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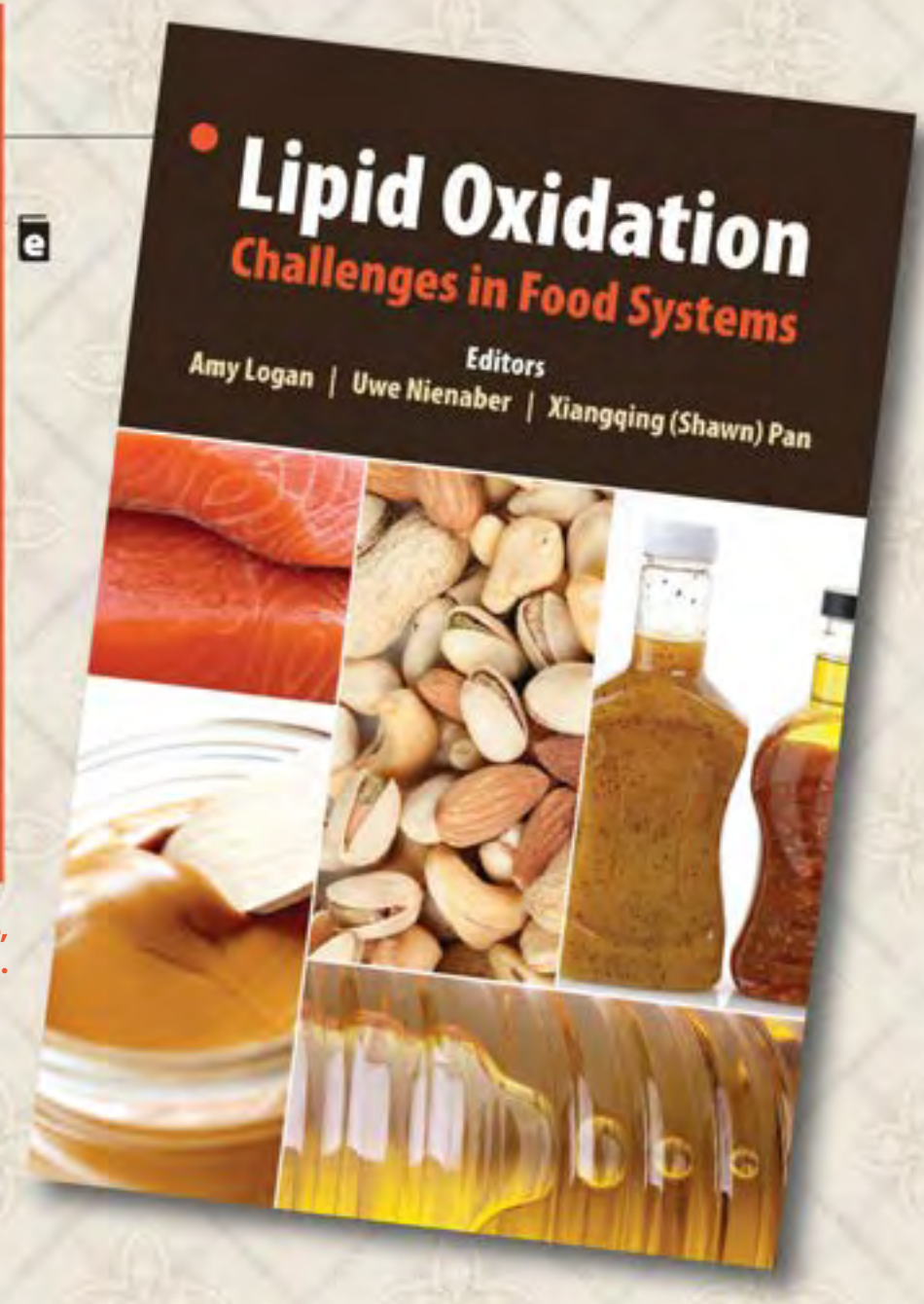

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FLUSH TO

CHRISTINE HERMAN

It is a matter of time before biofuels become essential for powering the planet. As the population grows and the fossil fuel supply continues to dwindle, we will need sustainable, bio-based fuels to take the lead in the energy market. But, biofuels, most notably ethanol, have received criticism because they often compete with food for land space, putting food stocks at risk.

• It sounds like the ideal scenario. Municipal wastewater—a rich concoction containing nitrogen and phosphorous—is fed to algae, which grow while simultaneously converting carbon dioxide into oxygen, treating the water, and creating biomass that can be harvested and converted to biofuel and other useful products.

• Seem too good to be true? Some experts think so, while others continue to hold out hope that this approach will help keep wastewater from wreaking havoc on the environment while reducing our dependence on fossil fuels.

• There may be a long road ahead for algal biofuels, but coupling algae growth with wastewater treatment could help pave the way. This feature presents examples of how researchers in both academic and industry settings are taking the lead in these efforts.

For this reason and others, algae have come into the spotlight in recent decades as a source of biomass for fuel. The term algae refers to a diverse group of photosynthetic, eukaryotic organisms, which includes unicellular forms, such as microalgae and cyanobacteria, as well as multicellular macroalgae, more commonly known as seaweed. (For simplicity, we will refer to microalgae simply as "algae" in this report). Algal biofuels avoid the "food versus fuel" conundrum since they can be grown on nonarable land—typically in open ponds or enclosed photobioreactors. And, given their incredible diversity, algae have enormous potential for helping solve the world's energy crisis.

"There are conservatively 100,000 different species of algae, and up to closer to a million if you really dig into the literature," says Ryan Davis, senior member of the technical staff at Sandia National Laboratories (Livermore, California, USA). "Not all of those are going to grow really fast, and not all of them will be oleaginous like we'd like for biodiesel, but there's definitely some opportunity there."

ALGAL BIOFUELS: THE PROMISE AND THE CHALLENGE

Algae have attracted the interest of biofuels researchers worldwide because of its high-oil content, which can be extracted and converted into biodiesel. Its other components—including proteins, carbohydrates, and metabolites—can be separately processed into valuable products using a variety of conversion technologies, including chemical, biochemical, and thermochemical processes. The process of extracting and isolating the individual components can be energy-intensive and difficult to optimize. So, others have developed methods to convert the entire algal biomass into biocrude, which can be further refined and blended in with petroleum as a "drop-in" fuel.

There are many strains of algae that naturally grow on marine water, and those that require freshwater can be grown on municipal wastewater, which contains nitro-

FUEL

gen and phosphorus—nutrients necessary for growth. Biofuel production from algae has the potential to be a nearly "carbon-neutral" process, meaning the amount of carbon released to produce and process it is roughly equal to the amount of carbon that was captured in the process of growing it (see http://tinyurl.com/algae-carbon-neutral (pdf)). Also, on average, algae have a higher oil yield than traditional oilseed crops—anywhere from 30 to 100 times more oil per acre than corn and soybeans.

"We and others have demonstrated that you can grow algae to produce 70% of their dry weight as lipids that can be converted to biodiesel in a one-step process," Davis says.

So why has biofuel made from algae not taken the energy market by storm? A large part of the explanation comes down to the economics.

Peter Pfromm, chemical engineering professor at Kansas State University (Manhattan, USA), says there are two major cost considerations: the infrastructure for growing and processing the algae, and the ongoing costs for operating the facilities. The facilities can be quite expensive, he says, but "then you hopefully will make that money back over time and make some more money by selling biodiesel in the marketplace."

The problem is that algal biofuels are not cheap to make, and there is only so much you can do to make the process more efficient. "There is not unlimited room for improvement because ultimately you have so many photons coming from the sun per square meter and time," Pfromm says. "That is not something you can change . . . That's the physics that limits the process."

Pfromm and his coworkers conducted a 2014 research study on the economic feasibility of algal biodiesel (see http://tinyurl.com/Pfromm-2014), in which they concluded that the success of algal biodiesel will require "significant and continued support" from the government in the form of regulations and financial incentives. The analysis was performed with optimistic assumptions about algae productivity, such as an estimated yield of 50 grams of biomass per square meter per day. The academic study was performed without external financial sponsorship of any kind, Pfromm says, emphasizing that since he and his col-

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FIG. 1. The process of creating algal biofuels involves four basic steps: growing, harvesting, dewatering, and extraction. Microalgae growth, which requires sunlight, nutrients, and carbon dioxide, can also be coupled with wastewater treatment to produce clean water, fertilizer, and other useful valuable products.

laborators do not perform research on algal biofuels themselves, they did not have a bias going into the analysis to favor one outcome over another.

But, the economic challenges aren't stopping researchers from trying to make algal biofuels a success—with or without supportive governmental policies.

Bruce Rittmann, a professor of environmental engineering at Arizona State University (ASU; Phoenix, USA), is working on ways to maximize the output of algal biomass grown in photobioreactors with the goal of increasing its overall production rate. "The No. 1 holdup is we can't get high enough productivity to make it economically viable," Rittmann says. "If we had that now, everybody would be doing it. But, we're doing various types of research and development to get over this hump."

Photobioreactors, since they are closed systems, allow the algae to grow in a highly controlled environment, which results in higher yields. But, they are about 10 times more expensive than traditional open pond systems. "There's no question that you can get better performance in a photobioreactor," says Jim Flatt, president of the Genovia Bio Division of Synthetic Genomics (La Jolla, California, USA). "The challenge has been that that incremental performance benefit hasn't usually been justified by the added capital investment."

For this reason, those interested in producing algal biofuels tend to lean toward open pond systems, which come with their own challenges, namely contamination and water evaporation. Researchers in the field seem to agree that photobioreactors will likely be reserved for higher-value algae-based products, such as nutritional and food ingredients, and the more affordable open pond systems will be used for generating biofuels.

Still, it's not enough for algal biofuels to simply sell for greater than the cost of making them. They have to be produced cheaply enough to compete with other fuels on the market.

"If you can get an energy return of greater than one, you're doing something," Sandia's Davis says. But at this point, petrochemicals are getting a much greater return on invested energy, so it's hard to compete.

Because of the tough technical challenges, Flatt says Synthetic Genomics is simultaneously pursuing algae for both intermediate- and high-value products in addition to a separate research effort with ExxonMobil (Irving, Texas, USA) on biofuels. In an effort to make algal biofuels economically feasible, the company is exploring various techniques, including genome editing and genetic modification, to improve photosynthetic efficiency and direct more of the carbon absorbed in the process to lipid production.

"Biofuels remain a strong interest for the company, but we also know they're very long-term," Flatt says. "The average selling price of fuel is still relatively low compared to many other commodity products. As such, it's really going to require the most mature technology available in order to be economical." In other words, in the near-term, the company plans to work its way up the learning curve with respect to growing, processing, and scaling up algal technologies while getting intermediate- and high-value algae-based products into the market. Then, when the economically competitive algal strains are ready for development into biofuels, the company will be have the know-how to cultivate and process them in an economical way, which will increase their chances of succeeding in the energy market.

Other companies, such as Muradel Pty Ltd (Whyalla, South Australia), are going full steam ahead toward using algae to create biofuels. Researchers at Muradel use a type of microalgal strain that is halophytic, meaning it can grow in shallow, open raceway ponds in water containing high salt concentrations—up to four times the level in seawater. "We knew from the beginning that to be sustainable we had to minimize the use of freshwater, which is a resource under enormous pressure in Australia," says Andrew Milligan, Muradel's business development manager. So, they have chosen an algal strain with demonstrated high productivity and high oil content that can grow in salinated water, such as seawater or the brine output of a desalination plant.

Since the company is based in Australia, which has an abundance of nonarable land, Milligan says that production of algal biofuels will not compete with food production. "We calculate that an area of 10,000 km^2 dedicated to microalgae production could provide all of Australia's liquid transport fuels," Milligan says. "That equates to less than 0.2% of Australia's total land area." Although the company is still "precommercial" (meaning it cannot yet compete on price with fossil fuels), it hopes to create a full-scale commercial and profitable operation, comprised of 1,000 hectares of algal ponds, by 2019. It is also looking into ways to create valuable byproducts of fuel production, which could help boost the bottom line.

