eye and brain function in adults, and help to alleviate symptoms of rheumatoid arthritis and inflammatory bowel disease (Jacobson et al., 2013). Such findings have led to a surge in the use of omega-3 oils in application segments such as dietary supplements, food and beverages, pharmaceuticals, and infant formula. The global market for omega-3 polyunsaturated fatty acids (PUFA) is expected to be over $4 billion by 2019.

A big challenge that comes with using fish oils is their oxidative stability. The term omega-3 (or n-3) denotes PUFA that have a double bond at the third carbon atom counting from the methyl terminus of the acyl chain. Table 1 (page 415) shows a list of omega-3 fatty acids. The number of double bonds in these fatty acids varies from 3 to 6; this high level of unsaturation greatly increases their susceptibility to oxidation.

Crude fish oil contains low amounts of endogenous enzymes and antioxidants that help protect marine organisms from damage due to reactive oxygen species (ROS) (Undeland et al., 1998). However, most of these intrinsic antioxidants are lost during the refining process; refining also exposes the oils to oxidation catalysts such as metals, water, and heat. Lipid oxidation gives rise to undesirable fishy off-flavors that make the oil and the food enriched with it unacceptable. It also reduces omega-3 PUFA levels and thereby sacrifices the nutritional value of these lipids.

The stability issue of omega-3 lipids can be addressed by adding exogenous antioxidants to the oil or food for extra protection. While the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ethylene diamine tetra acetic acid (EDTA) is very effective in inhibiting oxidation, consumers prefer clean labels, causing the food industry to seek natural alternatives.

Rosemary has been found to be one of the most effective natural antioxidants in many food systems. Major active constituents of rosemary are phenolic diterpenes such as carnosic acid and carnosol, and phenolic acids such as rosmarinic acid. These compounds donate hydrogen to quench free radicals, thus breaking the chain reaction and slowing down the oxidation (Berdahl et al. 2010).

CONTINUED ON NEXT PAGE
Figure 1, above. Impact of antioxidants on oil induction time of different fish oils (a-d) evaluated by oxidative stability index (OSI) at 80°C. RE – rosemary extract; MT – mixed tocopherols; AA – ascorbic acid. Concentrations of individual component in the (RE+AA+MT) blend range from 0.01% - 0.1%.

Figure 2, left. Impact of rosemary extract and tocopherol on oil induction time of algal oil. RE – rosemary extract; MT – mixed tocopherols; AA – ascorbic acid. Concentrations of individual component in the (RE+AA+MT) blend range from 0.01% - 0.1%.
Natural rosemary extract (RE) and unique combinations of rosemary extract with other functional antioxidants were recently tested in tuna, salmon, herring, and anchovy oils. The addition of natural rosemary extract extended induction time of omega-3 oils at 80°C by 67% to 136% (Fig. 1, a–e, page 413). The effectiveness of other antioxidants in addition to rosemary is matrix dependent. The addition levels of a combination of rosemary, mixed tocopherols (MT), and ascorbic acid (AA) were determined based on the active range of each component, and on the color and flavor impact on the fish oils; concentrations of individual components ranged from 0.01%–0.1%. The addition of low and high levels of this blend into various fish oils improved oil stability by a large extent, whereas MT and AA did not provide significant improvement in algae oil (Fig. 2).

The efficacy of natural antioxidants was also studied in emulsions and in food matrices containing omega-3 fatty acids. Most foods comprise different phases and components. This heterophasic nature makes the mechanisms of lipid oxidation in most foods more complex than those in neat oil systems, because oxidation reactions can be affected by a number of different factors. Emulsions are more prone to oxidation than bulk oils for two reasons: 1. water and prooxidants in water catalyze oxidation and 2. partitioning of substances between different phases increases the interaction between prooxidant and oxidizable substrates.

Enzymatically modified pea protein and green tea were evaluated by measuring their ability to inhibit hydroperoxide formation in oil-in-water emulsions. Enzymatically modified pea protein added at 0.01% showed excellent antioxidant effects in both emulsions with different ratios of fish oil and canola oil (Fig. 3a and b). Food protein-derived bioactive peptides have been shown to have strong antioxidant properties, with the ability to scavenge hydroxyl radicals (OH), superoxide anion radicals (O2−), and hydrogen peroxide (H2O2). Proteins may also have iron-binding capabilities that reduce the prooxidant activities of the metal. At the same concentration (0.01%), green tea extract also inhibited oxidation of the emulsions, yet to a lesser extent.

The use of omega-3 fatty acids in baked and extruded products also presents a greater challenge compared to bulk oils. First of all, these products use manufacturing processes that expose them to high temperatures and air. Secondly, most of these products are expected to be shelf stable and require extended shelf-life, sometimes for over a year. Therefore, the addition of omega-3 PUFAs needs to be paired with effective antioxidants to protect product integrity.

The effectiveness of rosemary extract in baked and extruded products fortified with omega-3 oils was assessed by formation of hexanal, which is a major marker for food rancidity, and by formal sensory tests. Adding 0.018% rosemary extract reduced hexanal formation in a flax seed-enriched cookie by 85% during a 12-week period of ambient storage (Fig. 4a). A combination of rosemary and mixed tocopherols showed similar antioxidant effects, with mixed tocopherols providing...
minimal additional benefits. A sensory study was also conducted using trained panels and the results showed reduced off-flavor and better retention of favorable aroma in the cookie (Fig. 4b). Rosemary also showed excellent antioxidant performance in extruded cereal formulated with 95% corn flour and 5% flax seed meal; it suppressed formation of hexanal and rancid aroma over a 20 weeks storage time at ambient temperature (Fig. 5a and 5b).

The increasing popularity of omega-3 enrichment calls for effective measures to maintain stability of the oils and food. With consumers’ increasing expectation for a “clean label,” finding natural solutions to conquer oxidation becomes more challenging. Given the complexity of food matrices, more effort needs to be dedicated to understand the roles of natural antioxidants so that this knowledge can be used to develop strategies for different omega-3 oil-containing foods.

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Jill McKeague is the product director for antioxidants at Kalsec, Inc. She has been with Kalsec since 2003 and previously held the role of senior scientist at Kalsec. She received her MBA from the University of Michigan in 2011.

Further reading


Table 1. A list of omega-3 fatty acids.

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Archer Daniels Midland Co. (ADM; Chicago, Illinois, USA) announced in late April that its Enderlin, North Dakota, USA, processing plant is now able to crush soybeans in addition to canola and sunflower seeds. In addition, the company has converted a canola processing line in Windsor, Ontario, Canada, to a flex line that can also process soybeans.

The European Commission has amended its regulation on methods of sampling and performance criteria for the methods of analysis for the official control of the levels of erucic acid in foodstuffs. The regulation (2015/705) was published on April 30 and repeals Commission Directive 80/891/EEC. See http://tinyurl.com/EC-erucic for the complete regulation.

NEWSMAKERS

Archer Daniels Midland Co. (ADM; Chicago, Illinois, USA) has named Greg Mills as president, Golden Peanut and Tree Nuts (GPTN; Alpharetta, Georgia, USA). In his new position, Mills will be responsible for all GPTN operations, business development, sales, marketing, and distribution activities. The company has 13 processing facilities in the United States, one in Argentina, and three in South Africa.

ADM also named Gregory Morris as senior vice president and president, Global Oilseeds. Morris has been with ADM for more than 20 years, serving on the Oilseeds team for over 17 years and as president of North American Oilseeds for more than two years.

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NEWS & NOTEWORTHY

Low-allergen soybean developed through conventional breeding

The World Allergy Association estimates that between 220 and 520 million people worldwide suffer from food allergies. Among the most problematic foods are the so-called Big Eight: cow’s milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybean.

Soybean allergy is one of the more common food allergies, according to Food Allergy Research & Education (FARE; www.foodallergy.org), especially among babies and children. Research indicates, however, that the majority of children with soy allergy will outgrow the allergy by the age of 10, notes FARE on its website.

Help may be on the way, thanks to a decade-long effort by University of Arizona (Tucson, USA) scientists Monica Schmidt and Eliot Herman and University of Illinois at Urbana-Champaign (USA) scientist Theodore Hymowitz. Their work has yielded a new soybean with significantly reduced levels of three key proteins responsible for both its allergenic and antinutritional effects. The

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Recycling olive mill wastewater

The European Union is the world’s largest producer of olives and olive oil, supplying an average of 2.2 million metric tons of olive oil every year. Spain, Italy, and Greece account for around 97% of that production, and small companies dominate the sector, with more than 12,000 olive mills in the EU-27 alone.

Producing 100 kilos (220 pounds) of olives requires about 50 liters (13 gallons) of water to wash the olives, which currently is discarded as wastewater. Now, the ALGATEC II project has found a way to reuse the wash water, cutting the use of potable water by 90% and removing the wastewater disposal problem. (See http://www.algatec2.eu.)

“We really think this can work,” said project coordinator Antonia Lorenzo of BIOAZUL, a company that facilitates research projects and is based in Málaga, Spain. “Growing global demand for olive oil is considerably increasing the use of water in countries where this resource is scarce and, moreover, there is significant waste, especially wastewater.”

CLEANING UP WASH WATER

Polyphenols are one of the reasons that olives and olive oil are healthful. Research has shown that these antioxidants, which are naturally present on the skin of olives, may offer protection from disease. However, when they are released into the environment in large volumes, such as in olive wash water, they can be harmful.

Olive wash water is traditionally left in large evaporation ponds. However, the ponds have a limited capacity—which in turn limits production—and must sometimes be emptied manually. Meanwhile, the odors and insects that accompany stagnant water are unpleasant for the olive mill’s neighbors.

The ALGATEC II approach first involves pumping the wash water to a photobioreactor, where microorganisms absorb the pollutants. In winter, when sunlight is lacking, solar panels can be used to heat the water, increasing the organisms’ growth to speed up the process.

The effluent then goes through a final “polishing step,” where it is passed through two membrane filters that remove any remaining pollutants. What is left can be used as drinking water or to wash more olives. It can also be discharged safely into sewers, whereas dirty wastewater cannot.

Although the solution cannot compete with evaporation ponds on cost, it removes many of the problems associated with the ponds and allows producers to expand production. The system can also be tailored to the producer’s needs given that the final filtering step is not necessary if drinking water is not required.

The additional cost attached to a liter of olive oil would be “almost nothing,” says Lorenzo, and the technology is cheaper than current alternatives on the market.

A demonstration plant is up and running at the University of Huelva in Spain, and the five ALGATEC II partners—small- and medium-sized enterprises from Spain, Italy, and Germany—are now working on a business plan to put the technology on the market.

The ALGATEC II project received €886,000 in funding under the EC’s Seventh Framework Programme for Research and Technological Development (2007–2013) as a demonstration project.

Cargill purchases OPX Biotechnologies

Cargill has acquired fermentation technology from OPX Biotechnologies (Boulder, Colorado, USA). OPX had been developing applications to produce 3-HP (3-hydroxypropionic acid) via fermentation, which is then converted in one step to biobased acrylic acid. Cargill and Novozymes have been collaborating since 2008 on technology for the production of acrylic acid from 3-HP through the fermentation of sugar using a bioengineered microorganism. The acquisition of OPX brings with it world-class capability in strain and metabolic engineering as well as a portfolio of target molecules including butanol, BDO, 3-HP, acetyl-CoA, malonyl-CoA, malonate semialdehyde, acrylic acid, PDO, malonic acid, ethyl 3-HP, propiolactone, acrylonitrile, acrylamide, and methyl acrylate.
Work is described in a paper published online in the journal *Plant Breeding* (see http://tinyurl.com/Triple-Null).

“We have created a low-allergen and low antinutritional inhibitor soybean using conventional breeding methods,” said Herman, a professor in the UA School of Plant Sciences.

In 2003, while with the US Department of Agriculture, Herman made national headlines when he and his colleagues named P34 as the soybean’s key allergen, and genetically engineered it out of the crop. Although the new soybean may have been less likely to cause allergic reactions, acceptance and further testing was impeded by its transgenic source, especially in key applications such as infant formula.

To circumvent the issue, Herman, Schmidt, and Hymowitz set out to create a similar soybean using conventional breeding methods. After screening 16,000 different varieties of soybean for the desired trait, they found one that almost completely lacked the allergen P34. The team stacked the P34 null with two varieties previously identified by Hymowitz that lacked soybean agglutinin and trypsin inhibitors, proteins that are responsible for the soybean’s antinutritional effects in livestock and humans.

“We really believed in this goal and wanted to produce an enhanced soybean that could be used,” said Herman. “That became the motivation for using conventional breeding rather than the transgenic approach.”

After nearly a decade of crossbreeding each variety to the soybean reference genome called Williams 82, the team has produced a soybean that lacks most of the P34 and trypsin inhibitor protein, and completely lacks soybean agglutinin. Beyond these characteristics, the soybean is nearly identical to Williams 82. The team has dubbed the new variety “Triple Null.”

“We think this will be embraced by many, whether they prefer conventional breeding or transgenic methods of food production,” said Herman.

CONTINUED ON NEXT PAGE

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**IN MEMORIAM**

Pericles (Perry) Constantine Markakis, a professor emeritus at Michigan State University (MSU; East Lansing, USA) and Fellow of the Institute of Food Technologists, died on May 3, 2015. He was 95.

Most of Dr. Markakis’ academic career was spent as a tenured professor of food chemistry in the MSU Department of Food Science and Human Nutrition. It was there that he served as academic advisor for two AOCS Fellows—Keshun Liu and Apostolos Paul Kiritsakis.

Among Dr. Markakis’ explorations were the promise of the cassava root as a major global food source, physical factors contributing to the optimal refinement of olive oil, and the role and extraction of natural food colorants.

“He was a true mentor,” writes Paul Kiritsakis. “His scholarly and personal guidance and friendship continued long after my graduation until his passing. [He was] a truly kind and gracious man and an outstanding scientist.”

Dr. Markakis is survived by his wife of 62 years, Georgia, three daughters and sons-in-law, and eight grandchildren. ■
Schmidt, an assistant professor in the School of Plant Sciences. “It can be grown organically, with pesticides, and although conventional itself it could be transformed [through genetic modification] to add other producer or consumer traits.”

In collaboration with scientists at Purdue University, tests are planned to evaluate the efficacy of the low-allergen soybean in swine. The Purdue team has bred a line of swine that develops a strong allergic response very similar to that of human infants who are allergic to soybean formula. The swine studies will enable testing of Triple Null and foster new approaches to mitigate soybean allergies in humans.

“Food allergy is a huge and growing problem for children,” explained Herman. “We hope this work will offer a new approach to developing low-allergen foods and help to bend down the curve of growing food allergy.”

Triple Null also has applications for livestock and agriculture. Soybean meal use is increasing in aquaculture, which produces more than 50% of consumed seafood, with this number expected to rise to 75% by 2030. Before soybean is used in feed, it must undergo a heating process to eliminate antinutritional proteins such as trypsin inhibitors and soybean agglutinin, which adds to the cost.

“All over the world, people are consuming more meat,” Herman said. “At the current rate, we’ll have to more than double the amount of animal feed by the year 2050. This means that several hundred million more tons of soybeans will need to be processed before it can be fed to animals.” By preemptively knocking out the antinutritional components of soybean, the researchers hope Triple Null can eliminate the need for extra processing and make creation of animal feed more efficient, potentially developing a raw soybean as animal feed.

“By the year 2050, animal feed needs are expected to rise 235%,” Schmidt said. “We’re hoping that our soybeans can help with this. It’s great to know that they can have an impact.”

WASDE: record soybean crop in Brazil in 2015/16


Global soybean production was projected at 317.3 MMT, almost unchanged from 2014/15, with gains for Brazil, India, Paraguay, and Ukraine offset by reductions for the United States, Argentina, and China. The Argentine soybean crop was projected at 57.0 MMT, down 1.5 million from 2014/15 with higher area but lower yields. The Brazilian soybean crop was projected at a record 97.0 MMT, up 2.5 million on higher area. China soybean production was projected at 11.5 MMT, down 0.85 million as producers shift to more profitable crops. Total oilseed supplies were up 2.8% from 2014/15. With crush projected to increase 2.3%, global oilseed ending stocks were projected at 107.4 MMT, up 8.3 million from the revised 2014/15 stock estimate.
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In late April 2015, India’s Minister for Agriculture Pocharam Srinivas Reddy laid the foundation for a new oil palm facility that aims to use oil palm sludge to produce biodiesel. The new factory, the second such to be built in the Apparaopeta region of Telangana state, is expected to become operational in 10 months, according to The Hindu newspaper.

Brazil recently raised its biodiesel mandate to 7%, which has reduced the need for diesel imports to power the country’s agriculture sector. This is according to Jorge Celestino Ramos of Petrobras, Brazil’s state-run petrochemical company, as quoted by the Platts news service. The increased mandate, ABIOVE said, may lead to an estimated production of 4.2 billion liters in 2015, as compared with 3.2 billion liters in 2014. ABIOVE (Associação Brasileira das Indústrias de Óleos Vegetais) is a trade association representing vegetable oil producers.

Gevo, Inc. stock more than doubled after the company’s announcement in May 2015 that Alaska Airlines will purchase Gevo’s renewable jet fuel and fly the first-ever commercial flight on alcohol-to-jet fuel (AJF). The test was expected to occur in mid-to-late 2015 after Gevo (Englewood, Colorado, USA) receives ASTM International certification for the fuel. Once approved, the fuel “can be seamlessly integrated into the existing distribution infrastructure and onto commercial aircraft,” noted Gevo in a statement. The company’s AJF is produced at its biorefinery in Silsbee, Texas, USA, using isobutanol produced at its Luverne, Minnesota, USA, fermentation facility, according to www.biofuelsdigest.com.

Pacific Ethanol, Inc. (PEI; Sacramento, California, USA), a producer and marketer of renewable fuels, has begun commercial production of corn oil at its Madera, California, plant utilizing Valicor’s proprietary VFRAC™ corn oil recovery system. Neil Koebler, the company’s president and CEO, stated, “Plans are underway for corn oil production to begin at our

CONTINUED ON PAGE 431
chemical precursors that can be used to make a wide range of everyday products from antibiotics to paints.

The authors acknowledge funding from the US Department of Energy, the Lawrence Berkeley National Laboratory, Howard Hughes Medical Institute, the National Science Foundation, and the National Institutes of Health.

Low-carbon fuel technologies to watch

A diverse range of novel low-carbon transport fuels are nearing market introduction, with many offering the potential for high greenhouse gas savings without using land-based feedstocks.

The UK Department for Transport (DfT) commissioned two London-based sustainable energy consultancies—E4tech and Ecofys—to investigate these fuels, as currently only biofuels are supported by the UK’s Renewable Transport Fuel Obligation (RTFO; see http://tinyurl.com/RTFO-UK).