COUPLING ALGAE GROWTH WITH WASTEWATER TREATMENT

Another potential avenue for making algal biofuels more profitable involves growing algae with the help of something that exists in great abundance all over the world: wastewater. Since wastewater is rich in nutrients that algae need to grow, some researchers think we need to start viewing it not as something to be discarded but as a resource to be exploited for the benefit of the environment.

FIG. 2. Lucas McGrath, a researcher at Sandia National Laboratories adjusts the nutrient injection ports on an algal tank mixing arm. The goal is to generate a source of biofuel that can be grown on nonarable land to avoid competition with food crops. Photo credit: Randy Montoya.

Using algae to treat wastewater is nothing new. Microalgae are well-known for their ability to remove nutrients, organic contaminants, and heavy metals from the water they are cultivated in. If not removed from wastewater prior to its release into the environment, these components can wreak havoc, causing harmful algal blooms and areas known as "dead zones," where uncontrolled algae growth causes oxygen depletion in the water, killing off fish and creating major ecological catastrophes, Sandia's Davis says. So, wastewater treatment with algae is important—but even better if you can then harvest the algae to create useful products, such as biofuel.

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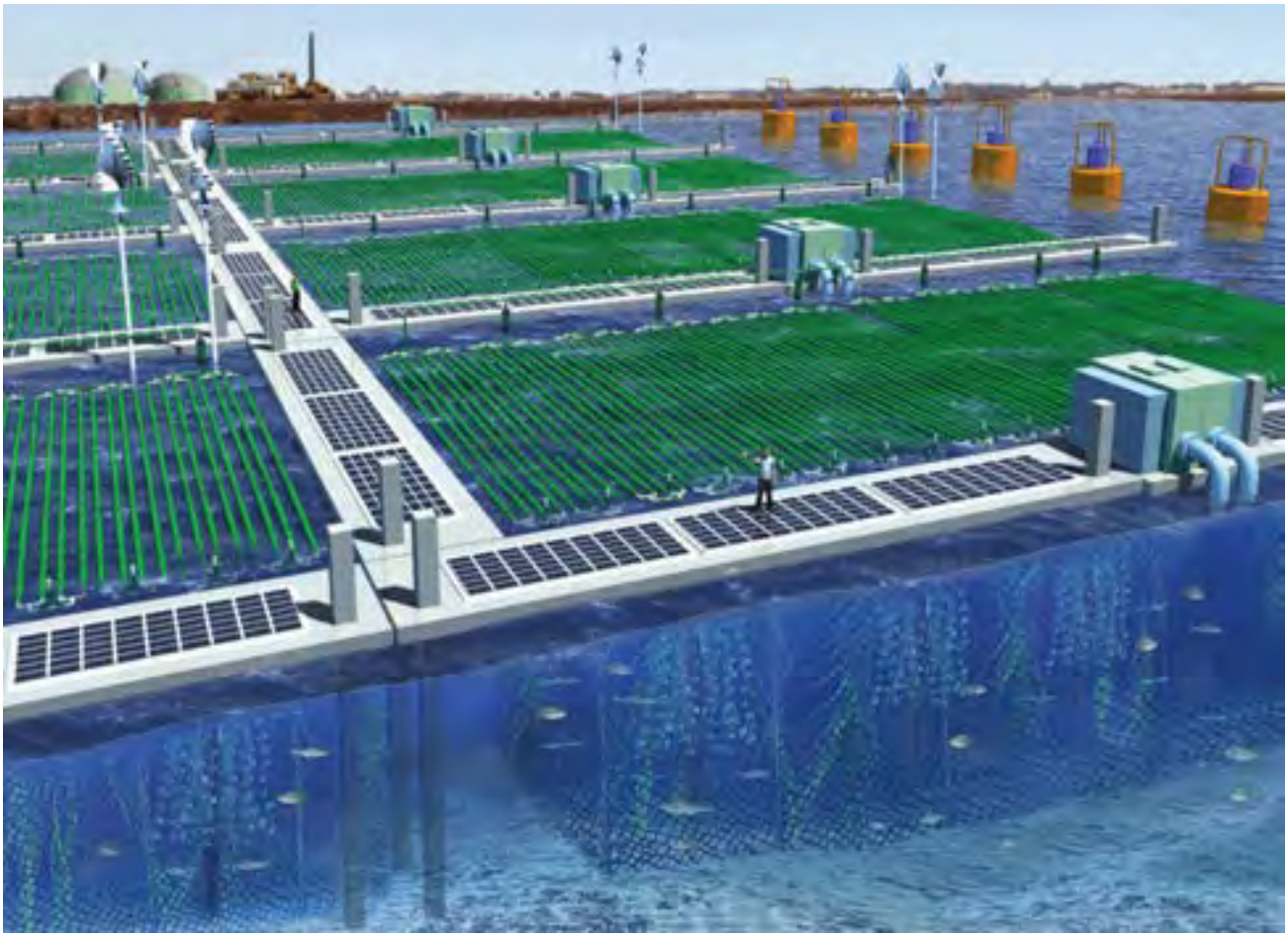

FIG. 3. The OMEGA approach. A team of scientists led by NASA's Jonathan Trent is developing a process for growing algae on municipal wastewater. The project, known as OMEGA, stands for offshore membrane enclosures for growing algae. Credit: T. Esposito, Top Spin.

"If we can start directly converting our waste . . . into fuels, then we're doing good on two ends," Davis says. "We're supporting our domestic independence from foreign fuel resources, we're diversifying our liquid fuels, and we're also solving the problem of uncontrolled nutrient release" into our water streams.

Jonathan Trent, adjunct professor at the University of California, Santa Cruz (USA) and a scientist with NASA (National Aeronautics and Space Administration; Ames Research Center, Moffett Field, California, USA), agrees. "There are huge amounts of useful nutrients in the waste streams from our cities and we're basically throwing it away," says Trent, who is working on a project known as OMEGA, which stands for offshore membrane enclosures for growing algae.

Currently, in San Francisco (California, USA), wastewater is processed by screening, settling, and biological nutrient removal to a level of cleanliness known as "secondary" treatment before it is released into the bay. Secondary-treated wastewater still has plenty of nutrients in it to grow algae. That is usually not a problem in San Francisco Bay, but it is a great resource for the algae grown in the OMEGA system, Trent says. By simultaneously growing algae and treating wastewater, companies can save money by not having to pay for other means of biological nutrient removal. Additional cost savings can be expected if the entire operation is moved off of land to an enclosed bay (a key component of the OMEGA vision for wastewater treatment and algae production). The water in the bay cools and mixes the algae with the wastewater to stimulate growth, and the offshore infrastructure can be coupled with aquaculture and energy-generating technologies, such as wind turbines and water-cooled solar panels. "As you start adding these value-laden additional activities, then the cost of the fuel, which is part of this overall system, comes down," Trent says. "The OMEGA project is about food, it's about water, and it's about energy from solar and from biofuels, but it's also about cleaning up the environment—all things we need to improve to sustain our lifestyle in a world of seven billion people and counting."

Trent and his colleagues have performed techno-economic analyses that suggest that if the OMEGA approach were scaled up to accommodate all 65 million gallons (or roughly 250 million liters) of wastewater produced by one of the treatment plants in San Francisco, the system would generate roughly 3 to 6 million gallons of biofuel a

year. Instead of trying to grow a pure culture and go through the process of dewatering and extraction to create biodiesel, Trent's team uses a chemical process known as hydrothermal liquefaction to convert the partially dewatered but wet algal biomass into a biocrude that can be further refined and blended with petroleum fuels. They are also working on methods to use an underwater anaerobic digester to convert the biomass into natural gas. The products of the anaerobic digester—methane and carbon dioxide—can be used to produce either syngas (a mixture of carbon dioxide, carbon monoxide, and hydrogen) or burned to create heat to produce energy by turning turbines. The carbon dioxide produced by both the digester and by burning the methane is pumped back into the OMEGA system to feed the algae, creating a closed system.

The team has also developed an efficient method to recover the wastewater from the algae cultures as "super-clean water" ready to be used for industrial purposes or even drinking water. This wastewater-recovery technology combines algae pretreatment with forward and reverse osmosis (see sidebar). "The OMEGA system is about looking at wastes as resources and creating an 'ecology of technologies' by optimizing everything the system and the environment has to offer," Trent says.

Not everyone thinks coupling algal biofuels with wastewater treatment is a good idea. ASU's Rittmann, whose research directions include algal biofuels and wastewater treatment, although not a combination of the two, writes in an email, "I can describe much better ways to revolutionize wastewater treatment while not 'messing up' microalgae technology." In an interview, Rittmann explained that wastewater treatment with algae is "a very old technology . . . and it's generally proven to be not very reliable in terms of producing very good quality wastewater treatment." The efficiency of the process plummets on days when the sun is not out or the temperature is not just right. Add in the fact that wastewater is a highly variable stream and the whole approach is "not really commensurate with having a high production system to produce a lot of algae." In short, if you try to both grow algae and treat wastewater, Rittmann doesn't think you will be able to do either one particularly well.

Trent agrees that algae can be finicky. "And, if you're trying to grow a certain species that is a high oil producer, you may be frustrated by trying to do it in a wastewater environment," he says. "But, if you're open to just [creating] biomass, and your system is a community that is being enriched by these incredibly high concentrations of nutrients," there are ways you can manage the community to keep it working optimally.

This is precisely the approach Algae Systems (Daphne, Alabama, USA) takes to ensure that the algae

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Algal biofuel technology helps convert wastewater to drinking water

Reverse osmosis of seawater is a technique commonly used to produce clean water and brine. The water is kept and the brine is considered waste, although it took as much energy to make the brine as it did to make the clean water. In a project known as OMEGA (offshore membrane enclosures for growing algae), NASA scientist Jonathan Trent and his colleagues use the waste brine from reverse osmosis to drive forward osmosis of algae-treated wastewater.

"The brine pulls algae-wastewater through forward-osmosis membranes, which cleans the water and dilutes the brine to about 1% salt," Trent says. "This is less than 1/3 the salt concentration in seawater, which is 3.5% salt. The forward-osmosis treated wastewater-brine is then processed by reverse osmosis to produce clean water and to recover the brine, which is used again."

The OMEGA wastewater, treated by algae, forward osmosis, and reverse osmosis, is recovered as clean water, and the process utilizes and detoxifies the brine, which is currently a problematic waste-product of reverse osmosis. In this way, the process of producing algae biomass with the OMEGA approach turns wastewater into drinking water, Trent says.

they grow on wastewater are able to thrive. "We measure the incoming wastewater for the qualities we're looking for" and adjust the levels of nutrients and water accordingly, says Matthew Atwood, the CEO. The question the company considers is: "'Do we want to treat more wastewater or grow more algae?' and depending on what we're trying to do, we can change the recipe mix, so to speak."

Atwood says Algae Systems was inspired by the OMEGA project to create offshore membrane enclosures to treat wastewater, although the technology it has developed is different and separate from what NASA has done. The company's aim is to "develop technologies at the water-energy nexus and to try to reconceptualize the way that municipal infrastructure is developed." It is currently operating a demonstration plant and plan to take the next few years to gather data that will help it optimize the productivity of algae growth and wastewater treatment under various climate conditions. The plan is to take that information and learn how to scale up the technology and tailor it so that it can be applied in different geographic locations to meet the unique needs of the local environment and community. "We need to demonstrate that we can operate the system safely for a long period of time and find partners that are willing to work with us to develop these systems and scale it up," Atwood says.