“Our report provides a much needed classification framework for the various types of fuels,” E4tech said in a news release. “It also highlights companies known to be developing these novel fuels, and crucially identifies potential regulatory and sustainability risks (and mitigation options), before discussing some of the practical implications of including them under the RTFO.”

The consultancies recommend adding two new categories to the UK’s RTFO guidelines: (i) low-carbon fuels from non-renewable sources, such as the production of fuels from used tires, waste, plastics, steel mill carbon monoxide, or stranded gas; and (ii) renewable fuels of nonbiological origin (RFNBO).

The definition of RFNBO includes hydrogen from renewable energy sources (where there are no carbon atoms in the fuel), as well as cases where hydrogen from renewable energy sources is combined with CO₂ (from waste/residue fossil carbon sources or atmospheric/naturally occurring carbon sources) to make various transport fuels.

The 29-page report, titled “Novel Low-Carbon Transport Fuels and the RTFO: Sustainability Implications,” is available for download at http://tinyurl.com/RTFO-DfT. See Table 1 for examples of technologies and companies highlighted by the report.

### BIO: RFS delays costly

Delays in rulemaking by the US Environmental Protection Agency (EPA) over the last two years have “chilled necessary investment” to the tune of an estimated $13.7 billion shortfall, according to a white paper by the Biotechnology Industry Organization (BIO; http://tinyurl.com/WP-BIO). BIO is a trade organization based in Washington, DC, USA.

Based on research published in 2009 by Bio Economic Research Associates (bio-era), more than $95 billion in cumulative capital investments would be needed by 2022 for construction of nearly 400 advanced biofuel biorefineries with the capacity to produce 23 billion gallons (87 billion liters) of advanced biofuel in order to meet the goals set by the EPA’s Renewable Fuel Standard (RFS).

BIO points out that as of April 2015, five commercial cellulosic biorefineries with a combined capacity of more than 50 million gallons are operating in the United States, along with several pilot and demonstration facilities. “Taking into account

### Table 1. Some novel low-carbon fuels currently in development

<table>
<thead>
<tr>
<th>Company</th>
<th>Fuel</th>
<th>Carbon source</th>
<th>Hydrogen source</th>
<th>Energy source</th>
<th>Process</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cynar</td>
<td>Diesel and jet</td>
<td>Waste plastics</td>
<td>Waste plastics</td>
<td>Waste plastics</td>
<td>Pyrolysis</td>
<td>Demonstration</td>
</tr>
<tr>
<td>PYReco</td>
<td>Semi-refined diesel</td>
<td>Waste tires</td>
<td>Waste tires</td>
<td>Waste tires</td>
<td>Pyrolysis</td>
<td>Pilot</td>
</tr>
<tr>
<td>Velocys</td>
<td>Gasoline, diesel, and jet</td>
<td>MSW or flared gas</td>
<td>MSW or flared gas</td>
<td>MSW or flared gas</td>
<td>Fischer-Tropsch</td>
<td>Demonstration</td>
</tr>
<tr>
<td>Algenol</td>
<td>Ethanol, diesel, jet, gasoline</td>
<td>CO₂ from point sources</td>
<td>Water</td>
<td>Fuel secretion from modified algae</td>
<td>Laboratory</td>
<td></td>
</tr>
<tr>
<td>Joule</td>
<td>Diesel, ethanol, jet</td>
<td>CO₂ from point sources</td>
<td>Water</td>
<td>Sunlight</td>
<td>Fermentation</td>
<td>Demonstration</td>
</tr>
<tr>
<td>Sunfire</td>
<td>Synfuels (gasoline, diesel, jet)</td>
<td>CO₂ from point sources</td>
<td>Water</td>
<td>Solar heat</td>
<td>Electrolysis of H₂O and catalytic synthesis</td>
<td>Laboratory</td>
</tr>
</tbody>
</table>

Abbreviations: MSW, municipal solid waste.

Source: Novel Low-Carbon Transport Fuels and the RTFO: Sustainability Implications; see http://tinyurl.com/RTFO-DfT.
additional renewable diesel producers deploying novel technologies, such as Altair, REG, and Diamond Green, the industry has reached the level of investment (roughly $3 billion) and production capacity (600 million gallons per year) that bio-era originally projected for 2011,” notes BIO. “We therefore estimate that the industry has a cumulative delay of four years, corresponding to a shortfall in investment of more than $20.6 billion, which can be attributed to policy instability, the general economic recession, and the challenges of scaling up new technologies.”

The EPA was on schedule in issuing Renewable Volume Obligation (RVO) rules under the RFS in 2011 and 2012. The agency was nine months late issuing the 2013 RVOs and currently is more than 18 months behind in issuing the 2014 rules.

China’s energy challenges and emission reductions

China’s government and other sources say that the country’s carbon-dioxide emissions flattened out between 2013 and 2014. The leveling-off was a remarkable feat that could set the country on a course to beating its own goals for lowering emissions. But this optimistic outcome hinges on China overcoming some serious energy challenges, according to an article in Chemical & Engineering News (C&EN; see http://cenm.ag/chinaemissions).

Steven Gibb, a senior editor at C&EN, reports that a number of factors could help explain the emissions plateau. China reduced its carbon-intensive coal consumption by 2.9% in 2014 after a decade of double-digit annual growth. It has invested $90 billion in renewable energy such as solar and wind. And it is shifting toward a more service-oriented economy.

China still faces daunting challenges in sustaining control over its emissions, however. In particular, the country’s strategy to reach its carbon reduction goals had called for building one new nuclear power plant every three weeks. The government shelved those plans after Japan’s 2011 Fukushima disaster. Whether China can find new ways to stay on its emissions-cutting course remains to be seen.

Boardman, Oregon, plant in the second quarter, at which time all four of PEI’s ethanol production facilities will be producing and benefitting from this high-value co-product.”

Joule Unlimited (Bedford, Massachusetts, USA; www.jouleunlimited.com) announced a $40 million private equity and venture debt financing deal after a round led by existing investors. Joule has raised a total of $200 million to date toward commercial-scale use of its CO2-to-liquids platform. The conversion “requires only sunlight, nonpotable water, and proprietary catalysts that are tailored to produce specific products, including ethanol and hydrocarbon fuels that are inherently compatible with existing infrastructure,” explains Joule on its website.

Argentina’s exports of biodiesel are expected to drop by 50% in 2015, year on year, to 800,000 metric tons (MT), according to Victor Castro, executive director of the Argentina Chamber of Biofuels (see http://tinyurl.com/Argentina-exports). Castro was interviewed by Thompson Reuters in May 2015. Exports in the first quarter of the year reached about 90,000 MT of biodiesel, which was 57.5% below the amount shipped in the first quarter of 2014. The article attributed the drop in exports to low fossil fuel prices.
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General Mills (Minneapolis, Minnesota, USA) has filed a patent for a process to remove surface oil from fried foods such as potato chips, taco shells, snacks, and pizza (WIPO Publication No. WO/2015/065465; published May 7, 2015). The process uses an air flow chamber, in which the food is placed on a moving, vibrating conveyor belt. Two air flow types—laminar and turbulent—remove and capture excess oil from the fried snack. The pressure inside the chamber is equal or less than ambient pressure, and temperatures are kept above 100°C. In 2014, rival snack food company Frito Lay (Plano, Texas, USA) was granted a US patent for a two-step oil removal process that relies on differential pressures and a vacuum centrifuge.

Italy’s olives are under siege, according to a news story in the May 8, 2015, issue of Science (http://doi.org/10.1126/science.348.6235.620). The culprit is a bacterium called Xylella fastidiosa, which is causing entire olive orchards to wither and die throughout the heel of the boot of Italy. As of October 2014, more than 23,000 hectares were affected by the blight. X. fastidiosa, spread by sap-sucking insects such as the spittlebug, multiplies in plant vascular tissue, clogging water transport systems, and eventually killing the tree. To keep the disease from spreading north, Italian authorities have established a three-kilometer-wide eradication strip and buffer zone that stretches across the peninsula. Within this area, workers are removing sick trees and spraying insecticides to kill sap-sucking bugs. Despite these efforts, infected trees were discovered in April 2015 about 30 miles north of the containment zone. Transport of most plants from the affected region has been banned by the European Union since February 2014.

Coffee byproducts are potent sources of antioxidant and antimicrobial compounds, according to a new study in Food Science and Technology (http://doi.org/10.1016/j.jlwt.2014.11.031, CONTINUED ON NEXT PAGE)

Understanding patterns of omega-3 incorporation in humans

Fatty acids from the diet are transported in the blood to the body’s cells and tissues, where they get incorporated into phospholipids of cell membranes, stored as triacylglycerols, or metabolized. Lipid pools at different locations in the body vary in fatty acid composition, and understanding how each pool changes with dietary intake of omega-3 fatty acids is no easy task. In an oral presentation at the 106th AOCS Annual Meeting and Industry Showcases, held May 3–6, 2015, in Orlando, Florida, USA, Philip C. Calder from the University of Southampton (United Kingdom) described human clinical trials investigating short-, medium-, and long-term incorporation of omega-3 fatty acids into various lipid pools.

According to Calder, changes in fatty acid composition influence cell and tissue metabolism, function, and responsiveness, ultimately impacting health and disease risk. Increasing the dietary intake of the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), widely thought to be beneficial to health, increases the DHA and EPA content of many cells and tissues in the body, with the effect being dose-, time-, and tissue-dependent.

In 2012 Calder and his colleagues reported a study of 210 subjects given different doses of EPA and DHA (American Journal of Clinical Nutrition, http://dx.doi.org/10.3945/ajcn.112.041343, 2012). The researchers analyzed the fatty acid composition of blood plasma lipids, blood cells, and adipose tissue from the volunteers at several time points. The researchers found a dose-dependent enrichment of EPA and DHA in all pools. However, the time taken for DHA and EPA to reach their peak concentrations differed greatly among pools. Plasma phosphatidylcholine EPA and DHA responded the most quickly to changes in EPA and DHA intake, making this pool the most suitable biomarker for short-term changes.

CONTINUED ON NEXT PAGE
According to lead author José A. Rufíán-Henares from the University of Granada (Spain), the coffee industry generates more than 2 billion metric tons of coffee byproducts annually. These include coffee silver-skin (the epidermis of the coffee bean that is removed during processing) and coffee grounds. Rufíán-Henares and his colleagues found that these byproducts are rich in fiber, phenols, and compounds called melanoidins. The coffee byproducts showed potent antimicrobial and antioxidant effects and could be new sources of food additives.

in intake. Platelets were a good medium-term marker of intake, and blood mononuclear cells were a marker of long-term (habitual) intake.

In the United Kingdom, nutritional guidelines recommend consuming one to four portions of oily fish per week to obtain sufficient amounts of EPA and DHA. In contrast to this sporadic consumption of fish (e.g., one or two times per week), most controlled intervention studies of omega-3 supplements use daily dosing schedules. Therefore, little is known about how a sporadic intake of EPA and DHA alters the fatty acid composition of various lipid pools compared to daily dosing.

To address this question, in 2014 Calder and his colleagues compared the short-, medium-, and long-term incorporation of DHA and EPA into plasma phosphatidylcholine, platelets, and mononuclear cells for people taking two larger doses of EPA and DHA per week (equivalent to two portions of oily fish) versus those taking smaller doses daily that added up to the same total amount of EPA plus DHA (5.6 g/week). Daily low-dose EPA and DHA consumption showed greater medium- and long-term incorporation of DHA and EPA (The Journal of Nutrition, http://dx.doi.org/10.3945/jn.113.186346, 2014). This finding may have implications for dietary guidelines that recommend 1–2 large doses (as oily fish portions) of EPA and DHA per week.

In unpublished data presented at the meeting, Calder investigated the identities of fatty acids that EPA and DHA replace in different pools. Although the identities and amounts varied with the pool being examined, the most common fatty acids being replaced were the omega-6 fatty acids arachidonic acid, dihomo-γ-linolenic acid, and linoleic acid and the omega-9 fatty acid oleic acid. Although the current thinking is that new lipids get incorporated into the membrane as new cells are made, Calder’s data suggest that lipid exchange may be taking place in mature cells in the circulation.

New insights into chocolate bloom

Chocolatiers dread the greyish haze that sometimes appears on the surface of chocolate. This so-called fat bloom affects the appearance and texture of the chocolate, resulting in consumer complaints and sales losses. Now a study in ACS Applied Materials & Interfaces provides new information on structural changes that occur during fat blooming, which could help chocolatiers prevent the process (http://dx.doi.org/10.1021/acsami.5b02092, 2015).

Structurally, chocolate consists of particles (cocoa solids, sucrose, and for milk chocolate, milk powder) embedded in a continuous fat phase of cocoa butter. Fat bloom results when lipids migrate to the surface of the chocolate and then recrystallize. However, the exact mechanism and preferred migration pathway of the lipids through chocolate are not well understood. For example, it is unknown whether the lipids migrate at the fat–particle interface, through the fat phase, or through a continuous network of particles.

Svenja K. Reinke at the Institute of Solids Process Engineering and Particle Technology (Hamburg, Germany) and colleagues used a technique called microfocus small-angle X-ray scattering (μSAXS) to study the migration pathway of fat through chocolate. In μSAXS, an X-ray beam passes through a sample, and nanometer-scale structures in the sample scatter the beam. The resulting scattering pattern, picked up by a detector, provides information on the microscopic structure of the sample. Because μSAXS is a nondestructive technique, the researchers can analyze the same sample continually over time.

The team found that lipids initially migrate through pores or cracks in the chocolate, probably driven by capillary pressure. Along the way, the lipids dissolve the crystalline structure of the solid cocoa butter into a liquid form, allowing the lipids to address this question, in 2014 Calder and his colleagues compared the short-, medium-, and long-term incorporation of DHA and EPA into plasma phosphatidylcholine, platelets, and mononuclear cells for people taking two larger doses of EPA and DHA per week (equivalent to two portions of oily fish) versus those taking smaller doses daily that added up to the same total amount of EPA plus DHA (5.6 g/week). Daily low-dose EPA and DHA consumption showed greater medium- and long-term incorporation of DHA and EPA (The Journal of Nutrition, http://dx.doi.org/10.3945/jn.113.186346, 2014). This finding may have implications for dietary guidelines that recommend 1–2 large doses (as oily fish portions) of EPA and DHA per week.

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to migrate through the fat phase of the chocolate. These findings suggest strategies to prevent fat bloom, such as reducing porosity and minimizing cracks in the chocolate structure. Also, reducing the content of noncrystallized liquid cocoa butter could minimize dissolution of the cocoa butter and subsequent chemical migration of the lipids.

**Walnut consumption slows colon tumor growth in mice**

A recent study in *The Journal of Nutritional Biochemistry* suggests that eating walnuts suppresses colorectal cancer in mice (http://dx.doi.org/10.1016/j.jnutbio.2015.02.009, 2015). Moreover, the study offers a potential mechanism for this protective effect: Fatty acids or other compounds in the nuts may alter the expression levels of certain microRNAs.

Walnuts contain the omega-3 fatty acids α-linolenic acid (ALA), docosahexaenoic (DHA), and eicosapentaenoic acid (EPA). Recent studies have shown an inverse relationship between nut consumption and colon cancer risk; however, the underlying mechanisms are not well understood. So Christos S. Mantzoros at Harvard Medical School and Beth Israel Deaconess Medical Center (Boston, Massachusetts, USA) and colleagues injected colon cancer cells into mice and then randomized the animals into two groups: a control group, and a group that consumed ground walnuts equivalent to two servings per day for humans.

After 25 days, the colorectal tumors of walnut-fed mice contained significantly higher amounts of ALA, DHA, and EPA, and 10 times the amount of total omega-3 fatty acids, compared to the control group. The walnut-consuming mice also had smaller tumors than mice in the control group. A smaller tumor size correlated with a higher percentage of omega-3s in the tumor.

Next, the researchers examined microRNA expression in tumor tissues from the mice. MicroRNAs (miRNAs) are small, noncoding RNAs that regulate gene expression. MicroRNAs can affect tumor growth by influencing processes such as inflammation, vascularization, proliferation, and apoptosis. The team found decreased expression of miRNAs 1903, 467c, and 3068 and increased expression of miRNA 297a* in the tumors of the walnut-fed group compared to those on the control diet. Although the relevance of these miRNAs to colon cancer currently is unknown, changes in their levels may affect gene transcripts involved in tumor growth.

It remains to be seen whether similar effects take place when people with colorectal tumors eat walnuts. If so, researchers may be able to develop new strategies for colorectal cancer diagnosis, prevention, and treatment.

This study was funded by the American Institute for Cancer Research and the California Walnut Commission.
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Chinese scientists produce beef high in omega-3s

Omega-3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) can help protect against obesity, cardiovascular disease, and neurodegenerative disorders. However, many people do not consume enough omega-3s in their diet to reap these benefits. Now a team of researchers has cloned the first cattle with beef rich in omega-3s, which could someday help people boost their dietary intake of the beneficial fatty acids (Biotechnology Letters, http://doi.org/10.1007/s10529-015-1827-z, 2015).

Fish are the best dietary sources of long-chain omega-3 fatty acids. In contrast, beef contains very few omega-3s but is higher in omega-6 polyunsaturated fatty acids, such as linoleic acid. An increased ratio of omega-6 to omega-3 polyunsaturated fatty acids in the diet has been linked to the development and progression of various diseases. An enzyme called fatty acid desaturase (FAD3) can convert omega-6s to omega-3s; however, humans, cattle, and other vertebrates lack this enzyme.

So Gong Cheng, Linsen Zan, and coworkers from Northwest A&F University in Yangling, China, used transgenic technology to introduce a gene encoding FAD3 (known as fat1) from the nematode Caenorhabditis elegans into a Chinese breed of cattle called Luxi Yellow. The team first modified the fat1 gene so that it could be expressed in mammalian cells, and then introduced the gene to fetal calf fibroblast cells. Using a technique called somatic cell nuclear transfer, the researchers transferred the modified fibroblasts into enucleated cow oocytes and stimulated the reconstructed embryos to develop. Finally, they implanted the embryos into Qinchuan cattle surrogates.

From 94 implanted embryos, 20 calves were obtained by caesarean section. Fourteen
of these calves tested positive for the fat1 gene, as determined by polymerase chain reaction. The researchers found that the fat1 gene was expressed in the liver, muscle, kidney, and lung of a transgenic-positive calf, but not in a transgenic-negative calf. When the team analyzed the fatty acid profiles of muscle tissue from some of the calves, they discovered that the total level of omega-3s increased by 2- to 5-fold in four transgenic calves compared to a nontransgenic control. The omega-6:omega-3 ratio decreased from 5.33:1 in a non-transgenic calf to 0.95:1 in a transgenic calf. Of the 20 calves obtained by embryo transfer, only three stayed alive with no obvious abnormalities. The other 17 died shortly after birth or sometime in the next 111 days. Two of the calves that remained alive were transgenic; one was nontransgenic. Autopsies of the 17 dead calves showed that inflammation and a type of bacterial infection called hemorrhagic septicemia were the main causes of death. According to the researchers, a high rate of neonatal mortality is a major problem for livestock produced by somatic cell nuclear transfer and may result from incomplete reprogramming of the somatic cells (in this case, fibroblasts) or from abnormal epigenetic modification of their DNA.

“There is much to learn about the best scientific techniques and the best husbandry required to make beef a rich animal source of omega-3 oils for human nutrition, but we have taken the first step,” says Cheng.

The intuitive appeal of GMO opposition

Why do many people oppose genetically modified organisms (GMOs), despite abundant evidence that GM crops are a safe and sustainable way to feed the world’s growing population?

The answer, according to a team of Belgian philosophers and plant scientists, lies in people’s intuitive expectations about the world (Trends in Plant Science, http://dx.doi.org/10.1016/j.tplants.2015.03.011, 2015).

Research has shown that GM crops do not pose specific health or environment risks, but can instead increase land productivity and bring financial benefits to local farmers. However, anti-GMO sentiment remains strong, particularly in Europe. “The discrepancy between public opinion and the scientific evidence requires an explanation,” write the authors, led by Stefaan Blancke at Ghent University, in Belgium.

The researchers take a cognitive approach to account for opposition to GMOs. “We argue that intuitions and emotions make the mind highly susceptible to particular negative representations of GMOs,” they write. When confronted with complex situations such as biotechnology, many people revert to intuition rather than reasoning. They may lack the time, resources, or desire to gain an understanding of the science behind GMOs, so they prefer easily understandable representations of GMOs that are in line with their intuitive expectations.

One intuitive preference is known as essentialism: the idea that organisms have an unchangeable core that determines their identity. DNA is considered the essence of the organism; therefore, putting a gene from one organism into another violates species boundaries and produces abnormal “Frankenfood.” For example, people may erroneously believe that putting a single fish gene into a tomato will make the tomato taste like fish.

Teleological and intentional intuitions can also influence people’s perceptions of GMOs. These intuitions may stem from religious beliefs or from a secular belief in the wisdom and inherent order of “Mother Nature.” They cause people to view genetic engineering as “playing God” or “against nature.”

AOCs MEETING WATCH


October 4–6, 2015. 25th Canadian Conference on Fats and Oils, co-organized by the Canadian section of the American Oil Chemists’ Society and the Consortium for Research and Innovation in Industrial Bioprocesses, Delta Hotel Québec, Québec City, Québec, Canada. http://tinyurl.com/CAOCS-meeting


May 1–4, 2016. 107th AOCs Annual Meeting & Expo, Calvin L. Rampton Salt Palace Convention Center, Salt Lake City, Utah, USA.

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Finally, emotions, particularly disgust, can interfere with people’s rational assessment of GMOs. They view gene modification as contamination, rendering the organism toxic and unfit for consumption. Not only do GM foods elicit disgust, but so do their producers and developers. Anti-GMO activists label such corporations as greedy, immoral, or exploitative of farmers.

The researchers acknowledge that there may be legitimate concerns about particular GM crops, but they need to be evaluated on a case-by-case basis, rather than condemning the entire technology. They say that education on the benefits of GMOs (for example, alleviating poverty and hunger in developing nations, or allowing farmers to produce more food on less land) can, to some extent, combat the intuitive appeal of GMO opposition.

Sweet potato is natural GMO

Anti-GMO activists may have one less talking point in their toolbox: the contention that transgenic food crops are “unnatural.” According to a recent paper in the Proceedings of the National Academy of Sciences USA, one of the most widely consumed food crops in the world, the sweet potato, is naturally transgenic (http://dx.doi.org/10.1073/pnas.1419685112, 2015).

Sweet potato (genus *Ipomoea*) is one of the oldest domesticated crops in the Americas. Archeological evidence from Peru suggests that humans have consumed the root vegetable for thousands of years. In the course of analyzing nucleic acid sequences from the cultivated sweet potato *Ipomoea batatas*, researchers led by Jan F. Krueze at the International Potato Center in Lima, Peru, discovered DNA sequences from a genus of bacteria called *Agrobacterium* within the sweet potato’s genome. *Agrobacterium* is well known for its ability to transfer its own DNA to plants, both in the wild and in the lab, and has therefore been used as a tool for the genetic engineering of crops. However, this is the first time that researchers have detected *Agrobacterium* DNA in a naturally occurring food crop.

The *Agrobacterium* DNA sequences embedded in the sweet potato’s genome encode several bacterial genes. Interestingly, these genes are expressed at low but detectable levels in multiple tissues of the sweet potato plant, including the leaf, stem, and root. The researchers currently do not know what evolutionary benefits, if any, the bacterial genes provided to the sweet potato. The team detected *Agrobacterium* genes in 291 sweet potato cultivars and also in several wild species.

These data provide evidence of an ancient gene transfer from an *Agrobacterium* to an ancestor of the modern sweet potato, the researchers say. Because the bacterial genes were present in all of the domesticated sweet potato plants tested, the researchers hypothesize that one or more of the transferred genes may have contributed to a beneficial trait that breeders selected for during sweet potato domestication thousands of years ago. “Our finding, that sweet potato is naturally transgenic while being a widely and traditionally consumed food crop, could affect the current consumer distrust of the safety of transgenic food crops,” the researchers write. ■
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Research that shows how to improve surfactant formulations for enhanced oil recovery has been recognized as the best paper of 2014 in AOCS’ Journal of Surfactants and Detergents (JSD). The American Cleaning Institute (ACI) presented the Distinguished Paper Award to senior researcher Jean-Louis Salager, former editor-in-chief of JSD, and his co-authors from the University of the Andes (Mérida, Venezuela) at the 106th AOCS Annual Meeting and Industry Showcases, held in Orlando, Florida, May 3–6, 2015. In a process known as chemical enhanced oil recovery, surfactant formulations are injected into oilfields, helping to free petroleum trapped in porous rock. Salager’s paper provides straightforward guidelines that could simplify how surfactants are used in chemical enhanced oil recovery (http://dx.doi.org/10.1007/s11743-013-1534-5, 2014).

Croda International (East Yorkshire, United Kingdom) has begun construction on a $170-million facility that will produce 100% sustainable nonionic surfactants from bioethanol. The new plant will be located near Croda’s existing Atlas Point facility in New Castle, Delaware, USA. The nonionic surfactants produced at the plant could be used in a broad range of personal care and household products, such as face cream, toothpaste, laundry detergent, and paint. The facility will begin production in 2017.

The Danish Environmental Protection Agency (EPA) has called for an EU-wide ban of methylisothiazolinone (MI) in personal care products because of the preservative’s potential to cause allergies. A powerful synthetic biocide, MI is widely used in personal care products such as shampoos and sunscreens. A new report by the Danish EPA evaluated concentrations of MI in personal care products on the Danish market, finding that consumers may be exposed to levels of the preservative that could cause allergies. In 2013, Cosmetics Europe advised members to stop using MI in leave-on skin products, and in 2014, the European Commission’s Scientific Committee on Consumer Safety issued an opinion that no concentration of MI in leave-on cosmetics is safe to prevent contact allergies.

Cleaner clothes at lower wash temperatures

Reducing the wash temperature during laundering could lower energy consumption by more than 65%. However, effectively removing stains—especially oily stains—from fabrics in cold water is challenging. Two oral presentations at the 106th AOCS Annual Meeting and Industry Showcases, held May 3–6, 2015, in Orlando, Florida, USA, addressed this important topic.

David A. Sabatini from the University of Oklahoma (Norman, USA) discussed optimized microemulsion systems to remove vegetable oils from fabrics at low temperatures. Vegetable oils are difficult to remove because of their hydrophobicity and bulky triglyceride structure. Surfactants can displace oily stains from fabrics into the wash water, but most work best at higher temperatures (around 40°C). A relatively new class of surfactant called extended surfactants has shown promise for removing oily stains at lower temperatures (25°C), but many of them require high salinity (4–14% sodium chloride), limiting their use in practical applications.

Extended surfactants have intermediate-polarity polypropylene oxide and/or polyethylene oxide groups inserted between the head and tail of the surfactant molecule. This chemical structure allows the surfactant tail to extend further into the oil phase of the emulsion droplet without compromising the droplet’s water solubility, providing a smoother transition between the oil and water phases. Extended surfactants can form microemulsions with very low interfacial tension between the wash solution and the oily soil, easing stain removal.

Using an extended surfactant in a mixture with a conventional surfactant (e.g., sodium dioctyl disulfosuccinate; also known as AOT)
can speed microemulsion formation and reduce the salinity requirement. In previous work, Sabatini’s group showed that a mixture of AOT and an anionic extended surfactant (C<sub>10</sub>–18PO–2EO NaSO₄) removed more than 90% of canola, jojoba, coconut, and palm kernel oils from fabric at temperatures above the oils’ melting points (−10, 9.7, 24.4, and 28.6°C, respectively), at a salinity of only 0.5% sodium chloride (Journal of Surfactants and Detergents, http://dx.doi.org/10.1007/s11743-014-1659-1, 2015). Detergency performance decreased at temperatures below the oils’ melting points, but was still superior to that of a commercial laundry detergent. However, relatively high concentrations of total surfactant (1000 ppm) were required.

In new work presented at the meeting, Sabatini found that another extended surfactant, C<sub>14</sub>–15-(PO<sub>3</sub>)₄–sulfate, in the absence of AOT or other surfactants, had greater than 90% detergency with canola oil at 25°C, 4% NaCl, and a surfactant concentration as low as 62.5 ppm. At 1% NaCl and 250 ppm surfactant, the extended surfactant performed better than a commercial laundry detergent at both 10 and 25°C. The researchers also found that calcium chloride was a more effective salt than sodium chloride, achieving greater than 90% detergency for canola oil at 0.025% CaCl₂, 250 ppm surfactant, and 25°C. At 10°C, the detergency decreased to about 85%. A CaCl₂ concentration of 0.025% corresponds to naturally occurring hard water, reducing or eliminating the need for added salt.

In another presentation, Ashish Taneja from BASF (Wyandotte, Michigan, USA) discussed strategies developed by his colleague Keith Gutowski to improve laundry cleaning performance at lower wash temperatures. According to Gutowski and Taneja, achieving superior performance at lower wash temperatures is highly challenging. Although cold-water detergents have been on the market since 2005, consumers haven’t widely accepted them because of performance issues.

To be effective, the detergent formulation must easily dissolve in cold water. In addition, the detergent must effectively remove particulates and oily soils from fabrics, as well as complex, organic food soils and stains. Oily soils are particularly challenging to remove in cold water because large triglycerides are not readily solubilized, especially from hydrophobic fabrics.

BASF has optimized a surfactant system for cold water. A mixture of branched non-ionic surfactants, BASF Lutensol XP40 and Lutensol CS6250, outperforms other cold water detergents. However, the high nonionic content of the surfactants in Unit Dose systems make them take longer to dissolve in cold water detergent. The polymer additive polyethyleneimine ethoxylate (PEIE) breaks the gel structure of undissolved clumps of detergent, providing a 30–50% reduction in cold-water dissolution time.

**Biobased surfactants roundup**

Biobased surfactants are attracting increased interest as sustainable, biodegradable alternatives to conventional petroleum-based surfactants. Doris de Guzman, author of the Green Chemicals Blog (http://greenchemicalsblog.com), posted a “Biobased surfactants roundup” on May 7, 2015, and an addendum on sophorolipids on May 8, which summarizes current producers and developers of the microbial-derived surfactants.

Chemical structures of the two best-known classes of biosurfactants, rhamnolipids (left) and sophorolipids (free acid form, right). *Inform*, April 2015, vol. 26 (4), p. 208

**RHAMNOLIPIDS**

Rhamnolipids, which consist of one or two rhamnose sugars covalently linked to two fatty acids, are naturally produced by *Pseudomonas* spp. and related gram-negative bacteria. These surfactants are oil-in-water emulsifying agents that reduce surface and interfacial tension, as well as conferring anti-aging and antimicrobial benefits.

Rhamnolipids are more expensive than petroleum-derived surfactants or sophorolipids (see below) because they are challenging to produce and purify at an industrial scale. According to de Guzman, market prices for rhamnolipids are $200–350 for 10 mg, at purities of 95–98%. “Rhamnolipid from China is around $11/kg for purity between 40% and 85% in barrel batch quantities, according to sources,” she writes.

Logos Technologies (Fairfax, Virginia, USA) produces its NatSurFact line of rhamnolipid-based surfactants by microbial fermentation using natural oil for feedstock. The company produces several grades of NatSurFact and can tailor them to applications in personal care, household cleaners, and chemical enhanced oil recovery. According to Logos Technologies, the rhamnolipids have high activity compared to conventional surfactants, with only milligrams per liter of rhamnolipids required to make water “soapy.” The company currently is producing rhamnolipids at a pilot scale of about 1 kg per week and is exploring partnering options to bring NatSurFact to market.

Like Logos Technologies, GlycoSurf (Park City, Utah, USA) produces rhamnolipids that are more than 95% pure. However, GlycoSurf makes rhamnolipids by chemical synthesis with rhamnose sugar as feedstock, rather than by microbial fermentation. GlycoSurf can attach different lipids to the sugar, tailoring the surfactant to customers’ needs. The company has been producing 1-kg batches, mostly for customer testing at the R&D level in anti-aging creams and sunscreens.

Other producers or developers of rhamnolipids include AGAE Technologies (Corvallis, Oregon, USA), Rhamnolipid Companies (St. Petersburg, Florida, USA), TensioGreen (Covina, California, USA), and Daqing Victex Chemical Co. (Daqing City, China).

**SOPHOROLIPIDS**

Sephorolipids consist of a sophorose sugar head group covalently linked to a hydrophobic fatty acid tail. These CONTINUED ON NEXT PAGE 461
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Attended the Annual Meeting
Imagine receiving a certified letter from the US Environmental Protection Agency (EPA) announcing that it plans to conduct an audit of your company’s facility in two weeks. The audit will focus on your company’s compliance obligations as a chemical manufacturer under the Toxic Substances Control Act (TSCA). Would you be prepared or are you unsure of what TSCA is and whether it applies to you?

Moving from bench to market
Many small companies or those new to chemical manufacturing are not aware of their obligations under TSCA. Most biobased chemical companies have likely operated under the research and development (R&D) exemption under TSCA Section 5(h) (and maintained all required paperwork). But as soon as the manufacturer is ready for a commercial launch, the R&D exemption no longer applies. To avoid hefty fines of up to $37,500 per day per violation, it is critical for companies to understand their obligations under TSCA Section 5 not only to avoid inadvertent violations, but also to avoid any undue delays in bringing their products to market.

A chemical manufacturer or importer of a substance for a TSCA use (that is, everything but a food, food additive, drug, cosmetic, medical device, pesticide, tobacco product, firearm, or nuclear source material) must comply with all aspects of TSCA. This article focuses on the rules related to chemical nomenclature, including how those rules could adversely impact a company’s ability to market new bio-based substances.

A company must ensure that any chemical substance it manufactures (or imports) is listed on the TSCA Chemical Substance Inventory (the Inventory) or be eligible for an exemption. If a substance is not listed on the Inventory (either as a public or confidential identity), the manufacturer must submit a premanufacture notice (PMN) to EPA 90 days prior to producing or importing that substance. TSCA applies to feedstocks, intermediates, microorganisms, enzymes, and other catalysts, in addition to final products.

The importance of identity
The first step in determining Inventory status is determining the appropriate Chemical Abstracts Index name for the substance. Note that the existence of a Chemical Abstracts Index name or Chemical Abstracts Service (CAS) Registry Number does not mean that a substance is listed on the Inventory. In fact, many CAS identities do not comport with the TSCA nomenclature rules.

For a single, defined substance (what EPA calls a Class 1 chemical), such as ethanol, the identity is straightforward.
and a search of the Inventory can easily reveal if a substance is listed and if there are any restrictions to its manufacture, processing, or use. Like many petroleum substances, many biobased substances are not single, defined substances. They are considered unknown or variable composition, complex reaction products, or biological materials (UVCB), or Class 2 chemicals. UVCB substances are typically identified by source and/or process and may include a definition in addition to the substance name. Triglyceride oils provide an instructive example of how the source is included in the substance identity.

Corn oil is listed on the Inventory as:

**Corn oil.**

**Definition:** Extractives and their physically modified derivatives. It consists primarily of the glycerides of the fatty acids linoleic, oleic, palmitic and stearic. (*Zea mays*). (CAS registry number 8001-30-7).

It is a distinct substance from other vegetable oils, such as:

**Soybean oil.**

**Definition:** Extractives and their physically modified derivatives. It consists primarily of the glycerides of the fatty acids linoleic, oleic, palmitic and stearic. (*Soja hispida*). (CAS registry number is 8001-22-7).

The definitions of these two oils are the same, except for the source names: *Zea mays* and *Soja hispida*. Even though the two oils have very similar fatty acid profiles and content, and are often used interchangeably, the different source designations mean that these are two different substances under TSCA. A manufacturer of one could not rely on the identity of the other for TSCA purposes. The source-based name may also extend into a downstream product. For example, a fatty acid methyl ester (FAME) biodiesel made by the transesterification of corn oil with methanol would be:

**Fatty acids, corn-oil, Me esters**

(CAS registry number 515152-40-6),

while the soy FAME would be:

**Soybean oil, Me ester**

(CAS registry number 67784-80-9).

These two identities are distinct and a biodiesel producer would have to be sure that the corresponding FAMEs were listed on the Inventory before making biodiesel from either corn or soybean oil.

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**TSCA reform gaining momentum**

Reform of the US Toxic Substances Control Act (TSCA), which has never been updated in the 39 years since it was passed in 1976, has limped along for years without meaningful action. Recent legislative activity, however, suggests reform may yet happen.

As *Inform* went to press, signs of renewed life included a unanimous vote on May 15 by the House Subcommittee on Environment and the Economy to send a revised draft bill (the TSCA Modernization Act of 2015) for full committee consideration. A similar bill was approved after modification by the Senate Environment and Public Works Committee in late April. Known as The Frank R. Lautenberg Chemical Safety for the 21st Century Act—named for the late US senator who championed TSCA reform for years—the bill has picked up a number of bipartisan sponsors, suggesting that a floor vote may be called soon. If the bill passes the Senate, it will move to a joint conference committee where the two versions (assuming the House passes its bill) will have to be reconciled. Then the conference report would go back to both chambers for new votes, after which it would have to be signed by the President. All of which is to say, reform is anything but a done deal.

Industry generally is in favor of both bills. Ernie Rosenberg, president of the American Cleaning Institute (ACI), noted in a statement, “Along with the progress on bipartisan legislation in the Senate, action in the House sets us further on the path to passing a more effective law to govern chemicals in commerce.