Even if algae can grow on wastewater in useful quantities, and the wastewater can be adequately treated for safe release into the environment, Davis, sees another limitation to coupling the two processes, at least in the United States.

"The scale of liquid fuel consumption in the United States is much larger than the amount of nutrients we could ever recapture in any kind of realistic way," Davis says. "So, yes, we have this synergy between wastewater and algae, but there are limits to how far we can go."

That wastewater is a resource available in any industrialized locale may seem like a good thing for algae growers. The problem, says KSU's Pfromm, is that to exploit all available wastewater for cultivating algae, many small facilities will need to be built, as opposed to a few large facilities (the cost of transporting wastewater long distances would quickly cancel out the benefits of any resulting biofuels). "That makes it less efficient by definition," he says.

But, Davis is hopeful that the development of micro-modules for processing algae grown on wastewater will help mitigate this problem. He and his coworkers are developing a technology for capturing, dewatering, and processing algae on a small scale. The scales will have to be adaptable, Davis says, and the techno-economics still have to be worked out to determine if the process is efficient enough to be viable. "It's a challenge, but I don't think it's insurmountable."

Even companies that are not trying to couple wastewater treatment with algae growth are looking into ways to exploit wastewater for biofuels. Using the same hydrothermal liquefaction technology that it uses to process algae, Australia's Muradel converts biosolids—one of the byproducts of wastewater treatment—into crude, and find that it has a high energy content that is suitable for mixing with petroleum fuels. "We are solving what was previously an intractable waste problem for wastewater treatment plants," Milligan says. "It's a win-win for both parties."

There are huge amounts of useful nutrients in the waste streams from our cities and we're basically throwing it away.

NASA's Trent thinks researchers and industries need to be persistent in working toward sustainable solutions if they are serious about putting a dent in fossil fuel consumption. "There are always going to be naysayers; these people who are arguing whether the cup is half empty or half full," he says. "But, the OMEGA project is saying no, no, no, you're using the wrong cup. We really need to move our thinking to how do we make an efficient system given that we need to make a [renewable] liquid fuel for the forseeable future . . . The right attitude is not to ask: 'What's wrong with the system?,' but 'How can we make this work and be sustainable?'"

THE WAY FORWARD

Now, Trent says his goal is to find people who embrace the OMEGA vision and want to make it work in their location. He hopes to serve as a resource in those efforts. Since the first three years spent on the project were full of false starts and mistakes, Trent says, "I can tell people what not to do." As he explained in a 2012 TED talk he gave on the OMEGA project (see http://tinyurl.com/Trent-TED), like Thomas Edison, he has learned many ways the system does *not* work. And now he says, "I'm willing to go anywhere in the world to make OMEGA work for the sake of future generations.

"It's really about creating a sustainable system for society," Trent says, "and failure is not an option." (Although Trent works for NASA, he was not interviewed as a NASA civil servant, and his views are not necessarily representative of NASA's opinion or that of the US government).

Algae Systems's Atwood understands there are challenges at the water-energy nexus but says he's determined to find a way forward. "We're as much a wastewater treatment company as we are a fuel producing company," he says. "We believe fundamentally that the future requires a different relationship to waste, and . . . we cannot continue to develop a municipal infrastructure or [wastewater treatment] plants that externalize the costs of releasing large amounts of nutrients into the ocean and losing them.

"We have to close those cycles, and algae [are] nature's way of doing that," he adds. "We can grow the algae in a closed system and gain the benefits of it by being smart about how we deal with it downstream."

Christine Herman is a science writer for AOCS.

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LIPIDS FROM ALGAE: NOVEL APPLICATIONS AND POWERFUL QUANTIFICATION METHODS

ANDREW DEAN AND JON PITTMAN

Algae are a large and diverse group of simple plants that use solar energy to fix carbon through photosynthesis. They form the basis of the food chain in lakes and oceans, range in size from giant seaweeds to tiny free-floating single cells, and are vital to the wellbeing of the planet. Algae use solar energy to convert CO_2 into a number of metabolites that can be used across a number of industries including personal care, drugs, food, health, materials, and energy. Such metabolites include oils and lipids, which can be used to produce food and health products, lubricants, biofuels, bioplastics, biosurfactants, and many other products.

- **There is an estimated 70,000 species of algae from as many as 15 different phyla, but the vast majority have not been characterized in terms of their lipid content.**
- **Many algal species have very low total lipid yields but they synthesize an abundance of lipids under conditions of stress. Consequently, to harness the full potential of algae, efficient methods are needed to screen the lipid content of strains under varying environmental conditions.**
- **This article explains how new spectroscopic techniques, such as Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy coupled with multivariate data analysis, can be used to rapidly screen strains for specific lipid profiles.**

Some algal species can produce high amounts of health-promoting long-chain polyunsaturated omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and species known to do this are being cultivated commercially. For example, *Schizochytrium* sp. accumulates neutral lipids that are rich in DHA, while *Phaeodactylum tricornutum* produces high amounts of EPA. Using algae to source DHA and EPA may have environmental as well as health benefits. Currently, the main source of omega-3 fatty acids is fish oils but there is concern over the long-term sustainability and environmental cost of these supplies, and algae may provide an alternative sustainable source.

Other algae produce high quantities of lipids (up to 70% of dry weight) with a high proportion of triacylglycerol (TAG). Extracted TAG can be converted into biodiesel by following the same transesterification procedure as for other vegetable oils. Species like *Nannochloropsis* sp. can accumulate high quantities of TAG that may not be as ideal for nutritional use, but are suitable for fuel conversion. Other algae, such as *Botryococcus braunii*, can produce high quantities of triterpenic hydrocarbons that can be more easily converted into fuels.

One challenge with using algae as a source of lipids for biotechnological applications is that these organisms are very diverse in number (estimates of over 70,000 species) and in characteristic (includes organisms from as many as

15 distinct phyla), and the vast majority have not been characterised in terms of their lipid content. There is thus an enormous number of algal species that may be important future sources of lipid products. Furthermore, although many algal species have very low total lipid yields, they may synthesize lipids to high abundance under conditions of stress, such as nutrient starvation. Therefore, to harness the full potential of microalgae, efficient methods for screening the lipid content of strains under varying environmental conditions are needed.

In recent years, a number of research groups have begun to demonstrate that spectroscopic techniques, such as Fourier transform infrared (FTIR) spectroscopy and Raman spectroscopy coupled with multivariate data analysis, are particularly powerful high-throughput methods for algal lipidomics, in particular for rapidly screening strains for specific lipid profiles. An advantage of the FTIR spectroscopy technique is that small sample sizes can be used with simple processing that does not require cell extraction. For example, in a typical experimental setup, small volumes (<0.5 ml) of up to 96 samples of algal cells can be harvested, centrifuged, and re-suspended in a small volume of water (~20 µl) and deposited on a silicon plate. The plate is then analysed using FTIR spectroscopy and the 96 rapidly generated spectra processed for the assessment of cellular lipids, proteins, and carbohydrates.

Figure 1 shows images of a green algae (*Chlamydomonas reinhardtii*) grown under conditions of nitrogen starvation (which induces lipid synthesis) in which lipid bodies can be clearly observed. FTIR spectra from such nutrient starved cells, together with spectra from nutrient sufficient cells, are shown in Figure 2 (page 16). These spectra clearly show increases in lipid biosynthesis as well as an increase in carbohydrate (starch) accumulation. This increase in lipid is evident from the bands at ~1,740 cm^{-1}, associated with the υ(C=O) of ester groups of triglycerides and fatty acids, and the bands at 3100-2800 cm^{-1} from υ(C-H) of lipids. In addition, the carbohydrate bands at 1,200-900 cm^{-1} (υ(C-O-C) of polysaccharides) are also clearly elevated when compared to the spectrum from the cell grown in nutrient-replete conditions. Bands attributed to υ(C=O) stretching of amides from proteins (amide I, ~1,655 cm^{-1}); δ(N-H) bending of amides from proteins (amide II, ~1,545 cm^{-1}) can also be clearly seen.

Relative quantification of the lipid bands can be provided by normalization of the spectrum. This can be accomplished by extended multiplicative scatter correction or by determining the height or area under the band in relation to that of a macromolecule that remains unchanged between treatments or strains, such as normalization to the to the amide I peak. Confidence in the quantification of neutral lipid by this method can be gained by comparing the FTIR-derived lipid values with neutral lipid concentration determined by other methods, such as through the use of the neutral lipid-specific fluorescent stain Nile Red. In the example shown in Figure 3 (page 17), a very strong correlation

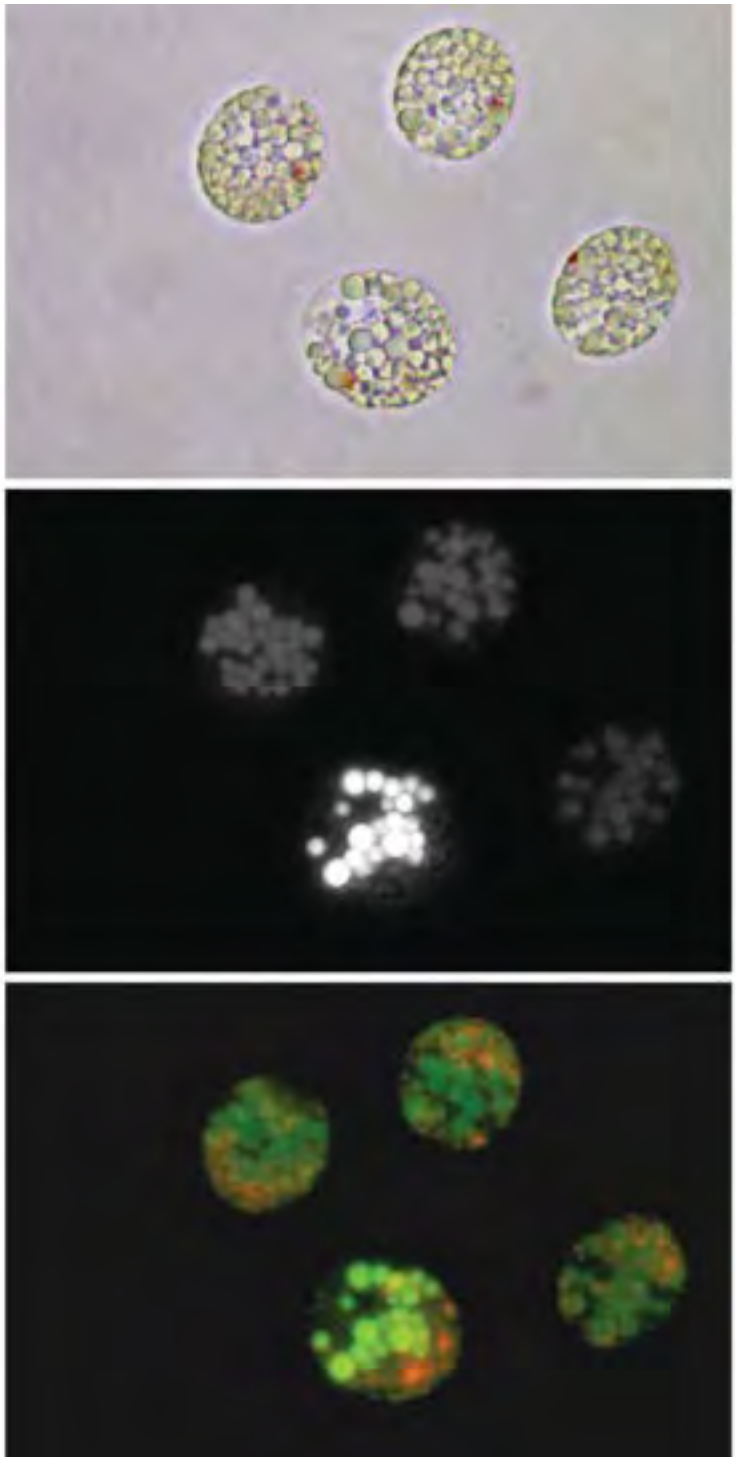

Figure 1. Neutral lipid localization in *Chlamydomonas reinhardtii* detected by Nile Red staining in response to N starvation. Epifluorescence images were taken 10 min. after addition of Nile Red to the cells. (A) Cells imaged using light microscopy, (B) Nile Red fluorescence alone and (C) Nile Red fluorescence (yellow/green) with chlorophyll fluorescence (red). Cells are approximately 10 µm in diameter.