“A stronger federal chemical law should reflect progress in science and technology and advance further innovations. A well designed, updated law can further enable our industry’s ongoing work to develop . . . more sustainable cleaning products.”

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**Feedstock flexibility**

Clearly this source-based nomenclature system for UVCB substances is complicated. In fact, EPA and industry recognized that this nomenclature system would be a barrier to manufacturers that use a variety of oil sources to produce derivatives, such as surfactants. EPA and the Soap and Detergent Association (SDA) (now the American Cleaning Institute) developed a source-agnostic nomenclature system based on alkyl ranges and substance type. The SDA nomenclature procedure covers 35 natural sources of fats and oils and their synthetic (i.e., petroleum) equivalents.
For example, either corn oil fatty acids or soybean oil fatty acids could be identified as:

**Fatty acids, C16-18 and C18-unsatd.**

**Definition:** This substance is identified by SDA Substance Name: C16-C18 and C18 unsaturated alkyl carboxylic acid and SDA Reporting Number: 11-005-00. Consult SDA Substance Identification Procedure.

(CAS registry number 67701-08-0).

These SDA names allow feedstock flexibility all along the supply chain: from triglyceride producers, to intermediate fatty acids, to final products, such as fatty acid ethoxylates. For example,

**Fatty acids, C16-18 and C18-unsatd., ethoxylated.**

**Definition:** This substance is identified by SDA Substance Name: C16-C18 and C18 unsaturated alkyl carboxylic acid ethoxylate and SDA Reporting Number: 11-017-00. Consult SDA Substance Identification Procedure

(CAS registry number 68989-58-2)

could be produced from fatty acids derived from any of the 13 vegetable oils listed in the SDA Substance Identification Procedures [2] that are identified as predominantly “C16-18 and C18-unsaturated.”

**Feedstock Inflexibility**

This long-standing nomenclature system has provided manufacturers substantial feedstock flexibility while relieving EPA of the burden of reviewing hundreds, if not thousands, of substances that have nearly identical properties, but differ only in the original plant source—but only if the plant source is one of the original SDA species. The SDA nomenclature specifically states: “Alkyl groups derived from other natural sources are not covered by this procedure.” This statement is especially problematic for companies that have developed triglycerides from other natural sources. For example, oils from jatropha or algae may match existing alkyl ranges, but because they are not on the list of fat and oil sources in the SDA Substance Identification Procedures, they are not eligible to be named using SDA nomenclature. This presents a burden on the oil producers, as they must file a PMN, and, perhaps more critically, it also presents a burden on customers looking to replace one of the SDA-eligible oils or other triglyceride sources, since they also must submit a PMN for their downstream product.

For example:

- A producer of an algal oil that is not listed on the Inventory would file a PMN for the triglyceride.
- That company’s customer plans to saponify the algal oil to make algal oil fatty acids and would be required to file a PMN for the algal fatty acids.
- Buyers of the fatty acids must file PMNs for each of the products they plan to manufacture, such as the sodium salt (to make an algae-based soap), methyl ester (algal biodiesel), ethoxylate (an algae-based detergent), and a polymer with neopentyl glycol and adipic acid (an algal-based polyester polyol).

**Call to action**

The SDA nomenclature system was specifically developed to reduce the reporting burden on industry in cases where additional information is unlikely to improve EPA’s ability to protect human health and the environment. Assuming that 1. EPA’s review of the algal oil finds no unreasonable risk to human health or the environment for the oil itself, and 2. its fatty acid profile fits one of the SDA categories, EPA should have the discretion to allow the downstream products from the algal source to use the SDA nomenclature, and avoid submissions of PMNs for downstream products. In order to do this, the rules must be changed. Opening the SDA nomenclature system to other organisms would either require statutory changes in TSCA or for EPA
to undertake rulemaking. Fortunately, TSCA reform is on Congress’s agenda in 2015, giving the biobased chemical industry a rare opportunity to update the nomenclature rules to level the playing field between legacy sources and new products. Even so, final legislation and implementing regulations are far off. Industry should convince EPA of the need to undertake rulemaking now in order to allow new oil sources to use SDA nomenclature once the new oils have been reviewed to allow the new oils to be smoothly integrated into the existing fatty acid supply chains. It is the proverbial win-win scenario—EPA has fewer PMNs to review without compromising its mission to protect human health and the environment; the innovative biobased products can move more seamlessly into the supply chain; and the increase in biobased/renewable chemicals benefits the global population as a whole.

Rich Engler is a senior policy advisor with Bergeson & Campbell, P.C. and The Acta Group. Previously, he worked for 17 years at the US Environmental Protection Agency (EPA), where he was a staff scientist in the Office of Pollution Prevention and Toxics. At EPA, he reviewed numerous chemicals under TSCA, led the Green Chemistry Program, including the Presidential Green Chemistry Challenge, and worked on many other projects, including the Risk-Screening Environmental Indicators, and Trash-Free Waters. Prior to joining EPA, Rich taught organic chemistry at the University of San Diego. He earned a Ph.D. in physical organic chemistry from the University of California, San Diego, USA.

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Feijoa, lúcuma, and goldenberry: novel fruit flavors from South America

Latin America Update is a regular Inform column that features information about fats, oils, and related materials in that region.

Leslie Kleiner

If there is a universal message regarding good food, it is that good food involves appealing flavors. Since we belong to a global community and many of us have grown up in culturally rich environments, it is natural to look for flavors that give us worldly experiences. In this article, I interviewed Pamela Oscarson, market and consumer insight lead at FONA International, Inc., to understand emerging flavors from South America and their application to the Latin American and US markets.

Oscarson pointed to three fruits: Feijoa fruit (*Feijoa sellowiana Berg* or *Acca sellowiana*), lúcuma (*Pouteria obovata*), and goldenberry (*Physalis peruviana* L.). Feijoa fruit (also called pineapple-guava) has a sweet-acidic and aromatic flavor, and it is the fruit of a perennial tree of the *Myrtaceae* family. Brazil is the natural habitat for Feijoa growth, but this tree is also grown in other South American countries, such as Paraguay and Argentina [1]. Lúcuma is a fruit with a tropical and exotic flavor that originates from the valleys of Perú, Ecuador, Colombia, and Chile. Lúcuma’s unique flavor makes it of interest not only in its original form, but for its pulp and flour, which can be used in many baked products and desserts [2]. Goldenberry (also called Cape-gooseberry) is a perennial semi-shrub found in subtropical regions, and its fruit has been found to have nutritional and bioactive components of interest [3]. I recently asked Oscarson about the current market for these fruit flavors, and their forecasted trends. Here is what she had to say.
Q: HOW ARE FLAVORS FROM SOUTH AMERICA, WHICH MAY BE OF INTEREST TO THE AMERICAN MARKET, DETERMINED?

A: Assertions about flavor trends can sometimes seem heavy on the intangibles and light on the data. To fill the gap between theory and fact, FONA developed Flavor Radar®, a flavor mapping methodology that trends flavors from novel idea to everyday pantry staple. Flavor Radar® is based on current data points from industry-renowned databases, and in-depth analysis on how a flavor trend is affecting the food and beverage industry. We are currently monitoring flavors from across the globe and how they can translate into trends from region to region. Novel flavors from Latin America that are on our flavor watch list include: feijoa, lúcuma, and golden berry.

Q: IN WHICH APPLICATIONS DO YOU FORESEE THESE FLAVORS BECOMING IMPLEMENTED IN THE US MARKET?

A: Taking a closer look at flavor trends in Latin America, we are seeing that food and beverage new product introductions have increased by 40% from 2010–2014, according to Mintel GNPD. The top growing product categories include baby snacks and juices, bean-based snacks, vegetable snacks, and rice snacks. We expect some of these categories to incorporate these novel flavors.

Q: WHAT ARE THE TRADITIONAL USES FOR FEIJOA, LÚCUMA, AND GOLDENBERRY, AND WHERE ARE THESE FLAVORS CURRENTLY IMPLEMENTED?

A: Feijoa is a fruit of edible flesh and seeds (the skin should not be eaten raw), and it is traditionally used as an ingredient in a variety of dishes, such as fruit salads, puddings, pies, jams, and jellies. We are seeing that 70% of all new feijoa flavored products were introduced in Asia Pacific, with top product categories being alcoholic beverages and juice drinks. At this time, only one product is available in the United States: New Belgium’s Lips of Faith series, Heavenly Feijoa Tripel Ale, which is brewed with Feijoa and Hibiscus.

Further reading


Lucuma, has long been used as a sweetener and a flavoring agent for foods such as ice cream. Widely available in powder form, lúcuma is often touted for its butterscotch flavor with sweet-brown undertones, as well as its rich source of nutrients. We see that 59% of all new product introductions in Latin America have lúcuma flavor, with top product categories being dairy-based frozen drinks, yogurt, and baked goods. North America accounts for 5% of all new products, with chocolate and beverage mixes as the top product categories.

Golden berry is often eaten raw like many other small berries, such as blueberry or raspberry. Because of its tangy sweetness and mouth-watering effect, goldenberry has been used as an excellent substitute for apples in pies and crumbles. In some cultures, such as India, goldenberries are commonly used to make jams and jellies. We are seeing that 44% of all new product introductions in Latin America have goldenberry flavor, with snack/cereal and energy bars as the top product category. North America accounts for 6% of all new products, with snack mixes the only product category.

As feijoa, lúcuma, and goldenberry flavors start permeating the US market, we look forward to tasting these South American fruits when shopping at the grocery store.
Fatty acid analysis before gas chromatography (abridged)

Today, fatty acids in natural or synthetic mixtures are measured and identified with gas chromatography (GC). A normal GC analysis can be completed in 30 minutes to an hour. High-speed GC can be done in minutes, while ultraspeed GC takes mere seconds to produce results.

Before gas-liquid chromatography equipment became commercially available, component acid analysis required about 15 working days and was conducted in three stages.

- Hydrolysis of the oil or fat and separation of the acids or their salts to fractions in which saturated, monounsaturated, and polyunsaturated acids were separately concentrated.
- Esterification of each subfraction to methyl esters and separation of these by chain length using fractional distillation under reduced pressure. Each ester fraction was weighed and its iodine value and saponification equivalent measured.
- Calculation of the results from these data and identification of major components by melting point and mixed melting point of solid acids or of solid derivatives of liquid acids.

This worked satisfactorily with oils containing only monoene and diene acids but additional procedures were necessary when the oil also contained linolenic acid. An older method involving reaction with thiocyanogen (CSN$_2$) was replaced by a more convenient method using alkali-isomerization. If an oil contained an acid of novel structure then additional procedures of isolation and degradation were required (i.e., the methods traditionally used by organic chemists at that time).

Saturated acids were frequently separated from unsaturated acids by lead salt crystallization. The acids and lead acetate were dissolved in hot ethanol and crystallized at around 15°C. The lead salts of saturated acids crystallize while the salts of unsaturated acids remain in solution. The solid and liquid phases are separated by filtration and the acids regenerated from each fraction. Typical results are shown in Table 1. An alternative procedure of crystallizing lithium salts from acetone was used to separate salts of saturated and monounsaturated acids from those of polyunsaturated acids.

Another separation procedure developed during the 1940s was crystallization of the acids themselves from organic solvents (methanol, ether, petroleum ether, or acetone) at temperatures down to −70°C using solid carbon dioxide as an external refrigerant. The method could be varied in many ways through the selection of solvent and of crystallizing temperature. Generally, three fractions were obtained in saturated, monounsaturated, and polyunsaturated acids, respectively, as in the example of groundnut oil set out in Table 2.

Table 1. Lead salt separation of fatty acids from an animal fat (pig) and a vegetable fat (*Allanblaikia parviflora*).

<table>
<thead>
<tr>
<th></th>
<th>Solid acids (% wt)</th>
<th>Liquid acids (% wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sat.</td>
<td>Unsat.</td>
</tr>
<tr>
<td>Animal</td>
<td>43.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Vegetable</td>
<td>53.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The balance is unsaponifiable material.
with a Perkin triangle, which allows collection tubes to be removed without losing the vacuum in the distillation column. The number of fractions (and the distillation residue) would be anything between five and 20 for each distillation. Each distilled fraction was weighed and its iodine value and saponification equivalent measured.

An example of the calculations required is given for a fraction of 3.95 g, IV 10.2, and SE 295.4. The saponification equivalent (295.4) lies between those for methyl palmitate (270.4) and methyl stearate (298.5), and the fraction is considered to be a mixture of esters of C₁₆ and C₁₈ acids on the assumption that the fractionation column can separate esters of C₁₄ acids from esters of C₁₈ acids and esters of C₁₆ from C₂₀ acids. The iodine value indicates the presence of an unsaturated component, which is considered to be methyl oleate. This can be calculated from the equation:

\[(\text{wt of oleate}) \times (\text{IV of oleate}) = (\text{wt of fraction}) \times (\text{IV of fraction})\]

i.e. \[\text{Ol} \times 85.6 = 3.05 \times 10.2\]
whence \[\text{Ol} = 0.36 \text{ g}\]

The remainder of the fraction is calculated as a mixture of methyl palmitate (P) and methyl stearate (St) by solving simultaneous equations based on (i) weight and (ii) saponification equivalent:

\[\frac{P + \text{St}}{270.4 + \text{St}/298.5} = \frac{3.05 + 0.36}{296.5}\]

Whence \[P = 0.28 \text{ g} \text{ and } \text{St} = 2.41 \text{ g}\]

Finally, the structures of the major acids had to be confirmed by the measurement of a melting point and a mixed melting point. For each of these, a distilled ester fraction rich in the acid to be identified was selected. Saturated acids were crystallized, oleic acid was oxidized with dilute alkaline potassium permanganate to erythro-9,10-dihydroxystearic acid, linoleic was brominated to give a 9,10,12,13-tetrabromostearic acid, and linolenic to a 9,10,12,13,15,16-hexabromostearic acid. When any of these checks failed, then a fatty acid of novel structure was present and a more complete investigation was needed. (The full version of this article is available at http://lipidlibrary.aocs.org/history/before-chrom/index.htm.)

Frank Gunstone, an AOCS Fellow since 1999, is the author or co-author of over 300 publications covering many aspects of lipid science. He has received the Lipid Award (AOCS, 1973); the Chevreul Medal (1990); the Normann Medal (1998); the Bailey Award (AOCS, 2005); and the Chang Award (AOCS, 2006). He can be reached at fdg1@st-andrews.ac.uk.

### Table 2 Separation of groundnut fatty acids into concentrates of saturated, monounsaturated, and polyunsaturated acids by crystallisation from acetone at (–50°C) and from diethyl ether at (–30°C).

<table>
<thead>
<tr>
<th>Composition (% wt)</th>
<th>Ether (–30°C)</th>
<th>Acetone (–50°C)</th>
<th>%</th>
<th>IV</th>
<th>Sat.</th>
<th>Mono.</th>
<th>Poly.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>insoluble</td>
<td>insoluble</td>
<td>21.3</td>
<td>3.3</td>
<td>94.4</td>
<td>5.1</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>soluble</td>
<td>insoluble</td>
<td>33.1</td>
<td>86.7</td>
<td>5.7</td>
<td>91.2</td>
<td>2.6</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>soluble</td>
<td>45.6</td>
<td>155.8</td>
<td>2.4</td>
<td>20.0</td>
<td>75.9</td>
</tr>
</tbody>
</table>

References

Enzyme-containing mini-emulsions
Hagenbucher, M., et al., Henkel and Co. KGaA AG, Max Planck Institutes, US883465, November 11, 2014

The present invention relates to a mini-emulsion which comprises at least one hydrolase, where the continuous phase of the mini-emulsion contains at least one oxidant, while the dispersed phase comprises at least one C6-60 carboxylic acid and optionally at least one reactant. Furthermore, the present invention relates to a method of preparing the mini-emulsion and to a process for the preparation of C6-60 percarboxylic acids and to a process for the preparation of an oxidized reactant, in each case using the abovementioned mini-emulsions.

Process for making a crystalline structurant comprising a molten hydrogenated castor oil

An oil-in-water emulsion comprising droplets of a molten hydrogenated castor oil, the droplets having a mean diameter of between 0.1 µm and 4 µm; and an anionic surfactant; and wherein the mean diameter is measured when the emulsion is at a temperature of between 85 °C and 95 °C.

Process for refining a triglyceride oil

A process for refining a triglyceride oil comprises: providing a triglyceride oil; bleaching the oil in the presence of an added antioxidant in a first bleaching step; bleaching the oil in a second bleaching step; and deodorizing the bleached oil, wherein the antioxidant comprises a rosemary extract.

Fatty acid dehydratases and uses thereof

The invention provides isolated nucleic acid molecules which encode novel fatty acid dehydratase family members. The invention also provides recombinant expression vectors containing dehydratase nucleic acid molecules, host cells into which the expression vectors have been introduced, and methods for large-scale production of long chain polyunsaturated fatty acids (LCPUFAs), e.g., SDA, EPA and DHA.

Peanut spread

Nut spreads having a stabilizer which is a blend of certain palm oil fraction stabilizers with certain cocoa butters and/or cocoa butter equivalents and/or replacements and/or extenders (with similar fatty acid profile to cocoa butter). When these fats are used in combination, particularly at ratios preferred herein, they impart a smooth creamy texture to the final nut butter or nut butter-based products and more importantly provide enhanced stability during storage, preventing oil separation in the final product during prolonged storage. The texture and spreadability of the final nut spread-based product achieved by using this blend is also superior to the texture and spreadability obtained by using conventional palm oil alone. Preferably the cocoa butters, equivalents, replacements and substitutes are not hydrogenated.

Process for producing dark chocolate
Bussmann, C., et al., Chocoladefabriken Lindt and Sprungli AG, US8906442, December 9, 2014

A process and a plant are proposed for producing dark chocolate which make it possible to produce dark chocolate, in particular also single-variety dark chocolate, having a high cocoa content without cocoa powder needing to be produced and/or added. By means of a partial defatting of the cocoa mass in a process step which is connected upstream of the mixing, grinding and/or conching, the fat content in the cocoa mass is reduced to the extent that dark chocolates can be produced having a high cocoa content without cocoa powder needing to be added. The partial defatting of the cocoa mass is preferably carried out using a decanter (50). The cocoa butter fraction (2) according to the invention occurs on decanting as a high-fat fraction which still contains a fraction of 17 to 21% bwt, a maximum of 30% bwt, of fat-free cocoa dry matter. In the novel process according to the present invention, the expenditure for producing a highly defatted cocoa powder is avoided, since the cocoa mass (11) is only defatted to the point that the mass produced therefrom can be comminuted without problem using rollers (30) and can subsequently be conched. The cocoa butter fraction is added during conching after the comminution in a ball mill.
Lipase-mediated lipid removal from propolis extract and its antiradical and antimicrobial activity


Propolis contains many antioxidants such as polyphenols and flavonoids. However, propolis-derived lipid components interrupt an efficient isolation of antioxidants from propolis extract. We examined the effectiveness of various lipase treatments for the removal of lipids from propolis extract and evaluated the biological features of the extract. Lipase OF and Novozyme 435 treatments did not reduce fatty acid level in propolis extract. However, Lipzyme TL IM-treated propolis extract showed a significant decrease in fatty acid level, suggesting the removal of lipids. Lipzyme RM IM also significantly decreased the fatty acid level of the extract, but was accompanied by the reduction of polyphenols and flavonoids, which are antioxidants. In Lipzyme TL IM treatment, an increase in active flavonoids, such as Artepillin C and kaempferide, was observed, with a slight increase of ferric reducing/antioxidant power (FRAP) radical-scavenging activity. In addition, antimicrobial activity towards skin health-related bacteria such as Staphylococcus epidermidis and Propionibacterium acnes was enhanced by Lipzyme TL IM treatment. Lipzyme TL IM treatment effectively removes lipids from propolis extract and enhances antibacterial activity. Therefore, we suggest that Lipzyme TL IM is a useful lipase for lipid removal of propolis extract.