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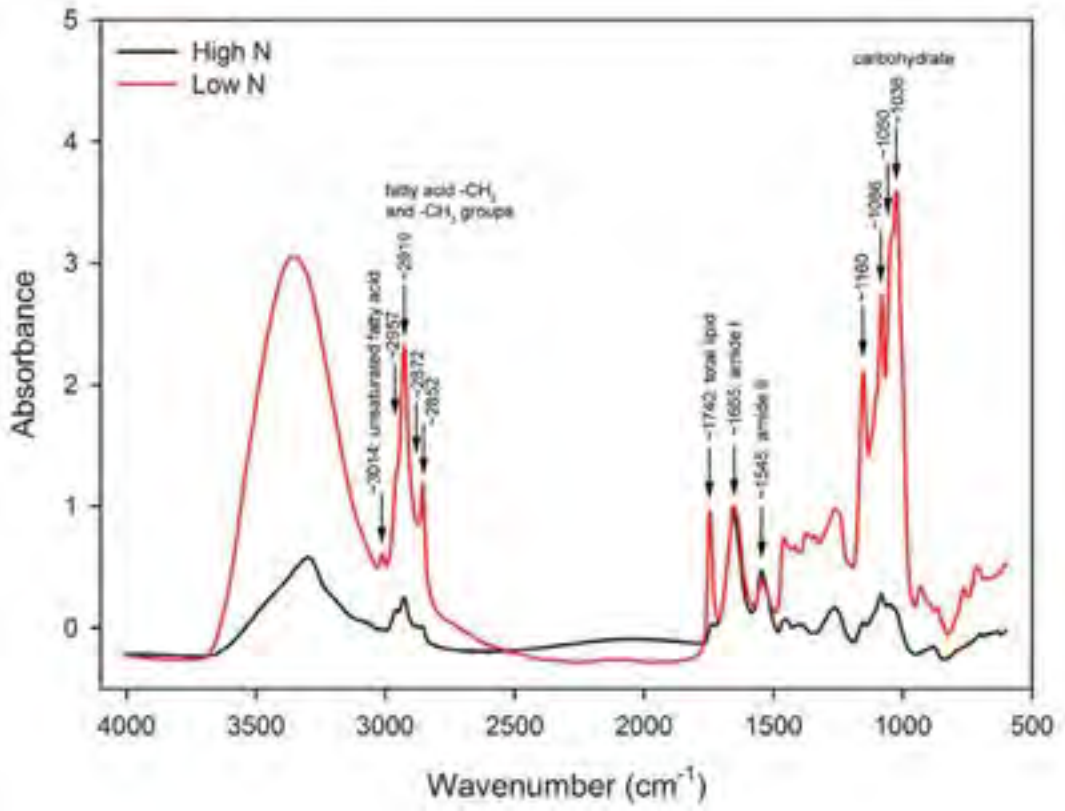

Figure 2. Typical FTIR spectra (4000–600 cm-1) taken from *Chlamydomonas reinhardtii* grown under nitrogen sufficient (black line) and nitrogen starved (red line) conditions. Both spectra are normalized to the amide I peak at 1655 cm-1.

between lipid:amide I ratio (band heights at 1,740 cm^{-1} and 1,655 cm^{-1}) and Nile Red fluorescence is seen.

Although the FTIR spectra can very readily provide detection and quantification of total lipid, particularly through quantification of the ~1,740 cm^{-1} band, the spectra can also provide more information about the lipid properties. For example, the C-H stretching of C=CH- chains at ~3,014 cm^{-1} can be used to determine unsaturation of lipids, while four band peaks between ~2,957–2,852 cm^{-1} are due to C-H stretching of symmetric and asymmetric $–CH_2$ and $–CH_3$ functional groups (Figure 2).

In our laboratory, we regularly use FTIR spectroscopy as a high-throughput methodology to screen large numbers of algal cultures for total lipid content. These cultures may comprise a range of different strains/species/mutants that have different lipid accumulation profiles, or a single species that has been cultivated under different stress conditions to induce maximal lipid synthesis. Once FTIR spectroscopy has identified algal strains or experimental conditions that provide the desired lipid characteristics, further detailed analysis of the lipids can be performed using other techniques, such as gas chromatography or liquid chromatography-mass spectrometry (GC or LC-MS).

Algae can be a potentially sustainable source of oils and lipids that be used in the future for a variety of applications, from foods to fuels and other high-value chemicals. But the development and use of analytical techniques, such as FTIR and Raman spectroscopy—in addition to the more conventional GC and LC-MS—are needed to realize this potential.

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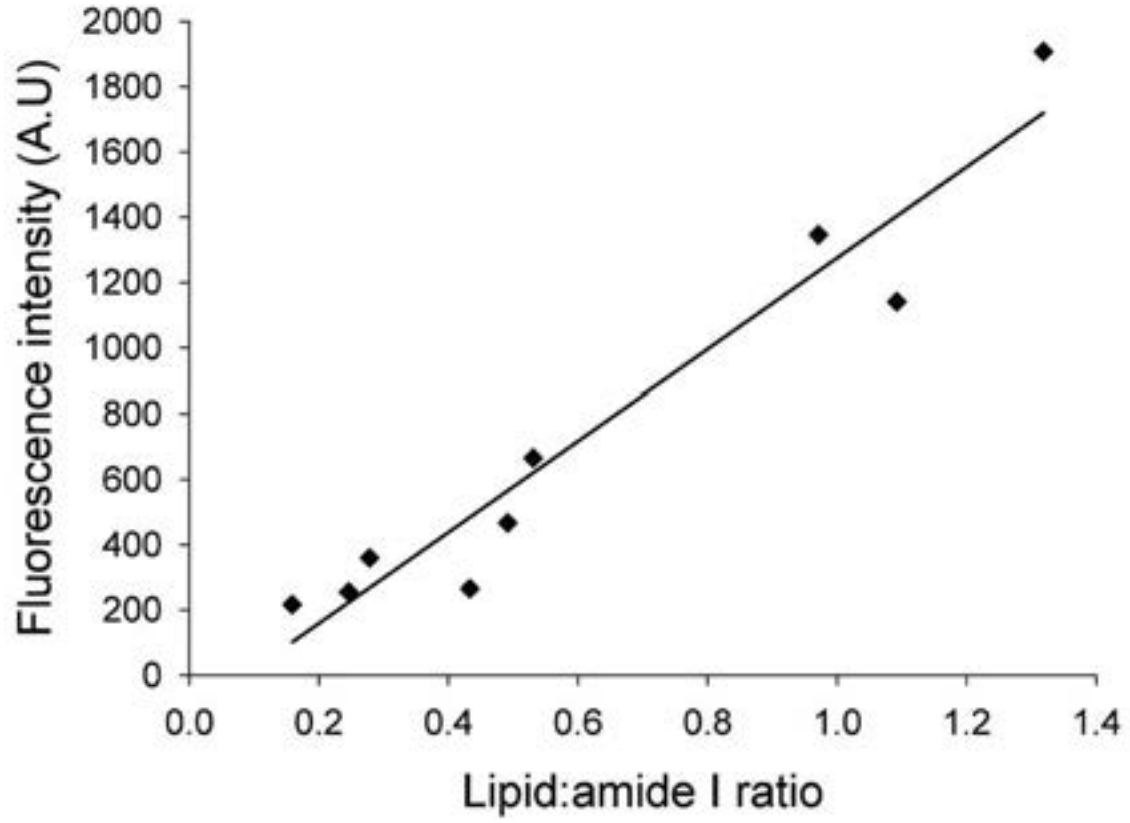

Figure 3. Correlation between total lipid values estimated by FTIR spectroscopy (1,740 cm^{-1} lipid : 1,655 cm^{-1} amide I ratio) and by Nile Red neutral lipid stain fluorescence.

AOCS MEETING WATCH

May 3–6, 2015. 106th AOCS Annual Meeting and Industry Showcases, Rosen Shingle Creek, Orlando, Florida, USA. http://annualmeeting.aocs.org

October 27–30, 2015. SODEOPEC2015, Hyatt Regency Miami, Miami, Florida, USA.

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

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

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

RUN & DONE

Adulteration detection just got easier.

MIDI, Inc.'s Sherlock® Software has been trusted for over 25 years to quickly and accurately identify fatty acids and is now expanding to include other industry-focused compounds from **edible oils**. Our proprietary software offers rapid automated analysis of sterols and triterpenes by GC and GC-MS and a library comparison to stored patterns of authentic and adulterated samples.

Olive Oil Adulteration Analysis by Sherlock

When fatty acid analysis alone cannot detect adulteration, additional compounds that may be used for automated adulteration pattern matching include: **4-Desmethylsterols, 4-Monomethylsterols, 4,4′-Dimethylsterols** and **Triterpene Diols.**

ß-Amyrin	Campesterol	24-Methylene-cycloartenol
δ-Amyrin	Citrostadienol	ß-Sitosterol
Δ^5-Avenasterol	Cycloartenol	Δ^7-Stigmastenol
Δ^7-Avenasterol	Erythrodiol	Stigmasterol
Brassicasterol	Lupeol	Uvaol

Example Library Match (95% EVOO, 5% Hazelnut Oil):

Library	Similarity Index	Entry Name
STEROL 1.00	0.971	EVOO 95%, Hazelnut 5%

Benefits of Sherlock Software:

- **System Suitability Monitoring**
- **Automated Library Matching**
- **Repeatable and Reproducible Results**
- **Adulteration Detection Under 10%**

Contact MIDI at oils@midi-inc.com to learn more about our software and how you can start analyzing edible oils faster and easier than ever before. Don't have the equipment? Use our service lab instead.

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BRIEFS

The United Nations' World Health Organization (WHO) is pushing for a total ban on dietary trans fatty acids (TFAs) in Europe (see http://tinyurl.com/trans-WHO). In the WHO European Food and Nutrition Action Plan 2015–2020, the agency suggests "removing TFAs from the food supply is one of the most straightforward public health interventions for reducing the risk of cardiovascular diseases and some cancer, and improving diet."

■■■

Archer Daniels Midland Co. (ADM; Decatur, Illinois, USA) is selling its global chocolate business to Cargill for $440 million, subject to regulatory approvals and a working-capital adjustment. Included in the sale are chocolate manufacturing operations located in Hazleton, Pennsylvania, USA; Milwaukee, Wisconsin, USA; Georgetown, Ontario, Canada; Liverpool, UK; Manage, Belgium; and Mannheim, Germany. Approximately 700 employees will transfer to Cargill with the sale.

■■■

NEWSMAKERS

Gerard Dumancas, an AOCS member since 2007 and former member of the *Inform* Editorial Advisory Committee, has been appointed as lead guest editor of two journal supplements to be published sometime during 2015 in *Bioinformatics and Biology Insights* and *Lipid Insights*. Dumancas is a consultant as well as a chemistry lecturer and laboratory stockroom manager at Oklahoma Baptist University in Shawnee, USA. ■

NEWS & NOTEWORTHY

California sets olive oil standards

The US state of California—through its Department of Food and Agriculture (CDFA)—has approved stricter standards for labeling and grading olive oil.