Analysis of lipophilic and hydrophilic bioactive compounds content in sea buckthorn (Hippophaë rhamnoides L.) berries


The aim of this study was to determine selected phytochemicals in berries of eight sea buckthorn (Hippophaë rhamnoides subsp. mongolica) cultivars, including lipophilic and hydrophilic compounds. In the experiment chromatographic analyses, GC (phytosterols and fatty acids), UPLC-PDA-FL, LC-MS (polyphenols), and HPLC (L-ascorbic acid), as well spectrophotometric method (total carotenoids) were used. The lipid fraction isolated from whole fruit contained 14 phytosterols (major compounds β-sitosterol > 24-methylene cycloartenol > squalene) and 11 fatty acids in the order MUFAs > SFAs > PUFAs. Carotenoids occurred in concentrations between 6.19 and 23.91 mg/100 g fresh weight (fw) (p < 0.05). The major polyphenol group identified in berries was flavonols (mean content of 311.55 mg/100 g fw), with the structures of isorhamnetin (six compounds), quercetin (four compounds), and kaempferol (one compound) glycosides. Examined sea buckthorn cultivars were characterized also by a high content of L-ascorbic acid in a range from 52.86 to 130.97 mg/100 g fw (p < 0.05).

Effects of chemical and enzymatic modifications on starch–oleic acid complex formation


The solubility of starch-inclusion complexes affects the digestibility and bioavailability of the included molecules. Acetylation with two degrees of substitution, 0.041 (low) and 0.091 (high), combined without or with a β-amylase treatment was employed to improve the yield and solubility of the inclusion complex between debranched potato starch and oleic acid. Both soluble and insoluble complexes were recovered and analyzed for their degree of acetylation, complexation yields, molecular size distributions, X-ray diffraction patterns, and thermal properties. Acetylation significantly increased the amount of recovered soluble complexes as well as the complexed oleic acid in both soluble and insoluble complexes. High-acetylated debranched-only starch complexed the highest amount of oleic acid (38.0 mg/g) in the soluble complexes; low-acetylated starch with or without the β-amylase treatment resulted in the highest complexed oleic acid in the insoluble complexes (37.6–42.9 mg/g). All acetylated starches displayed the V-type X-ray pattern, and the melting temperature generally decreased with acetylation. The results indicate that starch acetylation with or without the β-amylase treatment can improve the formation and solubility of the starch–oleic acid complex.

Lipidomic changes of LDL in overweight and moderately hypercholesterolemic subjects taking phytosterol- and omega-3-supplemented milk


The benefits of dietary phytosterols (PhySs) and long-chain n-3 PUFA (ω3) have been linked to their effects as cholesterol- and triglyceride (TGL)-lowering agents. However, it remains unknown whether these compounds have further metabolic effects on LDL lipid composition. Here, we studied the effects of PhyS- or ω3-supplemented low-fat milk (milk) on the
LDL-lipidome. Overweight and moderately hypercholesterolemic subjects (n = 32) were enrolled in a two-arm longitudinal crossover study. Milk (250 ml/day), enriched with either 1.57 g PhSyS or 375 mg ω3 (EPA + DHA), was given to the participants during two sequential 28 day intervention periods. Compared with baseline, PhSyS-milk induced a higher reduction in the LDL cholesterol (LDLc) level than ω3-milk. LDL resistance to oxidation was significantly increased after intervention with PhSyS-milk. Changes in TGL and VLDL cholesterol were only evident after ω3-milk intake. Lipidomic analysis revealed a differential effect of the PhSyS- and ω3-milk interventions on the LDL lipid metabolite pattern. Content in LDL-glycerophospholipids was reduced after PhSyS-milk intake, with major changes in phosphatidylcholine (PC) and phosphatidylserine subclasses, whereas ω3-milk induced significant changes in the long-chain polyunsaturated cholesteryl esters and in the ratio PC36:5/lypoPC16:0, associated to a reduced inflammatory activity. In conclusion, daily intake of milk products containing PhSyS or ω3 supplements induce changes in the LDL-lipidome that indicate reduced inflammatory and atherogenic effects, beyond their LDLc- and TGL-lowering effects.

Plasma phospholipid very-long-chain saturated fatty acids and incident diabetes in older adults: the Cardiovascular Health Study


Circulating saturated fatty acids (SFAs) are integrated biomarkers of diet and metabolism which may influence the pathogenesis of diabetes. In epidemiologic studies, circulating levels of palmitic acid (16:0) are associated with diabetes; however, very-long-chain SFAs (VLSFAs), with 20 or more carbons, differ from palmitic acid in their biological activities, and little is known of the association of circulating VLSFAs with diabetes. By using data from the Cardiovascular Health Study, we examined the associations of plasma phospholipid VLSFA levels measured at baseline with subsequent incident diabetes. A total of 3179 older adults, with a mean age of 75 y at study baseline (1992–1993), were followed through 2011. We used multiple proportional hazards regression to examine the associations of arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0) with diabetes. Baseline levels of each VLSFA were cross-sectionally associated with lower triglycerides and LDL and increasing HDL.

Effects of hydroalcoholic extract of dill (Anethum graveolens) on the serum levels of blood lipids (cholesterol, triglycerides, LDL and HDL) in male NMRI mice


Increasing blood lipids, particularly cholesterol and triglyceride levels are dangerous factors causing cardiovascular disease and myocardial infarction in humans. The aim of this study was to investigate the effect of drinkable hydroalcoholic extract of dill on the serum cholesterol, Triglycerides, LDL and HDL levels. This study was conducted on 18 male NMRI mice. The mice were divided into 3 groups (n=6). The first group was given tap water as the control group. The second group, the extract at a dose of 250 mg / lit and the third group extract at a dose of 500 mg / lit were given in drinking water for 30 days. After the period of treatment under complete anesthesia, blood samples were taken from animals and serum cholesterol, triglycerides, LDL and HDL levels were measured. The result showed that consumption of hydroalcoholic extract of dill results in significant reducing of cholesterol, triglycerides and LDL in both extract - receiving groups compared to the control group (P<0.001). Also, the extract caused a significant increase in HDL levels in both treatment groups compared to the control group (P<0.001); meanwhile, these changes were dependent on the dill extract concentration. Results obtained in this study suggesting that administration of hydroalcoholic extract of dill via drinking water has effective concentration-dependent effect in decreasing cholesterol, triglycerides and LDL and increasing HDL.

Omega-3 and sleep: new insights from the DHA Oxford Learning and Behaviour (DOLAB) study


Does dietary intake of omega-3 DHA influence children’s sleep? Sleep problems affecting 40% of UK schoolchildren aged 7–9 years were reported in a recent University of Oxford study and lower blood concentrations of omega-3 DHA predicted more serious sleep problems in these otherwise healthy, normal children. In a randomised controlled trial, dietary supplementation with omega-3 DHA (docosahexaenoic acid) for 16 weeks
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Lipid-lowering and anti-inflammatory effects of palmitoleic acid: evidence from preclinical and epidemiological studies


In preclinical and human epidemiological studies, the monounsaturated fatty acid, cis-palmitoleic acid, has shown anti-inflammatory and lipid lowering effects linked to prevention of metabolic syndrome including cardiovascular disease and insulin resistance associated with diabetes and obesity. Randomised, double-blind, placebo-controlled trials are needed to clarify its role in these conditions.

Food sources of fat may clarify the inconsistent role of dietary fat intake for incidence of type 2 diabetes


Dietary fats could affect glucose metabolism and obesity development and, thereby, may have a crucial role in the cause of type 2 diabetes (T2D). Studies indicated that replacing saturated with unsaturated fats might be favorable, and plant foods might be a better choice than animal foods. Nevertheless, epidemiologic studies suggested that dairy foods are protective. We hypothesized that, by examining dietary fat and its food sources classified according to fat type and fat content, some clarification regarding the role of dietary fat in T2D incidence could be provided. A total of 26,930 individuals (61% women), aged 45–74 y, from the Malmö Diet and Cancer cohort were included in the study. Dietary data were collected by using a modified diet-history method. During 14 y of follow-up, 2860 incident T2D cases were identified. Total intake of high-fat dairy products (regular-fat alternatives) was inversely associated with incident T2D (HR for highest compared with lowest quintiles: 0.77; 95% CI: 0.68, 0.87; \( P \)-trend < 0.01). Decreased T2D risk at high intake of high- but not low-fat dairy products suggests that dairy fat partly could have contributed to previously observed protective associations between dairy intake and T2D. Meat intake was associated with increased risk independently of the fat content.

Fatty acid moieties have little effect on cholesterol-lowering potency of plant sterol esters


Plant sterol esters are the popular cholesterol-lowering dietary supplements. In order to understand the role of fatty acid moieties in the cholesterol-lowering activity of plant sterol esters, the present study was to: (i) compare the cholesterol-lowering activity of sterol esters of sunflower oil (SSF) with that of sterol esters of canola oil (SCO); and (ii) to systematically investigate the effect of SSF and SCO on the gene expression of sterol transporters, enzymes, receptors and proteins involved in cholesterol metabolism in hamsters fed a high cholesterol diet. GC analysis showed that SSF and SCO had similar sterol profile but they had different fatty acid moieties. Results showed both SSF and SCO could equally reduce plasma cholesterol in a dose-dependent manner. Both SSF and SCO could inhibit the cholesterol absorption and enhance the excretion of bile acids. In the similar ways, dietary SSF and SCO could up-regulate hepatic cholesterol-7α-hydroxylase (CYP7A1) and down-regulate intestinal sterol transporter Niemann-Pick C1-like 1 (NPC1L1), acyl coenzyme A: cholesterol acyltransferase 2 (ACAT2) and hepatic 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase). It was concluded that the fatty acid moieties had little effect on the cholesterol-lowering activity of SSF and SCO.

Functional monoesters of jojoba oil can be produced by enzymatic interesterification: reaction analysis and structural characterization


Long-chain monounsaturated fatty acid ethyl esters (LMFAEE) and fatty alcohol acetates (LMFAA) are important functional compounds in industry, and new sources are needed. In this study, jojoba oil was interesterified with ethyl acetate (EA) for the combined production of LMFAEE and LMFAA. Lipozyme RM IM and Novozym 435 were employed as the biocatalyst and their efficiencies were compared. Aliquot removal and subsequent \(^1\)H NMR analysis were used to monitor reaction progress and determine the interesterification yield (\( \text{IY}, \% \)). Optimization of this reaction was conducted for both lipases with respect to four experimental parameters: temperature, EA content, immobilized lipase load, and reaction time. The highest \( \text{IY} \) in the presence of Novozym 435 was 97%, which
was achieved after 9 hours of reaction at 37°C with 75 wt% EA and 5.00 wt% lipase load at 300 rpm, whereas the IY was 76% in the case of Lipzyme RM IM. The interesterified product was purified by preparative TLC and characterized by ¹H NMR, FT-IR and GC-MS, indicating the main components are ethyl cis-11-eicosenoate (44%), cis-11-eicosenyl acetate (25%) and cis-13-docosenyl acetate (22%). Novozym 435 exhibited excellent operational stability over 17 interesterification cycles with ca. 12% IY decrease.

Intake of farmed Atlantic salmon fed soybean oil increases hepatic levels of arachidonic acid-derived oxylipins and ceramides in mice


Introduction of vegetable ingredients in fish feed has affected the fatty acid composition in farmed Atlantic salmon (Salmo salar L.). Here we investigated how changes in fish feed affected the metabolism of mice fed diets containing fillets from such farmed salmon. We demonstrate that replacement of fish oil with rapeseed oil or soybean oil in fish feed had distinct spillover effects in mice fed western diets containing the salmon. A reduced ratio of n-3/n-6 polyunsaturated fatty acids in the fish feed, reflected in the salmon, and hence also in the mice diets, led to a selectively increased abundance of arachidonic acid in the phospholipid pool in the livers of the mice. This was accompanied by increased levels of hepatic ceramides and arachidonic acid-derived pro-inflammatory mediators and a reduced abundance of oxylipins derived from eicosapentaenoic acid and docosahexaenoic acid. These changes were associated with increased whole body insulin resistance and hepatic steatosis. Our data suggest that an increased ratio between n-6 and n-3-derived oxylipins may underlie the observed marked metabolic differences between mice fed the different types of farmed salmon. These findings underpin the need for carefully considering the type of oil used for feed production in relation to salmon farming.

Disruption of microalgal cells for lipid extraction through Fenton reaction: modeling of experiments and remarks on its effect on lipids composition


A novel cell disruption technique, based on the use of Fenton reaction, for the improvement of lipid extraction from microalgae has been recently proposed in the literature. The experimental results have shown that, when disruption is performed under suitable operating conditions, the amount of lipids subsequently extracted from Chlorella vulgaris is greatly increased with respect to the case where no pretreatment was performed. Specifically, the use of Fenton reactant leads to a corresponding increase of the extracted lipids from 6.9 to 17.4 %wt/wt. Moreover, it is observed that the treatment provokes a significant improvement of the quality of fatty acids methyl esters (FAMEs) obtained by transesterification of extracted lipids. These results, together with the extreme simplicity, low cost and energy consumption of the proposed technique, are very promising in view of its industrial transposition. To this aim, the use of suitable mathematical models might represent a valuable tool to design and control possible industrial size reactors. Along these lines, a simple but exhaustive model to quantitatively describe the effects of contact time and reactants concentration on the amount of lipids extractable from microalgae is proposed in this work. The model takes into account the effect of the OH• species on both cell wall breakage and lipid peroxidation phenomena. Model results and experimental data are successfully compared in terms of lipids extracted from wet microalgae previously subjected to the disruption treatment under different operating conditions.

INDUSTRIAL APPLICATIONS

A novel recovery process for lipids from microalgae for biodiesel production using a hydrated phosphonium ionic liquid


The use of a hydrated phosphonium ionic liquid, [P(CH₂OH)₄]Cl, for the extraction of microalgae lipids for biodiesel production, was evaluated using two microalgae species, Chlorella vulgaris and Nannochloropsis oculata. The ionic liquid extraction was compared to the conventional Soxhlet, and Bligh & Dyer, methods, giving the highest extraction efficiency in the case of C. vulgaris, at 8.1%. The extraction from N. oculata achieved the highest lipid yield for Bligh & Dyer (17.3%), while the ionic liquid extracted 12.8%. Nevertheless, the ionic liquid extraction showed high affinity to neutral/saponifiable lipids, resulting in the highest fatty acid methyl esters (FAMEs)-biodiesel yield (4.5%) for C. vulgaris. For N. oculata, the FAMEs yield of the ionic liquid and Bligh & Dyer extraction methods were similar (>8%), and much higher than for Soxhlet (<5%). The ionic liquid extraction proved especially suitable for lipid extraction from wet biomass, giving even higher extraction yields than from dry biomass, 14.9% and 12.8%, respectively (N. oculata). Remarkably, the overall yield of FAMEs was almost unchanged, 8.1% and 8.0%, for dry and wet biomass. The ionic liquid extraction process was also studied at ambient temperature, varying the extraction time, giving 75% of lipid and 93% of FAMEs recovery after thirty minutes, as compared to the extraction at 100 °C for one day. The recyclability study demonstrated that the ionic liquid was unchanged after treatment, and was successfully reused. The ionic liquid used is best described as [P(CH₂OH)₄]Cl·2H₂O, where the water is not free, but strongly bound to the ions.

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Furthermore, the possible chemical physical mechanisms underlying the improvement of FAMES composition, observed after the disruption treatment, is discussed. Finally, potential capabilities of the model to contribute to the industrial scale transposition of the proposed technique are presented.

Mannosylerythritol lipids: production and applications


Mannosylerythritol lipids (MELs) are a glycolipid class of biosurfactants produced by a variety of yeast and fungal strains that exhibit excellent interfacial and biochemical properties. MEL-producing fungi were identified using an efficient screening method for the glycolipid production and taxonomical classification on the basis of ribosomal RNA sequences. MEL production is limited primarily to the genus Pseudozyma, with significant variability among the MEL structures produced by each species. Outside of Pseudozyma, one recently isolated strain, Ustilago setiformis, has been shown to exhibit abundant MEL-B production from sugarcane juice. Structural analyses of these compounds suggest a role for MELs in numerous cosmetic applications. MELs act as effective topical moisturizers and can repair damaged hair. Furthermore, these compounds have been shown to exhibit both protective and healing activities, to activate fibroblasts and papilla cells, and to act as natural antioxidants. In this review, we provide a brief summary of MEL research over the past few decades, focusing on the identification of MEL-producing fungi, the structural characterization of MELs, the use of alternative compounds as a primary carbon source, and the use of these compounds in cosmetic applications.

Direct production of aviation fuels from microalgae lipids in water


In this contribution, we confirmed that aviation fuels could be synthesized directly from microalgae lipids in water over a Pt/C catalyst without additional hydrogen. After decarboxylation at 330 and 370 °C for 120 min, the oxygen content in the microalgae lipids was significantly reduced and the heating value of produced aviation fuels was greatly increased. The reaction mechanism of direct decarboxylation of microalgae lipids to aviation fuels was further investigated using each of the representative compounds in microalgae lipids, such as methyl laurate, methyl eicosanoate, methyl stearate, ethyl stearate, and tristearin as the starting material in separate reactions under the same conditions. Those reaction conditions, solvent, water loading, catalyst loading and reactant loading, were optimized. It was concluded that among the tested solvents, water was the most favorable for the selective decarboxylation of methyl stearate, that the catalytic decarboxylation rate of fatty acid esters with larger carbon numbers in water was faster than those with smaller carbon numbers, and that the Pt/C catalyst retained its activity through its third use. These results provide new insights for the direct decarboxylation of microalgae lipids to aviation fuels.

Steam explosion as a fractionation step in biofuel production from microalgae


In this study, various pretreatment methods (autoclaving, ultrasound, microwave and steam explosion) were compared to determine the most efficient method for the extraction of lipids from three different samples of microalgae (Nannochloropsis gaditana, Chlorella sorokiniana and Phaeodactylum tricornutum). Among the studied methods, steam explosion gave the highest lipid extraction yields for the three microalgae species. Therefore, the method was further studied and the application of acid catalysed steam explosion pretreatment was investigated for simultaneous lipid extraction and sugar release from microalgae. The effect of different variables, including temperature and acid concentration, was analysed. The experimental results demonstrate the efficacy and feasibility of the acid catalysed steam explosion pretreatment, followed by n-hexane lipid extraction. Remarkable sugar yields up to 96% were achieved under the pretreatment conditions of 1.7% sulphuric acid concentration and a temperature of 150 °C during steam explosion. Besides, this study verified high efficiencies in the extraction of lipid of exploded microalgae using n-hexane against the low efficiencies obtained for the untreated microalgae.

Extraction of natural compounds using supercritical CO₂: going from the laboratory to the industrial application


Despite industrial application for almost four decades, there is reluctance in some world regions to adopt supercritical (sc) CO₂ extraction because of the wrong perception that it is not fully competitive. To refute this misconception, this manuscript analyzes economics of scCO₂ extraction of vegetable oil from prepressed seeds. Selection of this application was due to the availability of a predictive mathematical model of the extraction process applicable for simulation purposes; inner microstructural changes of oilseeds during prepressing allow their extraction according to a shrinking core hypothesis. The predictive model has as its single parameter a particle-size and scCO₂-condition-independent microstructural mass transfer factor that can be best-fitted to laboratory extractions, existing literature correlations to estimate other model parameters, such as the axial dispersion in packed beds operating with supercritical fluids, and the solubility of vegetable oils in scCO₂. On the other hand, there is a need to correlate literature data for the film mass transfer coefficient to unveil the factors responsible for experimental data scattering. Because laboratory or pilot plant runs in single-extraction-vessel units cannot produce the simulated countercurrent contact in an industrial plant having ≥3 extraction vessels, mathematical simulation provides the relationship between oil yield and extraction.
time that can anchor precise estimations of extraction cost. Analysis of results unveiled differences in optimal extraction time (for minimal extraction cost) between production costs estimated in this work and the operational costs informed before. Because the operational cost does not include the capital cost of the industrial plant, the need appears to reduce its contribution to the total cost by increasing plant productivity. This is achieved reducing extraction time, which negatively influences oil yield. To make further progress in the optimization of industrial scCO₂ extraction processes, this manuscript proposes refining the mathematical simulation approach, and studying those technical constraints whose manifestations become more prevalent on scale-up. Mathematical simulation can be adapted to alternative, sample-pretreatment dependent mass transfer mechanisms in the solid matrix. It can be refined also to account for the size distribution of the substrate, radial changes in superficial scCO₂ velocity, axial changes in pressure, and radial/axial changes in temperature resulting from heterogeneous packing, pressure drop, and/or heat transfer from/to extraction vessel walls that may influence large-scale extractions. Large-scale experiments will allow studying these phenomena, as well as technical constraints to the decrease in particle size, increase in scCO₂ velocity, and decrease in extraction time imposed by the agglomeration and decrease in packed bed permeability of the substrate, and thermal effects during reconditioning of extraction vessels. The latter effects should be included as restrictions in the optimization of the extraction process, which may limit the extraction rate and the size or number of extraction vessel that impact economics positively. Close collaboration with industry will facilitate tackling large-scale problems, as well as refining estimates of plant cost as a function of its size and/or configuration.

Simultaneous treatment (cell disruption and lipid extraction) of wet microalgae using hydrodynamic cavitation for enhancing the lipid yield


Simultaneous treatment (combining with cell disruption and lipid extraction) using hydrodynamic cavitation (HC) was applied to Nannochloropsis salina to demonstrate a simple and integrated way to produce oil from wet microalgae. A high lipid yield from the HC (25.9–99.0%) was observed compared with autoclave (16.2–66.5%) and ultrasonication (5.4–26.9%) in terms of the specific energy input (500–10,000 kJ/kg). The optimal conditions for the simultaneous treatment were established using a statistical approach. The efficiency of the simultaneous method was also demonstrated by comparing each separate treatment. The maximum lipid yield (predicted: 45.9% and experimental: 45.5%) was obtained using 0.89% sulfuric acid with a cavitation number of 1.17 for a reaction time of 25.05 min via response surface methodology. Considering its comparable extractability, energy-efficiency, and potential for scale-up, HC may be a promising method to achieve industrial-scale microalgae operation.

Simultaneous extraction of oil- and water-soluble phase from sunflower seeds with subcritical water


In this study, the subcritical water extraction is proposed as an alternative and greener processing method for simultaneous removal of oil- and water-soluble phase from sunflower seeds. Extraction kinetics were studied at different temperatures and material/solvent ratios in a batch extractor. Degree of hydrothermal degradation of oils was observed by analyzing amount of formed free fatty acids and their antioxidant capacities. Results were compared to oils obtained by conventional methods. Water soluble extracts were analyzed for total proteins, carbohydrates and phenolics and some single products of hydrothermal degradation. Highest amount of oil was obtained at 130 °C at a material/solvent ratio of 1/20 g/mL after 30 min of extraction. For all obtained oils minimal degree of hydrothermal degradation could be identified. High antioxidant capacities of oil samples could be observed. Water soluble extracts were degraded at temperatures ≥100 °C, producing various products of hydrothermal degradation.

New biofuel alternatives: integrating waste management and single cell oil production


Concerns about greenhouse gas emissions have increased research efforts into alternatives in bio-based processes. With regard to transport fuel, bioethanol and biodiesel are still the main biofuels used. It is expected that future production of these biofuels will be based on processes using either non-food competing biomasses, or characterised by low CO₂ emissions. Many microorganisms, such as microalgae, yeast, bacteria and fungi, have the ability to accumulate oils under special culture conditions. Microbial oils might become one of the potential feed-stocks for biodiesel production in the near future. The use of these oils is currently under extensive research in order to reduce production costs associated with the fermentation process, which is a crucial factor to increase economic feasibility. An important way to reduce processing costs is the use of wastes as carbon sources. The aim of the present review is to describe the main aspects related to the use of different oleaginous microorganisms for lipid production and their performance when using bio-wastes. The possibilities for combining hydrogen (H₂) and lipid production are also explored in an attempt for improving the economic feasibility of the process.
Comparison between several methods of total lipid extraction from *Chlorella vulgaris* biomass

The use of lipids obtained from microalgae biomass has been described as a promising alternative for production of biodiesel to replace petro-diesel. It involves steps such as the cultivation of microalgae, biomass harvesting, extraction and transesterification of lipids. The purpose of the present study was to compare different methods of extracting total lipids. These methods were tested in biomass of *Chlorella vulgaris* with the solvents ethanol, hexane and a mixture of chloroform:methanol in ratios 1:2 and 2:1. The solvents were associated with other mechanisms of cell disruption such as use of a Potter homogenizer and ultrasound treatment. The percentage of triglycerides in the total lipids was determined by the glycerol-3-phosphate oxidase-p-chlorophenol method (triglycerides monoreagent K117; Bioclin). Among the tested methods, the mixture of chloroform:methanol (2:1) assisted by ultrasound was most efficient, extracting an average of 19% of total lipids, of which 55% were triglycerides. The gas chromatographic analysis did not show differences in methyl ester profiles of oils extracted under the different methods.

SYNTHETIC BIOLOGY

Influence of anionic dietary fibers (xanthan gum and pectin) on oxidative stability and lipid digestibility of wheat protein-stabilized fish oil-in-water emulsion


The influence of two anionic dietary fibers (xanthan gum and pectin) on the oxidative stability and lipid digestibility of fish oil emulsions stabilized by wheat protein (gliadin) was investigated. Lipid oxidation was determined by measuring lipid hydroperoxides and TBARS of the emulsions during storage, while protein oxidation was measured using fluorescence spectroscopy. Lipid and protein oxidation was faster at pH3.5 than at pH7, which may have been due to increased iron solubility under acidic conditions. Xanthan gum inhibited lipid and protein oxidation, which was attributed to its ability to bind iron ions. Conversely, pectin promoted oxidation, which was attributed to the presence of endogenous transition metals in the polysaccharide ingredient. In vitro digestion was carried out to evaluate the digestibility of oil droplets in emulsions with or without polysaccharides. Both xanthan gum and pectin significantly increased the rate of lipid digestion, which was attributed to their ability to inhibit droplet aggregation under gastrointestinal conditions. These results have important implications for designing emulsion-based functional foods with improved oxidative stability and lipid digestibility.

Lipid production from diverse oleaginous yeasts from steam-exploded corn cobs


Corn cob hydrolysate was used as substrate for growth and lipid accumulation viaoleaginous yeast species. A mass based suspension of 10 g 100 g-1 corn cob hydrolysate contained 26.0 g L-1 glucose, 8.5 g L-1 xylose. The inhibitor concentrations were 0.16 g L-1 acetic acid, 1,50 g L-1 formic acid, 0.48 g L-1 HMF and 0.06 g L-1 furfural. These conditions reduced the cell growth of non-adapted yeast. Successful adaptation of the tested yeasts over several generations in corn cob hydrolysate was performed. The adapted yeast *Candida lipolytica* produced 19.4 g 100 g-1 lipids in relation to the dry weight in 7.5 g 100 g-1 dry matter corn cob hydrolysate in fed batch mode. The scale up was done up to a volume of 2.5 liters – here lipid accumulation up to 17.5 g 100 g-1 was demonstrated with the quantitative GC/FID analyses. Predominantly oleic acid, palmitic acid, linoleic and palmitoleic acid were produced. This lipid spectrum is suitable for biodiesel production.

Metabolic engineering strategies for microbial synthesis of oleochemicals


Microbial synthesis of oleochemicals has advanced significantly in the last decade. Microbes have been engineered to convert renewable substrates to a wide range of molecules that are ordinarily made from plant oils. This approach is attractive because it can reduce a motivation for converting tropical rainforest into farmland while simultaneously enabling access to molecules that are currently expensive to produce from oil crops. In the last decade, enzymes responsible for producing oleochemicals in nature have been identified, strategies to circumvent native regulation have been developed, and high yielding strains have been designed, built, and successfully demonstrated. This review will describe the metabolic pathways that lead to the diverse molecular features found in natural oleochemicals, highlight successful metabolic engineering strategies, and comment on areas where future work could further advance the field.

Engineering *Ashbya gossypii* for efficient biolipid production


*Ashbya gossypii* is a filamentous fungus that naturally over-produces riboflavin. Indeed, engineered strains are currently used for the industrial production of riboflavin, replacing the chemical
Nanoparticle formation from amylose-fatty acid inclusion complexes prepared by steam jet cooking


Starch-based nanoparticles are of increasing interest for use as biobased fillers in composites with rubber and other polymers. Different methods have been reported for producing them, many requiring lengthy or complicated procedures. The purpose of this study was to determine whether the previously reported formation of spherulites by slowly cooling jet cooked dispersions of amylose inclusion complexes could be modified for nanoparticle synthesis. High-amylose cornstarch combined with oleic acid was jet cooked and then cooled at different rates ranging from 110 min to 10 s. Dynamic light scattering and SEM analysis showed that nanoparticles with diameters from 63 to 375 nm were obtained. X-ray diffraction analysis confirmed that they were comprised of V6 amylose complexes. Cooling rate and starch concentration affected yield of nanoparticles and their tendency to aggregate. Large quantities of starch-based nanoparticles can be prepared using this scalable method for further characterization and application development.

**Extracts & Distillates is compiled by Robert Moreau (US Department of Agriculture). Bryan Yeh (Intrexon) contributes references in the areas of industrial applications and synthetic biology. Extracts & Distillates is seeking a regular contributor with expertise in surfactants and detergents. If you are interested, please contact Kathy Heine at kheine@aocs.org.**

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**Home & Personal Care (cont. from 442)**

biosurfactants, which are produced by fermentation in yeast, reduce surface tension and have foaming and antimicrobial properties. Sophorolipids are currently more widely used than rhamnolipids because of their higher production yields and simpler purification, which translates to lower prices. Whereas rhamnolipids can only be produced at levels up to 100 g per liter, production of sophorolipids can exceed 400 g per liter.

Sophorolipids have been commercialized and are already used in some cleaning products. These biosurfactants are mixtures of a lactone and the corresponding acidic form, with the ratio influencing the sophorolipid’s properties and performance. Evonik Industries (Essen, Germany) has tailored the acid-to-lactone ratio in its Rewoferm SL446 sophorolipid to achieve better performance in foaming, degreasing, and tolerance to water hardness than other surfactants on the market. According to Evonik, its sophorolipids are ideal for applications such as hand dishwashing and hard surface cleaning. Evonik is ready to produce sophorolipids at a commercial scale beginning in Europe (following REACH approval) and then in the United States (after US registration).

Other companies that produce or develop sophorolipids include Ecover (Antwerp, Belgium); Henkel (Düsseldorf, Germany); Soliance (Pomacle, France); Saraya (Osaka, Japan); MG Intobio (Incheon, South Korea); Allied Carbon Solutions (Tokyo, Japan); and Synthesyme (Rensselaer, New York, USA).
Di-2-ethylhexyl phthalate (DEHP) is an organic compound with the formula C₆H₄(C₈H₁₇COO)₂. It is the most common compound in the class of phthalate plasticizers. The colorless viscous liquid is soluble in oil, but only very slightly in water. Due to its suitable properties and low cost, DEHP is widely used as a plasticizer in the manufacturing of articles made of polyvinyl chloride (PVC). Some plastics may contain 1% to 40% DEHP, which can be absorbed by food and water. Higher levels have been found in milk, cheese, and other fatty food products. The compound can also leach into a liquid that comes into contact with the plastic.

Recently, deliveries of packaged extra virgin olive oil (EVOO) from Victoria, Australia, to China have been rejected for containing levels of DEHP above 2.0 ppm. Random testing in similar products detected the presence of DEHP in levels ranging from 0.5 ppm to 3.0 ppm—even though no plastic containers with known quantities of DEHP were used in the production process.

The situation prompted researchers in the commercial olive and olive oil laboratory at the Australian olive company Modern Olives to begin to investigate where and how this contamination takes place, so that customers who want to export to China could take appropriate actions along the production chain to prevent future export problems and health concerns.

Tracking the origin and movement of DEHP through production

Our research group at Modern Olives analyzed a wide range and number of finished olive oil tanks from different grower types, environments, and varieties across the state of Victoria. These included tanks from two large growers from central and northern Victoria, two medium-size growers from central western and central eastern Victoria, and a couple of small growers from southern Victoria.

We then collected samples of fruit from two separate locations as well as samples of oil throughout the production chain to determine how much DEHP, if any, could be added during any of
those steps. Additionally, we analyzed and studied possible DEHP content and migration patterns from the most common products used to produce, harvest, manipulate, store, and bottle olive oil that may contain some levels of DEHP.

Since the analysis of DEHP is not a part of standard quality and authenticity analysis of olive oil, ancillary tests conducted on different olive oil batches would result in additional costs for growers and trading companies. Furthermore, uncertainty over how many tests need to be done to understand the pattern of contamination could increase the potential costs of the overall exploratory project, which was being co-funded by Modern Olives and the state government in Victoria.

Information and technical publications regarding contamination of olive oil with DEHP are virtually non-existent. Technical references to migration tests involving the limited range of materials used during extra virgin olive oil production (transport, storage, and packaging) are also very scarce.

Consequently, this project is generating innovative data and completely new information regarding the influence of different environments on DEHP contamination of olive oil, analyzing the different production steps to determine their impact on final levels of DEHP, as well as evaluating the most commonly used materials with respect to their role in DEHP transfer to olive oil.

Water as a potential source of DEHP contamination

As it can be seen in the table below, our analysis of more than 40 finished tanks with Victorian EVOO of different sizes confirmed our initial information regarding the consistent presence of DEHP in almost all tanks but one. The distribution and content of DEHP in those tanks was completely erratic and randomly distributed. Nonetheless, there was a rather clear tendency for the smaller tanks to have consistently higher levels of DEHP than the larger ones (Table 1). Overall, 61% of the samples had DEHP levels below 2 ppm and 39% of the tanks showed levels higher than this limit.

When we analyzed the oil obtained at different stages of the production process in two separate locations and with three different types of fruit—straight from the fruit at lab level, post decanter (after washing and malaxing), post separator (after centrifugation) and final tank—we observed that there was not only an initial and variable level of DEHP in the fruit but also that the levels of DEHP increased incrementally after each step of the process (Fig. 1, page 464).

Based on these results, we hypothesized that fruit was arriving to the processing plant with some inherent level of DEHP, and that the water added during processing may contribute to

CONTINUED ON NEXT PAGE

<table>
<thead>
<tr>
<th>Tank Type</th>
<th>Average</th>
<th>Median</th>
<th>Maximum</th>
<th>Minimum</th>
<th>STDEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10KL</td>
<td>3.673</td>
<td>2.291</td>
<td>11.547</td>
<td>0.000</td>
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<td>200kl</td>
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<td>1.340</td>
<td>26.304</td>
<td>0.074</td>
<td>6.090</td>
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<tr>
<td>TOTAL</td>
<td>2.808</td>
<td>1.668</td>
<td>26.304</td>
<td>0.000</td>
<td>2.510</td>
</tr>
</tbody>
</table>

TABLE 1. Summary of DEHP analysis on 41 tanks of different capacities
the continual boosting of those DEHP levels—possibly due to the preferential solubility of DEHP in oil vs. water. To test this hypothesis, we processed in the lab two different types of fruit from separate locations. A control group was washed with the normal tap water used on those facilities and also with distilled water to see if there was a difference (Fig. 2).

Our results confirmed that while the levels of DEHP were variable in the fruit, particularly on the skin, the water used during the production process added more DEHP to the final product. Indeed, DEHP levels in the oil samples produced by washing and processing the olives with distilled water were six times lower than the levels of DEHP in the fruit prior to washing, and the DEHP content of the water was approximately double what it was before washing.

Following these trials, we analyzed several municipal water samples from a variety of processing facilities at different times during the season. The results are presented in Fig. 3.

The results not only confirm that water could be an important source of DEHP contamination, but they also explain the erratic nature of those readings. Theoretically, such variations could be related to the nature of the piping system, the water source, the water treatment plant, how long the water resided in the system, and so on.

Another interesting aspect that we analyzed was the evolution of DEHP content during storage. The data collected from two different tanks over a period of five months is presented in Fig. 3.