The new standards (see http://tinyurl.com/CDFA-Standards) went into effect on September 26, 2014, for California producers of at least 5,000 gallons (about 19,000 liters) per year.

"The California olive industry will now be able to distinguish itself as the authentic, premium-quality, extra virgin olive oil producer to American consumers," said Jeff Columbini, chair of the Olive Oil Commission of California (OOCC), in a news release. "Consumers will now be able to know that

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Olive Oil: A Field Guide debuts

You are out to dinner with a sophisticated friend who tastes the olive oil on offer and pronounces it to be a poor quality Picual with notes of fustiness and mustiness. In times past, you would have discreetly Googled the terms to figure out what your friend was saying. Now, you can take along the pocket-sized *Olive Oil: A Field Guide* for help.

This indispensable reference covers everything you wanted to know about olive oil from how to taste it to definitions of olive oil grades and label terms. Learn about varieties, what influences olive oil quality, how to plan a festive olive oil tasting event . . . and more. Each of the guide's 88 pages is packed with information from around the world, minus any commercial slant. Whether you are a consumer wanting to know how to buy and use quality oil or a professional needing to do detailed assessments and make purchasing decisions, this handy book will serve as your guide.

The book is co-published by AOCS Press and the Extra Virgin Alliance (Petaluma, California, USA), an international nonprofit association of olive oil producers, purveyors, and experts. It is available in the AOCS Store at http://tinyurl.com/OO-FieldGuide for $7.95 (members) or $9.95 (nonmembers). Discounts are available for orders of 24 or more books (email orders@aocs.org for more information).

IN MEMORIAM

Frederick P. Simonian, 81, of Western Springs, Illinois, USA, died on August 29, 2014. An AOCS member since 1981, Simonian worked for Dial Corp. and was director of engineering before he retired in 1997. He is survived by his wife, Doris, two sisters, two nieces, three children, and nine grandchildren. Among other activities, Simonian was a board member of the Armenian Missionary Association of America and the Armenian Evangelical Union of North America.

■■■

One of the early and continuing critics of the demonization of saturated fat, Mary G. Enig, died on September 8, 2014. Enig, who was 83, was a long-time member of both AOCS and the American College of Nutrition. She received her Ph.D. in nutritional sciences in 1984 from the University of Maryland. "She continued throughout her lifetime to make the general public aware that dietary trans fats, not saturated fats, were the real culprits in the fat/heart disease dynamic," said her colleagues Harry G. Preuss and Beverly G. Teter in a written eulogy. "[She] will be long remembered for her perseverance and courage to fight for her principles." ■

when they are purchasing and consuming California extra virgin olive oil, it truly is 100% extra virgin olive oil." OOCC is made up of growers and millers and is a part of the CDFA.

The new standards allow the use of two methods of analysis for determining quality and freshness (pyropheophytins, or PPPs, and relative amounts of 1,2- and 1,3-diacylglycerol content, or DAGs) in accordance with International Organization for Standardization (ISO) 29841 and ISO 29822, respectively, and have been found to meet the precision requirements of ISO 5725. Further, the allowable level of free fatty acids in the new standards is 0.5%, which is below the standard of 0.8% set by the Madrid-based International Olive Commission (IOC). The level of campesterol allowed (expressed as a percentage of total sterols) is 4.5%, compared with 4.0% in the IOC standards.

The new California standards ban the use of labeling terms such as "light"—which must now read "refined olive oil"—and "pure"—which must now be labeled as "olive pomace oil." In addition, the standards set out new grading definitions and suggest not using the words "olive oil" when referring to refined olive oil blends or olive pomace oil.

"Consumers and the trade need to understand the important quality difference between extra virgin/virgin olive oils, 'the oils as they come from the olive,' compared with the lower grade refined and pomace oils, 'industrial manufactured olive oils.' The proposed California olive industry standard does this better than any of its many predecessors," testified Paul Miller, president of the Australian Olive Association, speaking at a CDFA hearing held in July 2014.

Mauro Battocchi of the European Union Delegation to the United States testified against the new standards at the same July 2014 hearing: "While the proposed standards will only apply to California producers and handlers over a certain amount of production, the EU remains deeply concerned about possible implications to trade in the short and long term."

Also speaking against the standards was Eryn Balch, executive vice president of the North American Olive Oil Association. "It appears that supporters of the standard intend to favor the only grade commercially sold by local producers, extra virgin olive oil, by attempting to attach negative-sounding technical verbiage to lower grades," testified Balch.

Richard Cantrill, AOCS' chief science officer and technical director, also provided testimony for the July 2014 hearing. In it, he noted that AOCS has worked with and will continue to work with both individuals and organizations to provide a venue for open and frank discussion.

"AOCS has no olive oil grades or standards it wishes to enforce or preserve. The mission of AOCS is to provide analytical accuracy and open the door to innovation and new approaches to solve problems encountered in the oils and fats arena. AOCS is proud that the OOCC has reached out to ask for support in their endeavors, just as other regional entities have worked with us on different projects with global implications."

Alibaba goes public

Alibaba, the Hangzhou-based group of e-commerce businesses, set a record for its $21.8 billion initial public offering in September 2014. The company's market capitalization and sheer volume of business—$8.5 billion in sales and $3.8 billion in net income for its 2013–2014 fiscal year—dominate companies such as Facebook, eBay, and Amazon.

The company's business-to-business online web portals deal in just about everything including oilseeds and oilseed-related equipment. An October 2014 search on "oilseed supplier" netted a total of 1,572 offerings, of which 1,514 were in India. A search for "oilseed extractors" brought back 1,310 vendors ready to give free price quotations on everything from a small cold press avocado oil extractor for the home to a "soybean oil production line and edible oil refinery plant" with an annual capacity of between 5,000 and 30,000 metric tons.

"I am sure none of the big companies around the world would consider looking at anything from Alibaba or Amazon," noted Larry Paukert of 3T Veg Oil Consulting, LLC, in Crowley, Texas, USA. "But the small backyard entrepreneurs who are just chasing an idea would go there because the price is so cheap and even freight to the United States is cheap. Most of these entrepreneurs will fail or lose interest in the idea, but the sale was made, connecting a buyer and seller."

Report outlines opportunities in the emerging bioeconomy

The US Department of Agriculture (USDA) has released a literature-based, 34-page report that summarizes opportunities in the emerging bioeconomy. The report—"Why Biobased?"—was created as a precursor for a more comprehensive economic study by the USDA BioPreferred program on the economic impacts of the biobased products industry.

SUSTAINABILITY WATCH

Target and Wal-Mart Stores team up

The two largest discount retailers in the United States—Target and Wal-Mart Stores, Inc.—co-hosted a meeting in September 2014 in Chicago, Illinois, to urge beauty and personal care suppliers to be more open about the chemicals in their products and to better explain what constitutes a sustainable product.

Hershey releases new palm oil sourcing policy

The Hersey Co. (Hershey, Pennsylvania, USA) has released a new palm oil sourcing policy and has joined The Forest Trust (TFT). TFT is a global not-for-profit group that works with companies to develop more sustainable sourcing. Hershey said it expects to achieve traceability to the mill level by the first quarter of 2015. See http://tinyurl.com/Hershey-TFT.

Bridgestone opens biorubber research center

Bridgestone Americas, Inc. (BSA; Nashville, Tennessee, USA), a subsidiary of Bridgestone Corp., has opened a Biorubber Process Research Center in Mesa, Arizona, USA. The 10-acre research and innovation campus is the center of Bridgestone's efforts to extract natural rubber from guayule, a shrub native to the southwestern United States. See http://tinyurl.com/BSA-center.

WBCSD publishes guide to life cycle metrics

The World Business Council for Sustainable Development (WBCSD; Geneva, Switzerland) has published a guide designed to help chemical industry customers and stakeholders make more informed, sustainable choices.

Titled "Life Cycle Metrics for Chemical Products," the guidance document is the result of collaboration among leading chemical companies that are part of the WBCSD's Reaching Full Potential project. (Access the guide at http://tinyurl.com/WBCSD-Guidance.)

Focused on life cycle assessment methods, a key objective of the new guide is to provide and communicate material information about the environmental footprint of products that customers and stakeholders can trust and compare.

WBCSD member companies that participated in the project include AkzoNobel; BASF; DSM; CEFIC (the European Chemical Industry Council); Eastman Chemical; Evonik Industries; Henkel; SABIC; SCG Chemicals Company; Solvay; and Mitsubishi Chemical Holdings Co., supported by PricewaterhouseCoopers. □

Synthesizing findings from existing government, academic, and nongovernmental organizations, the new report explores how government policies and industry business-to-business sustainability programs are driving the biobased economy. The report further demonstrates that the biobased economy is, in fact, growing and offers great potential for increased job creation in numerous sectors across the United States.

For instance, one report cited concludes that biobased chemicals are expected to constitute over 10% of the chemical market by 2015. Another report in the study concludes that there is a potential to produce two-thirds of the total volume of chemicals from biobased materials, representing over 50,000 products, a $1 trillion annual global market.

The voluntary "USDA Certified Biobased Product" label is designed to promote the broad-scale marketing of biobased products to consumers. As of September 2014, USDA had certified over 1,940 biobased products in more than 97 product categories for the label. Certified and designated products include construction, janitorial, and grounds keeping products purchased by federal agencies, as well as personal care and packaging products used by consumers.

The Biotechnology Industry Organization, a trade organization based in Washington, DC, USA, has estimated that US-based jobs for the renewable chemicals sector will rise from

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Happy 50th volume, *Lipids*!

AOCS has planned a number of events to celebrate the 50th volume of the journal *Lipids*, which begins with the January 2015 issue.

These celebratory events include a special *Lipids* 50th Anniversary Symposium at the 2015 AOCS Annual Meeting and Industry Showcases as well as a set of reviews in the journal on plant lipid biochemistry. In addition, *Lipids* Editor-in-Chief Eric J. Murphy has chosen a list of five papers representing the broad spectrum of highly cited work published over the years. These papers will be featured on the *Lipids* website and in the Publications section in future issues of *Inform*.

When *Lipids* debuted in 1966 under founding Editor A. Richard Baldwin, the list of associate editors included researchers from five European countries as well as Japan. Today, the list of reviewers and editors (see http://tinyurl.com/EditBoard) is ever more diverse, both geographically and in terms of areas of specialty, indicating the importance of international networks to lipids researchers.

approximately 40,000 jobs in 2011 to more than 237,000 jobs by 2025. This employment level would represent approximately 20% of all jobs in the chemicals sector.

To access the Why Biobased? report, visit: www.biopreferred.gov/files/WhyBiobased.pdf.

Green Chemistry Challenge Awards presented

Three of the five Presidential Green Chemistry Challenge Awards presented in December 2014 recognized innovations of interest to oils and fats chemists. The awards, given annually for the past 19 years, are administered by the US Environmental Protection Agency's (EPA) Green Chemistry Program. They recognize achievements in five categories: academic, small business, greener synthetic pathways, greener reaction conditions, and designing greener chemicals.

Amyris Inc. of Emeryville, California, USA, received the award in the small business category for engineering yeast to make a renewable fuel replacement for petroleum diesel. Making and burning this bus and truck fuel could reduce 82% of greenhouse gas emissions as compared to petroleum diesel, according to EPA. "Since carbon pollution increases our costs in health care and other impacts, this technology could save tens of thousands of dollars each year," the agency said in a news release.

In the "greener reaction conditions" category, Solazyme, Inc., of South San Francisco, California, USA, was recognized for developing novel oils from sugar and engineered algae in a way that significantly reduces the environmental effects that typically occur in producing and processing petroleum-based or plant-based oils. "Soaps, laundry detergents, food products, fuels, and industrial products can now be produced with greatly reduced energy, water, and waste, saving money," noted EPA, adding that the company's palm-oil equivalent "can help reduce deforestation and greenhouse gases that can occur from cultivation of palm oil."

The Solberg Co. of Green Bay, Wisconsin, USA, won the Designing Greener Chemicals Award for developing a safer foam—using surfactants and sugars—that can fight fires better than traditional foams that rely on persistent, bioaccumulative, and toxic chemicals. Solberg created a line of halogen-free foam concentrates that are a blend of hydrocarbon surfactants, water, solvent, sugars, a preservative, and a corrosion inhibitor. The presence of complex carbohydrates gives the foam significantly more capacity to absorb heat than fluorine-containing foam, the company noted in its award submission materials.

For more information about the awards, visit http://www2.epa.gov/green-chemistry. ■

BRIEFS

IncBio, an engineering company based in Porto, Portugal, has signed a contract with SPA Renewables S.A. to supply a fully automated 8,000-metric-ton-per-year biodiesel plant in Corinth, Greece. The plant will use IncBio's ultrasonic reactors to produce biodiesel from locally collected used cooking oil. IncBio expects the plant to be completed by the end of February 2015.

■■■

United Biscuits (UB; Hayes, Middlesex, UK) is using waste cooking oil to power 12 of its trucks. Up to 500 metric tons of the waste vegetable oil from the manufacturing of snacks will be processed each year, UB said in a news release.

■■■

Wide-scale deployment of emerging technology that converts used, non-recycled plastics (NRP) into oil could generate up to $9 billion in annual economic output in the United States and create more than 40,000 domestic jobs, according to a report released by the American Chemistry Council (see http://tinyurl.com/ACC-PTO). The report assessed the economic potential of a rapidly developing pyrolysis technology for converting NRP to synthetic crude oil or other types of fuel oil by means of thermal treatment.

■■■

The Southwest Research Institute (SwRI; San Antonio, Texas, USA) announced in September 2014 the addition of a custom-designed circulating fluidized bed (CFB) to convert heavy crude oils or biological feedstock such as corn into refined fuel samples that clients can assess for quality and profitability. SwRI's system produces samples at about a half-liter per hour, allowing more tests to be run in a shorter time. The CFB system converts biomass to organic liquids using fast pyrolysis, a thermal conversion of organic material in the absence of oxygen. It also can emulate a fluidized catalytic cracking unit to convert lower-value feedstock to higher-value products such as gasoline or diesel. See http://tinyurl.com/SwRI-CFB for more information. ■

ENERGY

POET-DSM's cellulosic ethanol plant opens in US

The biggest news in the bioenergy sector during the last quarter was the opening, on September 3, 2014, of POET-DSM's cellulosic ethanol plant in Emmetsburg, Iowa. The facility, dubbed "Project Liberty," is the first commercial-scale facility in the United States to use corn waste (also known as stover) as feedstock. With two more stover-based US cellulosic ethanol facilities from DuPont and Abengoa expected by the end of 2014, the prospects are looking up for second-generation ethanol.

Project Liberty is not the first cellulosic ethanol facility in the United States, however. That honor goes to INEOS Bio's Indian River BioEnergy Center in Vero Beach, Florida. Developed through a joint venture between INEOS Bio and New Planet Energy, the 8-million-gallon-per-year facility (about 30 million liters per year) uses a unique hybrid of gasification and fermentation technology to convert wood scraps, grass clippings, and other waste materials into transportation fuels as well as energy for heat and power. The project was originally developed with DOE support dating back to the 1990s and came online in 2013.

The problems that can beset fermentation are illustrated by the INEOS Bio project. The company's gasification process produced too much hydrogen cyanide (HCN), "which in turn contaminated the synthesis gas [syngas; a mixture of carbon monoxide and hydrogen] the plant fed to its anaerobic bacteria," according to ICIS News. After sharply reducing output for much of 2014, the company installed three towers to scrub

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and strip HCN from the syngas. Production resumed in September 2014, also according to ICIS.

PROJECT LIBERTY

Grand opening ceremonies in September 2014 for the 20-million-gallons-per-year POET-DSM plant drew the King of the Netherlands, US Secretary of Agriculture Tom Vilsack, Deputy Undersecretary Michael Knotek of the Department of Energy (DOE), and many other dignitaries. The plant cost $275 million to build, with $100 million of that coming from the DOE, $2.6 million from the US Department of Agriculture, and $20 million from the state of Iowa.

"Some have called cellulosic ethanol a 'fantasy fuel,' but today it becomes a reality," said Jeff Broin, POET founder and executive chairman. "With access now to new sources for energy, Project Liberty can be the first step in transforming our economy, our environment, and our national security."

The facility converts baled stover—corn cobs, leaves, husk, and stalk—into renewable fuel via enzymatic hydrolysis followed by fermentation. At full capacity, DSM said it would convert 770 short tons (700 metric tons) of biomass per day. The company plans to manufacture enzymes onsite as one way to bring the per-gallon price down.

Feike Sijbesma, chief executive officer and chairman of the managing board of Royal DSM said, "This is an historical day in the development of plant-residue-based cellulosic ethanol as a viable, commercially attractive alternative to gasoline as we are moving from the fossil-age to the biorenewable-age. For DSM, this is a strategic investment, applying our proprietary technology to convert agricultural residue on a commercial scale, allowing it to be replicated at other facilities globally as we are ramping up our cellulosic ethanol licensing business."

TWO MORE US FACILITIES PLANNED

Abengoa's (Seville, Spain) new cellulosic ethanol plant in Hugoton, Kansas, opened on October 17 and is expected to produce 25 million gallons of cellulosic ethanol and 21 megawatts of electricity annually from corn stover. The Kansas project received a $140 million cooperative investment from the DOE. A company spokesperson told www.ethanolproducer.com in June 2014 that it hopes "to be able to produce cellulosic ethanol at an operational cost between $2 and $2.30/gallon by 2015."

A DuPont cellulosic ethanol project was not far behind, with a $275 million commercial-scale facility in Nevada, Iowa, scheduled to open by the end of 2014. That 30-million-gallon-per-year plant will also produce a solid fuel that could be used in place of coal. Both DuPont and POET-DSM are offering global technology licensing, including a technology package, equipment supply, technical support, biocatalyst supply, feedstock supply consulting, and co-product energy solutions.

RENEWABLE FUEL STANDARD

Will cellulosic ethanol be viable in the US energy market?

The answer to that question depends in great measure on the provisions of the US Environmental Protection Agency's (EPA) Renewable Fuel Standard (RFS2) for 2014—already much delayed as this issue of *Inform* went to press. The RFS program was implemented in 2005 (RFS1) and expanded in 2007 (RFS2), setting volume mandates for renewable fuels. RFS1 required 7.5 billion gallons of renewable fuel to be blended into gasoline by 2012. EPA increased the volume requirements from 9 billion gallons in 2008 to 36 billion gallons by 2022 under RFS2.

In its proposed rule for 2014, EPA set cellulosic biofuel volumes at 17 million ethanol-equivalent gallons, compared with the final 2013 level of 810,185 ethanol-equivalent gallons. Cellulosic biofuel producers argue that the EPA used a newer methodology in the 2014 RFS "that lowered the cellulosic goals based on concerns about inadequate infrastructure to produce the fuels," according to InsideDefense.com (see http://tinyurl.com/RFS-Suit). Producers stated publicly that the 2014 goals should be higher to drive investment and, in September 2014, were considering filing suit against the EPA.

Other uncertainties remain. Will removing stover from farms negatively affect erosion and the quality of soil? Will stover collection infrastructure deliver a steady supply of feedstock to the new plants at an affordable price? Will the facilities be able to deliver cellulosic ethanol at a competitive price?

One partisan—Jeremy Martin, a senior scientist with the Union of Concerned Scientists (Washington, DC, USA)—sees only good things ahead for cellulosic ethanol produced from corn stover. "The amazing thing about cellulosic technology is that it allows biofuel production to expand without using any additional food crops. According to our analysis, Iowa has potential to expand biofuel production by a billion gallons—more than 25%—without using an additional kernel of corn."

US agencies invest $210 million in biorefineries

In September 2014, three US agencies awarded $210 million to three companies to build biorefineries for the production of cost-competitive, drop-in military biodiesel. The contracts were given as part of a 2011 Presidential directive and were made through the Department of Defense's (DOD) Defense Protection Act of 1950 by the Departments of the Navy, Energy (DOE), and Agriculture.

"Advanced biomass-based transportation fuels have the potential to provide a reliable and cost-effective alternative to traditional fuel sources," said Deputy Energy Secretary Daniel Poneman. "By advancing technologies that reduce our carbon emissions, this multi-agency partnership is demonstrating that by protecting our energy and environmental security, we will enhance our national security as well."

In total, the three projects are expected to produce more than 100 million gallons (approximately 380 million liters) of military grade fuel beginning in 2016 and 2017 at less than $3.50 per gallon, on a weighted average. The drop-in alternative fuels can be blended at a 50:50 ratio with traditional fossil fuels, the DOD said in a news release.

The details of the contracts are as follows:

Emerald Biofuels. This company, based in Chicago, Illinois, USA, will build an 82-million-gallon-per-year refinery on the Gulf Coast utilizing waste fats to create military-grade fuel. The

CONTINUED ON PAGE 35

BRIEFS

Omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) currently do not have Dietary Reference Intake (DRI) values in the US and Canada, but these nutrients have now been selected by the US and Canadian Federal DRI Committees for DRI reviews, along with vitamin E, sodium, and magnesium. The government agencies also announced plans for a 2015 workshop that will address whether and how chronic disease outcomes can be incorporated into setting DRI values. Nutrients receive priority for DRI reviews when their benefits for the general population are backed by consistent, high-quality scientific studies.

■■■

A 2014 paper published in *Comprehensive Reviews in Food Science and Food Safety* analyzes the safety and toxicity of widely used preservatives, nutritional additives, and extracts, as well as coloring, flavoring, and texturizing agents (http://dx.doi.org/10.1111/1541-4337.12065). Some food additives "are still enveloped in controversy," fueled by the lack of uniformity in worldwide regulations coupled with conflicting scientific studies on their safety, write researchers Patricia Morales and Isabel C.F.R. Ferreira from the University of Madrid (Spain) and the Polytechnic Institute of Bragança (Portugal), respectively. The review also addresses future applications of nanoencapsulation, vaccines for intolerance to additives, and "smart" additives and packages.

■■■

Europe leads the world in taking action to eliminate trans fatty acids from foods, according to Zsuzsanna Jakab, the World Health Organization (WHO) director for Europe, who spoke at the 64th session of the WHO Regional Committee for Europe in Copenhagen, Denmark, in September 2014. In 2003, Denmark limited the amount of trans fatty acids in foods to less than 2%, and since then, other European countries, including Austria, Hungary, Iceland, Norway, and Switzerland, have set similar limits. "If more countries act, the benefits to health can be substantial across the whole region," Jakab said. ■

FOOD, HEALTH & NUTRITION

A foundational research study on the diet of Greenland Eskimos in the 1970s has been criticized for its failure to establish a link between fish oil and lower rates of heart disease. But the lead author of that study stands his ground.

1970s fish oil study criticized but original author says critics twisted facts to get attention

The 1970s studies that set the stage for decades of ongoing research on the heart benefits of omega-3 fatty acids have recently come under attack. A 2014 review article highlights the alleged flaws of the studies that revealed a correlation between a high-marine-fat diet, low serum lipid levels, and low incidence of heart disease among Greenland Inuits. But are the allegations true? One of the studies' original authors, Jørn Dyerberg, now an emeritus professor at the University of Copenhagen, says no.