Similar results have been obtained for oil stored in commercial packaging. After analyzing these results in combination with the previous observation that contamination with DEHP in storage could arise from some form of surface/volume phenomenon, we decided to analyze other potential sources of contamination apart from the water itself, which is used to wash the tanks.

The study of the impact of nitrogen injection, led us to study and compare a blank sample of water used in the storage tank farm with a sample of the same water where nitrogen from the on-site generator bubbled for 24 hours. Results (pictured...
in Table 2) show that a small incremental amount of DEHP could be attributed to the nitrogen system, presumably from the plastic components in the generator and delivery system.

We have also analyzed other potential sources of contamination for storage tanks and packaging with significant results. Those results, mostly obtained by migration analysis on the respective materials, are pictured in Table 3.

**Further investigations**

It is possible to conclude that most olives would arrive at the processing plant with some level of DEHP on their surface (presumably from spraying those olives with fertilizers and chemicals). This DEHP on the fruit provides a baseline level of contamination typically below 1 ppm in the final oil but it suffers an increment during the process of washing and extraction as a likely consequence of the DEHP content in the water. It is important to highlight that DEHP levels in the fruit and processing water are quite variable and erratic but combined can produce oil with varying levels of DEHP, typically from 0.1 to 1.6 ppm.

Following the extraction process, we could establish that storage conditions either at the tank or finished goods level have the potential to continue increasing the initial levels of DEHP in the oil. This secondary contamination at the tank level is likely to be linked to the water and chemicals used to wash the tanks, the plastic side glasses used to measure oil levels in the tank, and/or the nitrogen used to protect the oil from oxidation. In the case of packaged oil, the contamination is likely to occur through the plastic components of the packaging (lids, caps, epoxy resin in tins or PET bottles) as these all seem to have the potential to migrate DEHP over time.

Based on the results obtained in this exploratory phase, we believe that further investigation may provide opportunities to:

- understand where the initial contamination of the olives at the farm level occurs (spraying, water, chemicals, environment, etc.);
- understand the dynamics of DEHP in the water commercially used in processing facilities;
- understand how secondary contamination during storage occurs; and
- develop a specific technique for measuring migration of DEHP and different materials in olive oil.

**Claudia Guillaume and Leandro Ravetti are researchers at Modern Olives in Victoria, Australia. Guillaume can be contacted at c.guillaume@modernolives.com.au.**

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**TABLE 2. DEHP levels in water with and without nitrogen injection**

<table>
<thead>
<tr>
<th>Sample</th>
<th>DEHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (blank)</td>
<td>0.149</td>
</tr>
<tr>
<td>Water (with N2)</td>
<td>0.208</td>
</tr>
<tr>
<td>Increment</td>
<td>0.059</td>
</tr>
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</table>

**TABLE 3. Other potential sources of DEHP contamination**

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>STDEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pourer</td>
<td>0.278</td>
<td>0.207</td>
</tr>
<tr>
<td>Lids</td>
<td>0.313</td>
<td>0.108</td>
</tr>
<tr>
<td>PET bottle</td>
<td>0.488</td>
<td>0.000</td>
</tr>
<tr>
<td>Tins</td>
<td>0.287</td>
<td>0.128</td>
</tr>
</tbody>
</table>
A. Richard Baldwin Distinguished Service
This is the Society’s highest service award. It recognizes long-term, distinguished service to AOCS in positions of significant responsibility.

Nature of the Award: $2,000, a travel-and-expense allowance, and a plaque provided by Cargill.

Deadline: November 1

AOCS Award of Merit
This award recognizes productive service to AOCS: leadership in committee activities; service that has advanced the Society’s prestige, standing, or interests; and service not otherwise specifically recognized.

Nature of the Award: A plaque.

Deadline: November 1

AOCS Fellow
The status of Fellow is awarded to members of AOCS whose achievements in science entitle them to exceptionally important recognition or to those who have rendered unusually important service to the Society or to the profession.

Nature of the Award: Fellow membership status and a plaque.

Deadline: December 1

Supelco/Nicholas Pelick-AOCS Research
This award recognizes outstanding original research of fats, oils, lipid chemistry, or biochemistry. The recipient must have published the research results in high-quality technical papers regarding fats, oils, lipid chemistry, or biochemistry.

Nature of the Award: $10,000, a travel-and-expense allowance, and a plaque. The award is sponsored by Supelco, a subsidiary of Sigma Aldrich Corp, and Nicholas Pelick, past president of AOCS.

Deadline: November 1

Stephen S. Chang
This award recognizes a scientist, technologist, or engineer whose distinguished accomplishments in basic research have been used by industries for the improvement or development of products related to lipids.

Nature of the Award: $1,500 and a jade horse, provided by the Stephen and Lucy Chang endowed fund.

Deadline: October 15

AOCS Young Scientist Research
This award recognizes a young scientist who has made a significant and substantial research contribution in one of the areas represented by the Divisions of AOCS.

Nature of the Award: $1,000, a plaque, and a travel-and-expense allowance provided by the International Food Science Center AOS.

Deadline: November 1

The Schroepfer Medal
Originated by colleagues of George Schroepfer, this award recognizes a scientist who has made significant and distinguished advances in the steroid field. The work may represent a single major achievement or an accumulation of data.

Nature of the Award: An honorarium and a medal.

Deadline: October 15

ACI/NBB Glycerine Innovation
The Industrial Oil Products Division initiated this award to recognize outstanding achievement for research in new applications for glycerine with particular emphasis on commercial viability.

Nature of the Award: $5,000 and a plaque provided by the American Cleaning Institute and the National Biodiesel Board.

Deadline: November 1

Samuel Rosen Memorial
Milton Rosen and the Surfactants and Detergents Division initiated this award to recognize a surfactant chemist for significant advancement or application of surfactant chemistry principles.

Nature of the Award: $2,000 and a plaque.

Deadline: November 1

Herbert J. Dutton
The Analytical Division initiated this award to recognize an individual who has made significant contributions to the analysis of fats and oils and related products or whose work has resulted in major advances in the understanding of processes utilized in the fats and oils industry.

Nature of the Award: $1,000, a travel-and-expense allowance, and a plaque.

Deadline: November 1

Timothy L. Mounts
The Edible Applications Technology Division initiated this award to recognize research relating to the science and technology of edible oils or derivatives in food products, which may be basic or applied in nature.

Nature of the Award: $750 and a plaque provided by Bunge North America.

Deadline: November 1

Edible Applications Technology Outstanding Achievement
This award recognizes a scientist, technologist, or leader who has made significant contributions to the Division’s field of interest, or made contributions to the advancement of edible oils.

Nature of the Award: $500 and a plaque.

Deadline: November 1

CALL FOR NOMINATIONS
Each award has its own specific and unique nomination requirements. Please refer to the website for full details.

Nominations must be submitted through our online process and must include all required letters, forms, and references for consideration.

Self-nominations are welcomed and encouraged.
Call for Nominations

Ralph Holman Lifetime Achievement
The Health and Nutrition Division established this award to annually recognize an individual who has made significant contributions to the Division’s field of interest, or whose work has resulted in major advances in health and nutrition.

*Nature of the Award:* $500, a travel-and-expense allowance, and a signed orchid print.

*Deadline:* November 1

Processing Distinguished Service
The award recognizes and honors outstanding, meritorious service to the oilseed processing industry.

*Nature of the Award:* Travel-and-expense allowance and a certificate.

*Deadline:* December 1

Surfactants and Detergents Distinguished Service
The award recognizes outstanding, commendable service to the surfactants, detergents and soaps industry.

*Nature of the Award:* A plaque.

*Deadline:* December 1

Alton E. Bailey
This award is supported by the USA Section and recognizes research and/or service in the fields of fats and oils and related disciplines.

*Nature of the Award:* $750 and a plaque.

*Deadline:* November 1

Thomas H. Smouse Fellowship
This award was established by the Archer Daniels Midland Foundation and the family and friends of Thomas H. Smouse. The purpose of this graduate fellowship is to encourage and support outstanding research by recognizing a graduate student pursuing an M.S. and/or Ph.D. degree in a field of study consistent with the areas of interest of AOCS.

*Nature of the Award:* The Fellowship level is up to $15,000 ($10,000 Fellowship, $5,000 for travel and research expenditures related to the student’s graduate program).

*Deadline:* February 1

Ralph H. Potts Memorial Fellowship
This award recognizes a graduate student working in the field of chemistry of fats and oils and their derivatives. Qualifying research will involve fatty acids and their derivatives, such as long-chain alcohols, amines, and other nitrogen compounds.

*Nature of the Award:* $2,000, a plaque, and travel-and-expense allowance. The award is supported by AkzoNobel, Inc.

*Deadline:* October 15

Honored Student
This award recognizes graduate students in any area of fats and lipids. To receive the award, a candidate must remain a registered graduate student and must not have received a graduate degree or have begun career employment prior to the Society’s Annual Meeting.

*Nature of the Award:* Travel-and-expense allowance and a certificate.

*Deadline:* October 15

Hans Kaunitz
This award is supported by the USA Section and encourages studies in the sciences relating to fats, oils, and detergent technology. This award is open to graduate students within the geographical boundaries of the USA Section.

*Nature of the Award:* $1,000, a travel-and-expense allowance, and a certificate.

*Deadline:* October 15

AOCS Division Awards for Students
These awards recognize students at any institution of higher learning, who are studying and doing research towards an advanced degree in fats, oils, proteins, lipids, surfactants, detergents, and related materials.

The following student awards are currently being offered by these AOCS Divisions:

- **Analytical Division Student Award**
- **Biotechnology Student Excellence Award**
- **Edible Applications Technology Division Student Award**
- **Health and Nutrition Division Student Excellence Award**
- **Industrial Oil Products Division Student Award**
- **Lipid Oxidation and Quality Division Student Poster**
- **Processing Division Student Excellence Award**
- **Protein and Co-Products Division Student Poster**
- **Surfactants and Detergents Division Student Travel Award**

*Nature of the Award:* Awards can consist of $100 to $1,000 and a certificate.

*Deadline:* Varies from October 15 to January 15

See website.

AOCS Awards contact ➤ awards@aocs.org • www.aocs.org/awards
Discovery, engineering and application of enzymes in lipid modification

Uwe T. Bornscheuer

Enzymes and (engineered) microorganisms are used in three major areas in the lipid field: (i) the modification of fats and oils already available from renewable resources; (ii) the transformation of precursors, such as alkanes into fatty acids or derivatives; and (iii) the de novo synthesis of fatty acids, fats, or oils from renewable carbon sources such as glucose. In most cases, the use of isolated enzymes is preferred, as many are commercially available and relatively easy to apply.

The development of advanced genetic engineering methods and a variety of protein engineering tools have made it possible for enzymes to be tailor-designed for given applications. Finally, metabolic engineering using designed whole-cell microorganisms has become an important alternative in the last few years, as artificial pathways can now be introduced to achieve targeted production of valuable new compounds.

Lipase-catalyzed synthesis of structured triglycerides

A considerable range of enzymes can be used to modify fats, oils, and other lipids. This is due to the usually excellent chemo-, regio- and stereoselectivity of enzymes—particularly under mild reaction conditions compared to chemical methods.

Lipases are the most important enzymes in lipid modification, and their application is well documented in the literature; overviews are given in recent review articles [1,2]. Structured triglycerides (sTAG) have a defined distribution of different fatty
acids along the glycerol backbone and are important compounds for a range of applications in human nutrition. For example, sTAG containing medium-chain fatty acids at the sn-1- and sn-3-position, together with a long unsaturated fatty acid at the sn-2-position—such as 1,3-caproyl-2-oleoyl-glycerol (CyOCy)—are used to treat patients with pancreatic insufficiency. Such sTAG are also used as a rapid energy supply for athletes.

Another important example is Betapol™, which is used in infant nutrition. Betapol™ contains oleic acid at the sn-1- and sn-3-positions and palmitic acid at the sn-2-position (1,3-oleoyl-2-palmitoyl-glycerol, OPO). It is manufactured by interesterification of tripalmitin with high oleic sunflower oil using a lipase from Rhizomucor miehei (Novozyme RMIM). This simple one-step transesterification has been used frequently, but a major limitation is that various triglyceride species are produced, not just the desired one. For instance, Betapol™ obtained by interesterification contains only 65% palmitic acid in the sn-2-position, causing undesired side effects in infants due to the formation of calcium soaps.

To address this problem our research team developed a scalable two-step lipase-catalyzed process (Fig. 1). First, the triglyceride tripalmitin is subjected to alcoholysis with ethanol yielding sn-2-monopalmitin, which can be isolated by crystallization. In the second step this monoglyceride is esterified with oleic acid yielding the desired sTAG OPO (Betapol™).

Protein engineering to alter the selectivity and stability of lipases

Quite often, natural enzymes do not demonstrate the desired characteristics required for their efficient application in biocatalysis. They may be inadequate not only with respect to such features as substrate scope, regioselectivity, and pH- and temperature profile, but also with respect to stability (long-term process stability, tolerance of organic solvents, and so on).

Protein engineering allows such properties to be altered and hence represents a key technology in modern biocatalysis [4].

Typically, either rational protein design or directed (molecular) evolution are used to improve enzymes.

Our research group used protein engineering to create a lipase with distinct selectivity for trans-fatty acids [5], which are present in partially hydrogenated oil and had been identified as an important risk factor for coronary heart disease. Starting from lipase CAL-A (originating from Candida antarctica), which already exhibits some selectivity for trans-fatty acids, we performed computer modeling based on the 3D-structure of the lipase and inspected all residues comprising the ~30 Å long acyl binding tunnel of CAL-A. Such modeling helped us identify 12 positions along the entire fatty acid binding tunnel.

These 12 positions were then subjected to individual mutagenesis. After expression in deepwell plates, we screened about 5,000 clones with a high-throughput assay to identify variants with an improved rate in the hydrolysis of trans-over cis-fatty acids. Next, the best mutants were used in the hydrolysis of a partially hydrogenated soybean oil containing 18% trans-fatty acids with approximately 20 individual species. The results revealed that the CAL-A mutants T221H and I301H (Fig. 2, page 470)
exhibited excellent selectivity and solely hydrolyzed trans- and saturated fatty acids, but no cis-fatty acids could be detected and thus remained bound to the glyceride fraction. These CAL-A variants are hence well suited to remove trans-fatty acids from partially hydrogenated plant oils [5].

Enzyme cascade reactions in lipid modification

A current trend in biocatalysis is to combine several enzymes in so-called cascade reactions. The advantages are that no intermittent product isolation is required, unstable intermediates are directly further converted, reversible reactions can be driven to completion, inhibition is reduced or eliminated, investment costs can be lower, waste is considerably reduced, and higher overall yields can be achieved. However, this approach requires the availability of suitable enzymes having similar pH- and temperature profiles and preferentially also similar specific activities and stabilities. Usually, the enzymes are recombinantly expressed and used either as whole-cell systems or as crude cell extracts that cannot tolerate undesirable enzyme activities in the host’s background. α,ω-Dicarboxylic acids, ω-hydroxy carboxylic acids and ω-amino carboxylic acids are important starting materials for the synthesis of a variety of chemical products and intermediates, such asnylons and other polyamides, polyesters, resins, lubricants, plasticizers, and so on.

**FIG. 2.** Structure of CAL-A indicating positions T221 and I301, which had the most pronounced effect on the trans-fatty acid selectivity of this lipase.

**FIG. 3.** Enzyme cascade reaction to convert unsaturated fatty acids such as oleic acid into ω-hydroxycarboxylic acids or dicarboxylic acids; BVMO: Baeyer-Villiger monooxygenase [6].
In a joint project with J.B. Park (Seoul, South-Korea) a biocatalytic cascade to produce ω,ω-dicarboxylic acids and ω-hydroxy carboxylic acids from renewable fatty acids obtained from vegetable oils (such as oleic acid, ricinoleic acid) was created [6]. The process began by hydrating the internal double bond of the fatty acid with an oleate hydratase, followed by enzymatic oxidation of the hydroxyl group to the ketone by an alcohol dehydrogenase (ADH).

Based on our earlier discovery that certain Baeyer-Villiger monooxygenases (BVMO) show distinct regioselectivity in the formation of esters from ketones, we used two different BVMOs for the oxidation of the ketone into the two regioisomeric esters (Fig. 3). In the last step, an esterase-catalyzed hydrolysis yields the target compounds (ω-hydroxy carboxylic acid or ω,ω-dicarboxylic acid). This made the cascade reaction much more versatile as two different target products can be easily produced as demonstrated for a variety of fatty acids as starting materials [6].

In summary, biocatalysis is a mature technology. However, recently developed tools for protein engineering and the combination of enzymes have further boosted its broad application in lipid modification.

Further reading
Novel biobased poly(vinyl ether)s for coating applications

Prior to the availability of low cost petrochemicals, plant oil triglycerides were used extensively for surface coatings. The suitability of a plant oil for use as a protective or decorative surface coating is highly dependent on the polyunsaturated fatty acid ester content of the oil, since the bis-allylic hydrogen atoms in the polyunsaturated fatty acid esters play a primary role in the conversion of the liquid plant oil to an insoluble film during the process of autoxidation. For this reason, oils with a relatively high level of polyunsaturated fatty acid esters, such as linseed oil (LO), walnut oil, poppy oil, tung oil, perilla oil, and safflower oil, have been the primary oils used for surface coatings and paints.

The authors have developed a novel polymer technology that uses plant oil triglycerides to produce vinyl ether monomers possessing a plant oil-derived fatty acid ester group within the structure of the monomer. Using the appropriate cationic polymerization system, it has been demonstrated that monomers derived from plant oils possessing relatively high levels of polyunsaturated fatty acid esters can be readily polymerized without affecting the unsaturation derived from the plant oil. [1]. In fact, polymerization systems for plant oil-based vinyl ether monomers (POVEs) have been identified that provide for a living polymerization. This enables the production of linear polymers with narrow molecular weight distributions and polymer molecular weight to be precisely controlled by simply controlling the monomer-to-initiator ratio and the degree of monomer conversion. [2] The living characteristics of the polymerization have also been shown to enable the production of block copolymers by simple sequential monomer addition. [3] Fig. 1 shows the synthetic scheme for producing a polyPOVE derived from soybean oil (SBO).

As a result of the much higher number of bis-allylic hydrogen atoms per molecule associated with a polyPOVE as compared to a triglyceride, the extent of autoxidation needed to generate an insoluble, tack-free film is dramatically lower. To illustrate, Fig. 2 (page 474) provides a comparison of drying time for films derived from LO, and polyPOVEs derived from SBO and LO. The proper chemical names for the polyPOVEs derived from SBO and LO are poly[2-(vinylloxy)ethyl soyate] [poly(2-VOES)] and poly[2-(vinylloxy)ethyl linseedate] [poly(2-VOEL)], respectively. As shown in Fig. 2, the coating film derived from LO took 280 hours (11.7 days) to become dry-to-the-touch, while coating films based on poly(2-VOES) and poly(2-VOEL) only took 6.1 and 1.2 hours, respectively, to become dry-to-the-touch.