A SURPRISING FINDING ABOUT FATTY FISH

Nearly five decades ago, a team of Danish researchers set out to understand how the

CONTINUED ON NEXT PAGE

Inuit population in Greenland could have such a low incidence of heart disease and diabetes despite a diet with high levels of marine fat. Their initial findings, published in *The Lancet* in June 1971 (http://dx.doi.org/10.1016/S0140-6736(71)91658-8), surprised them: The average Greenland Inuit had lower serum cholesterol and lipid levels than the average Dane. Their levels were also lower than Inuits who lived in Denmark, suggesting an environmental, and not genetic, explanation. The findings begged the question: Could a diet high in oily coldwater fish actually be the secret to good heart health? The study did not claim to answer this question but rather proposed it as an area of future investigation.

To this day, that 1971 study continues to be cited as the first to show a possible connection between fish oil and heart health. Denmark's Aalborg Hospital North researchers Hans Olaf Bang, Jørn Dyerberg, and Aase Brøndum Nielsen analyzed the plasma-lipid pattern of 130 Greenland Inuits in the northern part of Greenland's west coast. The team determined the concentrations of plasma lipids, cholesterol, and triglycerides in both men and women age 30 and above, and compared them to the levels in a Danish control group. In nearly all categories, lipid concentrations were significantly lower among Inuits than Danes, and the net difference increased with age. This was the first study to thoroughly examine the serum-lipid pattern of both of these populations.

"In the [Inuits] the association of low level of most types of lipid (except α-lipoproteins) with very low incidence of ischaemic heart-disease is striking," the authors wrote, "but not necessarily causal . . . The food of the [Inuits] still living as hunters and/or fishermen consists mostly of meat of whales, seals, sea birds and fish . . . [which] is extremely rich in protein and fat." To explain how a diet so high in animal fat could coincide with low plasma cholesterol levels, the authors speculated that the large amount of polyunsaturated fatty acids in marine food could be protective against increasing plasma-cholesterol levels, and thus protective against heart disease—a view that followed the thinking of that time.

A series of follow-up studies published over the next decade revealed more detailed information about the Inuit diet and other health factors. The researchers did not collect their own data on the incidence of heart disease among the Inuits but rather received this information from reports compiled by the chief medical officer in Greenland.

A HARSH CRITIQUE ATTRACTS MEDIA ATTENTION

In April 2014, George J. Fodor, University of Ottawa Heart Institute professor, and coworkers, published a paper in the *Canadian Journal of Cardiology* titled "'Fishing' for the origins of the 'Eskimos and heart disease' story. Facts or wishful thinking? A review" (http://dx.doi.org/10.1016/j.cjca.2014.04.007, 2014) The critical review caught the attention of several mainstream media outlets, which ran stories with headlines such as, "The Fishy Origins of the Fish Oil Craze" (see http://tinyurl.com/fish-oil-Slate) and "Fish Oil May Not Prevent Heart Disease After All" (see http://tinyurl.com/fish-oil-WP).

In the article, the authors state their biggest issue with Bang and Dyerberg's studies is that they did not directly examine the cardiovascular status of Greenland Inuits, relying instead on data from the annual reports from the chief medical officer in Greenland. These reports, which show deaths due to coronary artery disease in the 1960s ranging from 8.5% to 11.8% of total deaths, "have limited validity," the authors claim. "These reports are based on death certificates and hospital admissions." However, about a third of the population lived in small settlements, far from hospitals and medical officers, both of which had "limited diagnostic facilities," leading the authors to conclude "the reported data are likely an underestimation of the true magnitude of the disease in this area.

"Bang and Dyerberg's seminal studies from the 1970s are routinely invoked as 'proof' of low prevalence of [coronary artery disease, or CAD] in Greenland Eskimos ignoring the fact that these two Danish investigators did not study the prevalence of CAD," Fodor *et al.* wrote. "Instead, their research focused on the dietary habits of Eskimos and offered only speculation that the high intake of marine fats exerted a protective effect on coronary arteries."

A RESPONSE FROM ONE OF THE ORIGINAL AUTHORS

It seems one of the things that bothers Fodor and his colleagues about the 1970s study is that, to this day, many researchers credit Bang and Dyerberg for finding out Inuits had low rates of heart disease. "Nobody even, obviously, read this paper!" Fodor told Slate. (Inform contacted Fodor, but he was not available for comment).

But it's not their fault that people misquote the decades-old papers as proof that fish promote heart health, Dyerberg told *Inform*. That was not their intention and they never made that claim. The studies were meant to generate hypotheses that could be further investigated with interventional studies, he said.

"We based our statement of the rare occurrence of acute myocardial infarction in Greenland on a meticulous review [of the data that were] available at that time," Dyerberg said. The data from the chief medical officer of Greenland were consistent with slightly older epidemiological data on medial deaths in northern Greenland.

"They're trying to disprove the story by saying that the epidemiological data from Greenland way back in the '70s and earlier were not correct, so the story is not correct, which is, of course, bogus," he said. "The only [way to] prove or disprove whether there's a connection between diet and disease pattern is by doing an intervention study. I've been a professor of human nutrition for many years and I know it's difficult, but that's the only way you can do it. And there have been several studies, some finding a positive and others finding not a positive association between fish intake and cardiovascular heart disease. But most of them are positive.

"The cardiovascular story is rather solid but has some weak points," Dyerberg added. "But then it has opened up for a lot of other interesting aspects of the story," such as the lack of long-chain omega-3 fatty acids in the Western diet and the medical consequences that have resulted.

Dyerberg pointed out several times in the 2014 review article where Fodor *et al.* twisted the facts and misquoted basic papers in an effort to make the data fit into their fish-oil-debunking narrative. For example, they cite another one of Dyerberg's papers, published in 1988 in the *International Journal of Epidemiology*, which found that Greenland Inuits have a similar prevalence of cardiovascular heart disease and a mortality rate twice as high as non-Inuit population, which is correct. But the authors failed to mention another significant finding from that report, which is that people living in Greenland settlements have lower incidence of ischemic heart disease. It wouldn't have fit into their narrative—that there is no evidence that fish have heart health benefits—so they left it out, Dyerberg said, which is a deliberate twisting of the facts in an attempt to gain attention. "It's nice to have attention, but you shouldn't use science for that."

Fodor *et al.* also cite data that shows Greenland Inuits do not have lower rates of cardiovascular heart disease and acute myocardial infarction today as evidence that the data from half a century ago is flawed.

This is a weak argument, Dyerberg said. That does not disprove that it was that way back then, "and we only had the data that we had. Of course, you can put a question mark on anything, but this doesn't disprove anything."

Dyerberg said his response to the review article by Fodor *et al.*, in a nutshell, is: "And so what?

"The only one[s] who didn't do any studies are Fodor and his companions," he said. "They just quoted the literature; they misquoted a lot of it . . . I'm not annoyed, I'm just bewildered why they do it."

But negative stories are always interesting, he said, which may explain why the article caught the attention of mainstream media, despite its flaws. Dyerberg said he was not contacted by any media outlet, other than *Inform*, for comment on this 2014 critique of his decades-old studies. He continues to stand by the recommendations of the American Heart Association and other international organizations that recommend two or three servings of coldwater, fatty fish a week because, he said, the evidence is sufficient that the long-chain omega-3 fatty acids found in fish are beneficial for health. ■

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BRIEFS

A new website allows users to find information about the relative risks of genetically engineered crops. The GENetic Engineering Risk Atlas (GENERA), created by Biology Fortified, Inc. (BFI, Middleton, WI, USA), opened to the public in August 2014 and includes a searchable database of peer-reviewed scientific studies conducted worldwide (see http://genera.biofortified.org). Each listing includes information about the study's authors, funding sources, and which crops and traits were studied. BFI, an independent nonprofit organization that does not accept funding from industry sources, seeks to strengthen the public discussion of issues in biology, with an emphasis on genetics and genetic engineering in agriculture.

■■■

A strain of camelina that has been genetically modified to produce omega-3 fatty acids has been harvested for the first time in Britain. The camelina seeds, modified by the insertion of a gene from algae, produce up to 14% docosahexaenoic acid (DHA) and 12% eicosapentanoic acid (EPA)—levels comparable to fish oil. The findings were reported at the 12th Euro Fed Lipid Congress in September 2014 by Olga Sayanova, a scientist at Rothamsted Research (Harpenden, Hertfordshire, UK), according to *NutraIngredients* (read more at http://tinyurl.com/GM-camelina).

■■■

In the first half of 2014, US industries and organizations opposed to labeling genetically modified organisms (GMOs) in foods spent $27.5 million on GMO-related lobbying—three times as much as was spent in all of 2013—according to an analysis by the Environmental Working Group (EWG; Washington, DC, USA). The spending totals nearly 15 times more than the $1.9 million spent by GMO labeling proponents in the same time frame. The analysis did not account for funds expended to lobby state legislatures (see http://www.ewg.org/research/anti-label-lobby). The EWG is a nonprofit organization that supports GMO labeling. ■

BIOTECHNOLOGY

Scientists have sequenced the genome of oilseed rape, which with more than 100,000 genes has the highest gene density of all sequenced organisms.

International research team sequences *Brassica napus* genome

Brassica napus, also known as oilseed rape, has one of the most highly duplicated genomes of all flowering plants. The journal *Science* reports that its genome has been sequenced by a team of scientists from more than 30 research institutions around the world. The 2014 paper, titled "Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome," can be found at http://dx.doi.org/10.1126/science.1253435.

The complexity of the *B. napus* genome made its sequencing no small feat. Over time, its genome has accumulated a great number of genes—more than 100,000—landing it in the number one spot in gene density for all sequenced organisms (by comparison, the human genome has roughly 20,000 genes). While most flowering plants originated when their genomes replicated themselves millions of years ago (a process known as polyploidization), *B. napus* formed about 7,500 years ago in the post-Neolithic era when two plant species, *Brassica rapa* (Chinese cabbage) and *Brassica oleracea* (a cultivar that includes broccoli, cauliflower, Brussel sprouts, and collard greens), combined in the wild. Since its relatively recent initial formation, most of the genes in *B. napus* have copied themselves, resulting in a single genome composed of sub-genomes from each of the two parent

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species, each with two identical or highly similar copies of every gene.

"The main difficulty for oilseed rape has been to differentiate its sub-genomes," said Boulos Chalhoub, lead author of the study and researcher at the French National Institute of Agricultural Research (Paris, France), in a news release. "This has been achieved by the development of an original sequencing strategy, bioinformatics tools, and the analysis of duplicated gene expression and their regulation." The researchers believe the duplicated genes play an important role in diversification, the emergence of new functions, ability to adapt to different environments, and the overall improvement of the species. Genes from the two rapeseed sub-genomes can also be exchanged with one another, a process that is known to lead to diversification, although the underlying mechanism remains largely unknown.

B. napus originated in Europe and is grown across much of Canada and parts of the United States. Knowing the sequence of the *B. napus* genome can lead to the improvement of the crop, said University of Georgia researcher Andrew Patterson (Athens, Georgia, USA) in a news release. "We can use this knowledge to tailor the plant's flowering time, make it more resistant to disease and improve a myriad of other traits that will make it more profitable for production." For example, the information will play an important role in the development of *B. napus* as a biofuel and assist researchers in their efforts to use genetic engineering or traditional breeding techniques to improve oil yields, increase seed production, or modify the fatty acid profile of canola for food and other industry applications.

The computational power and cyberinfrastructure that the research team used to complete the analyses were provided by the iPlant collaborative, a project funded by the National Science Foundation (Washington, DC, USA). The team used the Comparative Genomics (CoGe) platform powered by iPlant, a powerful tool for large-scale studies in computational biology that uses remote servers for computation, data analysis, and storage. iPlant made it possible for the researchers to access a thousand or more computers to complete the operations, which required hundreds of computing hours. Currently, CoGe and iPlant are being used to analyze 23,000 genomes from 17,000 organisms, according to the researchers.

Global production of canola, which includes *B. napus* as well as *Brassica juncea* and *Brassica rapa,* has increased over the past 40 years. Canola is now the second largest oilseed crop in the world, according to the US Department of Agriculture.