These coatings were simply blends of the oil with a drier package to catalyze autoxidation. The same drier package composition and concentration was used for each oil.

Deep Kalita, Ihor Tarnavchyk, David Sundquist, Satyabrata Samanta, James Bahr, Olena Shafranska, Mukund Sibi, and Bret Chisholm

- Historically, plant oils with relatively high levels of polyunsaturated fatty acid esters (linseed, walnut, poppy, tung, perilla, and safflower) have been the primary oils used for surface coatings and paints.
- This is because the bis-allylic hydrogen atoms in the polyunsaturated fatty acid esters play a primary role in the autoxidation process that converts liquid plant oils to insoluble films.
- This article describes a novel polymer technology that uses plant oil triglycerides to make vinyl ether monomers with the ability to produce polymers tailored for specific coating applications.
In addition to faster drying/curing, polyPOVEs can be produced that possess much lower color than drying oils, such as LO. To illustrate, Fig. 3 (page 475) displays an image of a vial containing LO as well as a vial containing a poly(2-VOES) sample. The lack of color associated with the poly(2-VOES) sample can be attributed to the ability to purify the 2-VOES monomer by vacuum distillation and the mild conditions used to polymerize the distilled 2-VOES. It has been demonstrated that essentially colorless poly(2-VOES) can be produced using cationic polymerization at room temperature. As expected, colorless polymers and resins for coating applications is very important, especially for the production of clear coatings as well as white and other pale-colored coatings.

Probably the most important aspect of the POVE technology is the ability to produce copolymers. Copolymerization is an enormously important tool for tailoring polymer properties for a specific application. For example, acrylic polymers represent the largest market segment for polymers used for coating applications. As a result of the need to tailor fundamental polymer properties, such as glass-transition temperature (Tg), essentially all acrylic polymers used for coatings are copolymers. A variety of comonomers have been used in conjunction with POVEs to enhance their utility for coating applications. For example, a number of comonomers have been used to increase polymer Tg, which has resulted even shorter time periods needed for freshly applied coatings to become dry-to-the-touch. [4] In addition, hydrophilic comonomers have been used to create surface-active polymers that can be dispersed in water without the need for surfactant. These hydrophilic comonomers have also been shown to enable POVE copolymers that are useful for application as polymeric surfactants in shampoos. [5]

The main classes of polymers/resins used for coatings are polyesters, acrylics, alkyds, polyurethanes, epoxies, and amino resins. Of these, alkyd resins are the most similar to the POVE polymer technology since alkyd resins also possess fatty acid ester moieties in their structure, which are utilized to provide crosslinking/curing via autoxidation. Unlike POVE polymers, which are produced by a chain-growth polymerization process, alkyd resins are produced by a step-growth polymerization process. This basic difference in polymerization process results in some important differences between POVE polymers and alkyd resins for coating applications. With the step-growth polymerization process used to produce alkyds, very low molecular weight species such as dimers, trimers, tetramers, etc. are typically present in the resin, which have

CONTINUED ON NEXT PAGE
deleterious effects on drying/curing time and film properties, such as hardness and chemical resistance. With a chain-growth polymerization, the presence of these low molecular species can be avoided. To illustrate the dramatic difference in coating drying-time and film properties that can be obtained with a POVE polymer as compared to a conventional alkyd resin, Table 1 provides data comparing a clear coating derived from an SBO-based POVE copolymer to an analogous coating produced using a commercially available SBO-based long oil alkyd resin. Tg, Young’s modulus, tensile strength, and elongation at break were obtained from free standing films, while tack-free time, pendulum hardness (ASTM D4366-95), solvent resistance (ASTM D5402-93), impact resistance (ASTM D2794), and conical mandrel bend (ASTM D 522) were measured using coated steel panels.

As shown in Table 1, the Tg of the POVE-based coating film was more than double that of the alkyd film and the Young’s modulus and tensile strength were higher by a factor of 288 and 14.8, respectively. Thus, the cured POVE-based film possessed dramatically higher stiffness and strength than the cured alkyd film. For coated steel panels, the POVE copolymer coating was essentially tack-free as soon as the solvent evaporated from the film, while the alkyd coating required 10.9 hours to become tack-free. The solvent resistance and pendulum hardness of the POVE copolymer coating was far superior to that of the alkyd coating, while still maintaining good impact resistance and flexibility.

In summary, the POVE polymer technology briefly described in this document provides novel plant oil-based polymers that exhibit several advantages for use in coating applications. First, similar to alkyd resins, the polyunsaturated fatty

![FIG. 2. Drying time data for coatings derived from LO, poly(2-VOES), and poly(2-VOEL).](image)

**TABLE 1.** A comparison of the properties of a POVE copolymer-based coating and a coating based on a commercially available SBO-based long oil alkyd. The coatings were simply the POVE copolymer or alkyd resin dissolved in toluene along with a drier package to catalyze curing by autoxidation. The drier package composition and concentration was the same for both coating solutions.

<table>
<thead>
<tr>
<th>Property</th>
<th>POVE coating</th>
<th>Alkyd coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg (°C)</td>
<td>98</td>
<td>40</td>
</tr>
<tr>
<td>Young’s Modulus (MPa)</td>
<td>776.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Tensile Strength (MPa)</td>
<td>23.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Elongation (%)</td>
<td>4.1</td>
<td>59.0</td>
</tr>
<tr>
<td>Tack-free time (hours)</td>
<td>0.02</td>
<td>10.9</td>
</tr>
<tr>
<td>Pendulum Hardness (sec.)</td>
<td>115.5 ± 14.5</td>
<td>18.0 ± 5.6</td>
</tr>
<tr>
<td>Solvent Resistance (double rubs)</td>
<td>750</td>
<td>64</td>
</tr>
<tr>
<td>Direct Impact Resistance (in-lb)</td>
<td>137.2</td>
<td>168.6</td>
</tr>
<tr>
<td>Reverse Impact Resistance (in-lb)</td>
<td>117.6</td>
<td>160.7</td>
</tr>
<tr>
<td>Conical Mandrel Bend (% Elongation)</td>
<td>37.3</td>
<td>35.4</td>
</tr>
</tbody>
</table>
acid ester pendant groups in the polymers enable crosslinking/curing using autoxidation. Compared to other crosslinking/curing mechanisms used for coatings, oxidative curing has the advantage of enabling one-component coatings that can be cured at ambient conditions. Compared to alkyd resins, POVE polymers can be produced that are essentially colorless and void of low molecular weight species such as dimers, trimers, tetramers, etc. that can negatively affect drying/curing time and various film properties. Finally, the ability to utilize comonomers with POVEs enables polymer and coating properties to be tailored over a wide range to meet specific coating requirements.

Deep Kalita, Ihor Tarnavchyk, Satyabrata Samanta, James Bahr, Olena Shafranska, and Mukund Sibi, are with are at North Dakota State University in Fargo, ND, USA. David Sundquist is with Renuvix LLC, and the corresponding author, Bret Chisholm, is with both. He can be contacted at bret.chisholm@ndsu.edu.

The POVE polymer technology described in this document was developed with financial support from multiple federal and state agencies, specifically the North Dakota Soybean Council, US Department of Agriculture, National Institute of Food and Agriculture (grant 2012-38202-19283), Department of Energy (grant DE-FG36-08GO088160), and the National Science Foundation (grants IIA-1330840, IIA-1355466, IIP-1401801, and IIP-1416983). The authors thank these agencies for their financial support.

Further reading

So you want to get your manuscript published.

Five points to remember!

Michael K. Dowd

Journals reject research reports for many reasons, including inappropriate topic, lack of novelty, poor experiment design, limited results, incomplete analyses or discussion, unsupported conclusions, and poor presentation. While these problems can be difficult to resolve, many contributing factors can be avoided by following five simple points.

1. Write for your readers.

Ultimately, your readers will determine if you are successful as a researcher, so you need to understand their expectations. Your readers include a wide range of potentially interested industrial, academic, and government scientists; journal reviewers and editors; and possibly journalists looking for topics to present to the general public. Hence, you need to think carefully about what evidence you need to convince these groups that your work is a contribution worth publishing.

Successful authors start thinking about this early in the development of their project. If there are different ways to measure the same thing, a successful author will ask: “Which method will be the most acceptable to my scientific colleagues?” and “How much data will I need to prove my point?”

We all know colleagues who can generate a big story from three data points, but will this raise questions for reviewers? If non-routine instruments or tools are used, how much background information should be provided to ensure that your readers can comprehend the material? How much data replication will be necessary, and what statistics will be required to support your hypothesis? Today, many journals are grappling with the use of statistical approaches that provide preliminary information with minimal data. Will such an approach raise concerns for a knowledgeable reviewer? There is a lot to think about in developing a project, but thinking about it with an eye toward your readers will go a long way to avoiding problems during review.

2. Tell a story...and tell it well!

A research report must have a new story to tell. The basics of this story and its conclusions should be well ingrained in your mind before you start to write. Generally, it is best to start writing soon after completing your data analysis, when the work is fresh in your mind and your starting materials and equipment are still available. It is normal for data anomalies and unanticipated issues to crop up as you write. If you wait
until a critical piece of equipment becomes unavailable or the samples have aged, completing your study can get more difficult and could cost you the chance to publish.

A research report should have one main theme. Do not divert the reader’s attention with unrelated distractions or side issues. Mystery writers often introduce a diversion or two; short story writers do not, and a research report should be like a short story.

You must also tell your story clearly. Although the topic under discussion may be complicated, the writing should not be. Direct, straightforward communication is what is required. Maybe the most important thing is to tell your story in a logical, forthright manner. Be concise. Although words, sentences, and paragraphs are needed to describe your work, good technical writers know that verbosity is not a benefit. Avoid redundancy; organize the material so that you say things once. Avoid jargon, slang, and language that might not be familiar to a broad readership. And be absolutely sure that no ambiguities or inconsistencies exist that will introduce confusion.

3. Read and the follow the Instruction for Authors.

All journals have submission requirements that are explained in the Instruction for Authors. Read and follow these guidelines. Editors do not appreciate having to remind authors of these expectations. In fact, not following them will leave the impression that you were careless in preparing the report, and the editors may think that this carelessness extends to your science.

Reference formatting seems to be a particular problem and can be avoided by double-checking the references prior to submission. It is not unusual for an interested reviewer to look up a citation or two to check on specific points. I frequently receive requests from reviewers asking me to forward a reference they cannot readily access. If the reviewers cannot find an important reference or it is in error, you can expect the issue will be raised in the review.

Most author instructions provide reference formatting for journals, monographs, and patents, but many source materials may not be discussed. Guidance on the formatting of less common materials can be found in *The ACS Style Guide* [1]. In submissions, journal names are often abbreviated incorrectly. The Chemical Abstract Service Source Index (CASSI) provides correct abbreviations on its website [2]. You can also view recent issues of the journal for acceptable abbreviations.

4. Be ethical.

In addition to basic truthfulness, scientific authors have other publishing responsibilities. Authors must also acknowledge all parties contributing to a study and disclose all potential conflicts of interest, including sources for funding. They must also acknowledge significant prior contributions. If published methods were used in the report, they should be cited. If tables or figures from other publications were used, permission to reproduce these images is needed from the copyright holder (usually the publisher), and their original sources need to be properly cited.

Plagiarism is all too common in research submissions, and all journals are dealing with this problem to some degree. As a result, all manuscripts submitted to AOCS journals are reviewed with CrossCheck—a document screening service that searches its databases for duplicated text [3]. All of the major scientific publishers contribute their materials to the CrossCheck database, and plagiarism is now detected more frequently.

The most common form of plagiarism seen in submissions to AOCS journals is self-plagiarism, which involves authors copying their own material from already published work. Usually these manuscripts are returned to authors to be
rewritten before they are evaluated. If material is copied from other authors, the manuscripts are rejected outright.

5. Understand the review process.

Typically, the editor handling a submission will ask scientists working in similar areas to review the report. Getting researchers to review manuscripts is not easy, and editors do not always get the reviewers they want. Consequently, they frequently have to make alternative choices. Although authors may be asked to suggest possible reviewers, in reality authors have little control over this process. The best ways to handle this situation is to submit a well-written report that can be understood by a wide audience (per points 1 and 2). Because reviewers are donating their time to review your manuscript, they are unlikely to spend much time resolving parts of a paper that are not stated clearly.

Reviewers will likely provide comments on the novelty of the report, its importance to the field, the appropriateness of the methods, and the reasonableness of the results and discussion. Editors may also provide a review: This is expected of Associate Editors at the Journal of the American Oil Chemists’ Society (JAOCS). If things go right, the reviewers and editors will have carefully analyzed the manuscript. Likely, they will also comment on the quality of the presentation and note missing literature or issues that should have been addressed. Often, reviewers will provide a summary recommendation.

The editors will then evaluate the reviews and come to some conclusion about the submission’s suitability for publication. In general, it’s the specificity of the reviewer’s comments that help the editor judge the appropriateness of a review, and detailed reviews will carry more weight when deciding the fate of a report. Biased reviews are rare, but they do occur. Editors will generally discount overly critical comments that do not focus on the specifics of the manuscript. If the reviews that are returned are not sufficiently thoughtful, the manuscript will often be sent out for additional reviews.

Rarely does a submission get accepted without revision. Most manuscripts benefit (often greatly) by the independent comments of reviewers, who are likely to think about the subject matter a little differently. If there are many issues of concern, it is not unusual for a manuscript to go through multiple rounds of review and revision. Authors can reduce the likelihood of this by paying careful attention to their revisions not just with regard to the specific reviewer comments but to the report as a whole.

Most journals request a response to the reviewer comments. This response should be used to note changes made to a manuscript and reasons for not including requested changes.Verbose responses are rarely beneficial and will only make the editors feel that you are dancing around issues and wasting their time. Remember, it is the manuscript that is judged and not the author’s response. If comments were unaddressed or only addressed superficially in the manuscript, you stand a poor chance of acceptance.

What to do when things don’t go your way?

It takes time to learn what is expected by individual journals, so if you are new to scientific publishing, it is not unusual to receive a few rejections. Also, if you are in research long enough, it is inevitable that a few manuscripts will be rejected. Sometimes rejections can be harsh. (I was once told by a JAOCS Associate Editor not only that my submission was unacceptable but that I should not be a member of the AOCS.) When you receive a rejection, evaluate the comments carefully. For the most part, reviewers are not trying to be negative or discouraging but presumably have identified issues that may not have been thought about carefully. If comments regarding novelty are returned, be sure the literature has been thoroughly reviewed and that your thesis is original. If experimental issues are noted, a rethinking of the methods, experimental design, or data collection will be needed. If the data does not support the conclusions, then possibly the entire project may need to be rethought.

It is possible that after evaluating the comments, you will disagree with the decision. This can be a sign that the report was not written clearly enough for the reviewers to appreciate your points. Usually this calls for rewriting. Sometimes this occurs because the choice of journal was not ideal. For example, manuscripts with complicated statistical or mathematical methods might not be well received in a chemistry journal. If after considering the comments the thesis is still sound, consider submitting the report to another journal.

Michael K. Dowd is a chemical engineer at the US Department of Agriculture–Agricultural Research Service, and a senior associate editor for the Journal of the American Oil Chemists’ Society (JAOCS). He can be contacted at Michael.Dowd@ARS.USDA.GOV.

Further reading

If there was one word to describe the 106th AOCS Annual Meeting and Industry Showcases in Orlando, Florida, May 3–6, it was choice. For starters, those who attended the premier conference for professionals interested in oils, fats, and related materials could choose to visit any of three separate campuses—each with its own blend of interest areas, technical sessions, poster presentations, and industry showcases.

As guests circulated through the campuses, those interested in technical topics ranging from beverage emulsions to surfactants and solvents in enhanced oil recovery could not only choose from the lawn party was a big hit.

AOCS’ own technical program featured about 400 oral and 176 poster presentations on almost every conceivable topic, from oil stabilization in peanut butter, to the health effects of stearic acid, to wet wipe cleaning solutions, to formulating solutions for hair repair. Registrants could also attend joint sessions with the Society of Cosmetic Chemists that focused on areas common to both groups, such as lipid oils and skin health, surfactants and cosmetic science, and strategies in the advanced use of proteins and peptides. They also had access to the two SCC technical sessions, which were held on the Felix Paquin Campus.

2015 AOCS Annual Meeting and Industry Showcases

Kathy Heine

Registrants at the 106th AOCS Annual Meeting and Industry Showcases could visit three separate campuses divided by interest areas.

Five hot topics, lots of special sessions for academics, three joint sessions with the Society of Cosmetic Chemists (SCC), access to the SCC’s two technical sessions, and a sensory luncheon experience gave people plenty of options.

The lawn party was a big hit.
During the lawn party, some meeting registrants kicked off their shoes (sandals) to play beach volleyball, while others went ahead and played basketball in their three piece suits.

about 400 oral and 176 poster presentations in AOCS’ own technical program, they could also attend the three technical sessions that were held jointly with the Society of Cosmetic Chemists (SCC) or listen in on the two technical sessions held by the SCC on the Felix Paquin Campus.

Choice options for academics included the Lipids 50th Volume Symposium, “Lipid-binding proteins: fatty acid metabolism, trafficking, and signaling from gut to brain,” as well as special sessions on the statistical design of experiments and training for AOCS journal editors, reviewers, and authors; the challenges and rewards of the mentoring relationship; and enhancing one’s career by becoming a journal reviewer.

Deciding which of five hot topics to attend was not easy when faced with such choices as “High times for higher standards: Cannabis is creating new opportunities,” “Saturated fat in the diet: Where do formulators go from here?” “Combating scientific misinformation through scientific activism,” “Outlook on healthy oils: New policies, functions, and innovations,” and “Research on fatty acids in human health and function.”

There was even a choice for adventurous types. The ticketed luncheon event, “Understanding olive oil: The romance and the reality,” was a total immersion experience in which trained olive oil sensory experts introduced participants to official olive oil tasting protocol before leading them in a tasting of six olive oils that included top award-winners from around the world.

Although individuals had more options to choose from than ever, it was the event that drew the largest number of registrants together that everyone was talking about. Perhaps it was the tropical weather or the proximity to Disney World. Whatever the reason, during the lawn party on Tuesday evening, students, young professionals, board members, division and section leaders, and industry showcase partners alike really kicked off their shoes, let down their hair, and played. They spiked and assisted one another during beach volleyball, slammed dunked and blocked one another in basketball, and challenged each other to impromptu sandbag tosses and hobo golf. It was a rare chance to connect in a way that left them hungry for a rematch, May 1–4, 2016, in Salt Lake City, Utah, USA!

Kathy Heine is managing editor of Inform. She can be contacted at kheine@aocs.org.
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