USDA and EPA approve Enlist GM corn and soybeans

The US Department of Agriculture (USDA) has approved the use of Dow AgroSciences' (Indianapolis, Indiana, USA) new genetically modified (GM) corn and soybean crops. The new GM crops are resistant to Enlist Duo™, a new weed-killing formulation composed of a mixture of glyphosate and the choline salt of 2,4-dichloropenoxyacetic acid (2,4-D), which its creators say makes it less prone to volatilize, or evaporate, and drift.

Shortly after the USDA's announcement, the Environmental Protection Agency (EPA, Washington, DC, USA) issued a statement concluding that the Enlist Duo herbicide "ensures protection of human health, including infants, children." The statement also outlines a number of restrictions put in place to minimize pesticide drift, including a 30-foot in-field "no spray" buffer zone around the application area, no pesticide application when the wind speed is over 15 mph, and limiting its use to ground applications only.

The USDA predicts the crops could lead to a three- to sevenfold increase in the use of 2,4-D by the year 2020, according to a report by the Associated Press news service. Opponents of Enlist corn and soybeans are concerned that this increase could be damaging to the environment and nearby non-GM crops, as well as endangering public health, but Dow has stated the chemical is safe when properly used. The prevalence of "superweeds" that are resistant to the widely used weed-killer glyphosate has doubled since 2009, according to Dow, arguing that Enlist will help solve the problem and boost crop yields. But critics say the fix will be only temporary. "This is not the solution to our superweed problem and will only spur the evolution of yet more herbicide-resistant weeds, said Andrew Kimbrell, executive director for the Center for Food Safety (CFS, Washington, DC, US), in a statement. The CFS is a nonprofit environmental advocacy organization.

The USDA's decision applies to the Enlist corn, Enlist soybean, and Enlist E3™ soybean traits. Each of these varieties has tolerance to the Enlist Duo herbicide. Enlist E3, which is being developed through a collaboration between MS Technologies LLC (Westpoint, Iowa, USA) and Dow AgroSciences, has a three-gene, herbicide-tolerant trait stack, a molecular construction that "allows for more efficient breeding," according to the company's website.

The EPA's final regulatory decision document is available at www.regulations.gov, EPA docket: EPA-HQ-OPP-2014-0195. Regulatory approval in other countries, such as China, was still pending at the time *Inform* went to press.

Biotech crops preferred by US farmers

Farmers in the United States continue to prefer biotech crop varieties, citing environmental, economical, and societal

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BRIEFS

Swiss specialty chemicals manufacturer Clariant is building a new production facility at its Tangerang site in Indonesia to support regional demands in the personal and home care industry. The overall investment of approximately CHF 17 million ($18 million) in combined esterquat and methylquat production will add 12,000 metric tons of collective capacity for liquid and solid products used in consumer care products such as fabric softeners and hair conditioners. The new facility is expected to open in 2015.

Clariant also announced it has expanded its production facility in Roha, near Mumbai, India, at a cost of CHF 3.2 million (approximately $3.4 million). The expansion doubles the capacity at the facility for pigments and pigment preparations used in a wide range of applications, including interior and exterior coatings; packaging; personal, home, and fabric care products; seed coatings; and dispersions for printing.

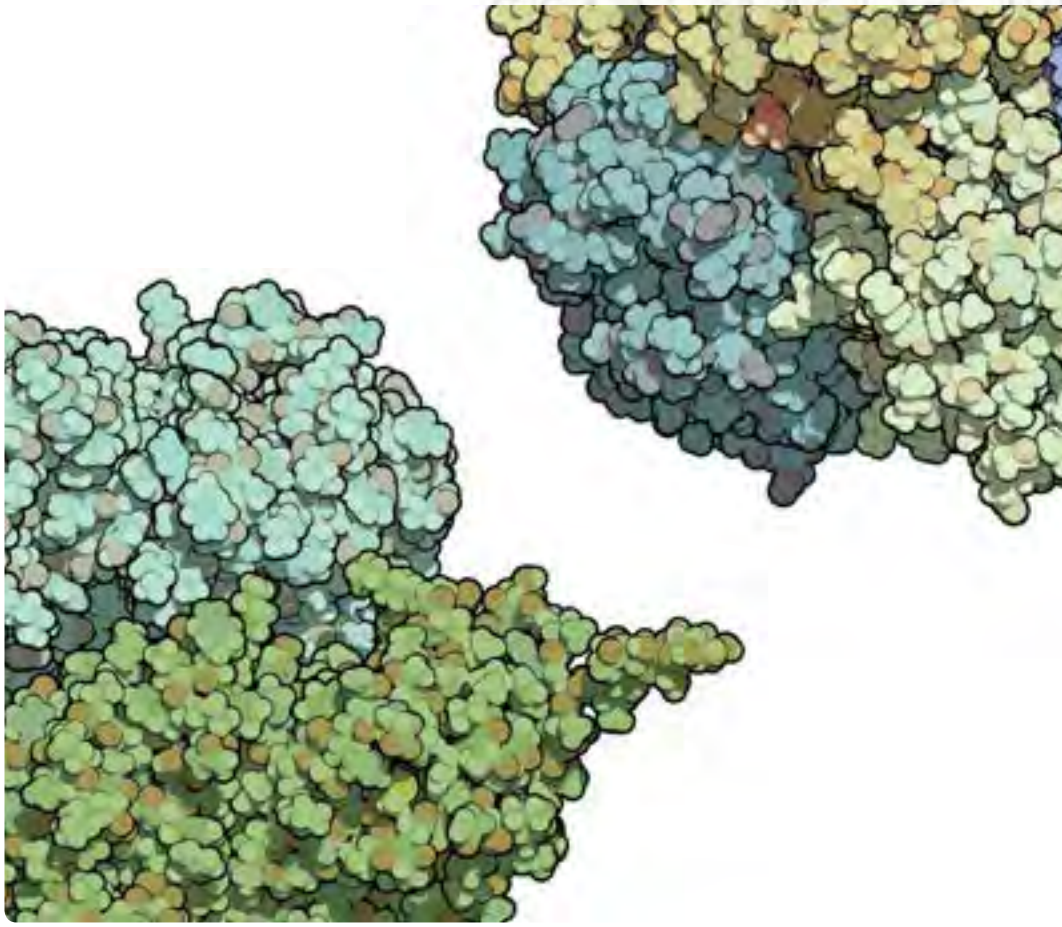
■■■

"Green" cleaning products company Method, a unit of Belgium's Ecover Group, has plans to create what it calls "the world's largest urban rooftop farm." The company, which is based in San Francisco, California, USA, is building a manufacturing plant in Chicago complete with a 75,000-square-foot (approximately 7,000 square meters) rooftop installation. "The greenhouses are being designed to grow up to 1 million pounds [about 500,000 kilograms] of fresh produce annually," according to www.forbes.com.

■■■

The HallStar Co., a specialty chemicals company based in Chicago, Illinois, USA, has acquired the Florasolvs® natural esters product line of specialty ingredients from FloraTech, a global provider of botanically derived ingredients for personal care products. The acquisition includes the FloraTech trade names and proprietary manufacturing technology for the Florasolvs-series brand natural esters. FloraTech is based in Chandler, Arizona, USA. ■

HOME & PERSONAL CARE



Enzymes that do not need water to function

New research by scientists at the University of Bristol has challenged one of the key axioms in biology—that enzymes need water to function. The breakthrough could eventually lead to the development of new industrial catalysts, according to a news release.

Findings published in *Nature Communications* (http://dx.doi.org/doi:10.1038/ncomms6058, 2014) show that water is not essential for enzymes to fulfil their catalytic role. This discovery could pave the way for the development of new thermally robust industrial enzymes that could be used in harsh processing conditions, with applications ranging from detergent technologies to biofuel production.

Adam Perriman and colleagues were able to circumvent the need for water by decorating the surface of the industrial enzyme lipase with long detergent molecules. In principle, what the team created was an enzyme with a built-in ability to exist as a liquid without any solvent. What astounded the researchers was that the solid chemical substrate could be dissolved directly by the liquid enzyme, which then went on to catalyze the chemical reaction, and continued to do so up to temperatures as high as 150°C.

Perriman said, "From our preliminary experiments, we knew that the molecular structure of the lipase was still intact after the modifications, even at 150°C.

"However, we were surprised and delighted to discover that the catalytic activity of the enzyme was still present. The ability to rationally design a self-contained reactive

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biofluid, where one can literally sprinkle a solid substrate onto it, and then observe a chemical reaction, represents a real fundamental scientific advance."

DuPont and P&G bring cellulosic ethanol into laundry detergent

DuPont and Procter & Gamble announced in early October 2014 that they would collaborate on using cellulosic ethanol in Tide® Cold Water brand laundry detergent in North America. The Tide Cold Water brand will be the first in the world to blend cellulosic ethanol in at commercial scale, the companies said. Conventionally produced ethanol (or ethyl alcohol) has long been a key ingredient in the Tide formulation, allowing for stability of the detergent formula and better washing performance.

DuPont will produce the cellulosic ethanol at the company's new biorefinery, currently under construction in Nevada, Iowa (see Energy on page 23). Once completed, the plant will be the world's largest bioethanol refinery, producing 30 million gallons (about 114 million liters) of cellulosic ethanol per year—via a process the companies say has zero net carbon emissions.

The amount of cellulosic ethanol used in Tide Cold Water in North America will re-purpose over 7,000 short tons of agricultural waste per year, according to a DuPont news release.

"We believe that actions speak louder than words in the area of sustainability and this partnership with DuPont demonstrates we are doing just that," said Gianni Ciserani, P&G group president of Global Fabric and Home Care. "As one of the world's largest laundry manufacturers, we have a responsibility to lead renewable sourcing in products."

"With this collaboration, DuPont is also taking the first step to diversify its markets for cellulosic ethanol beyond fuels," said James Collins, senior vice president, DuPont.

DuPont's James Collins and Procter & Gamble's Gianni Ciserani both spoke on October 7, 2014, at the AOCS World Conference on Fabric and Home Care in Montreux, Switzerland.

Clorox leaves Venezuela

The Clorox Co. (Oakland, California, USA) said in an October 2014 news release that its affiliate, Corporación Clorox de Venezuela S.A. (Clorox Venezuela), was "no longer viable." As a result, Clorox Venezuela discontinued its operations and was seeking to sell its assets.

"This is a very difficult situation for our company," said Chairman and CEO Don Knauss. "We are extremely proud of the men and women who did their very best to operate our business in the face of significant economic challenges. We are working to support them through this transition."

For nearly three years, Clorox Venezuela was required to sell more than two-thirds of its products at prices frozen by the Venezuelan government. During this same period, Clorox Venezuela experienced cumulative triple-digit inflation resulting in massive increases in Clorox Venezuela's input costs, including packaging, raw materials, transportation, and wages. As a result, Clorox Venezuela had been selling its products at a loss, causing continual operating losses.

Clorox Venezuela representatives met repeatedly with government authorities, according to the news release, to help them understand the rapidly declining state of the business, including the need for immediate, significant, and ongoing price increases and other critical remedial actions to address these adverse impacts. "Based on their representations, Clorox Venezuela had expected significant price increases would be forthcoming much earlier in 2014," the statement noted. "However, price increases subsequently approved by the government were nowhere near sufficient and would have caused Clorox Venezuela to continue operating at a significant loss."

INCPA welcomes new members

The International Network of Cleaning Product Associations (INCPA) welcomed two new member organizations—representing the Indian and Mexican marketplaces—as global cleaning product industry leaders gathered at the AOCS World Conference on Fabric and Home Care in October 2014 in Montreux, Switzerland.

INCPA also unveiled a new web portal—www.incpa.net—integrating the websites of the world's leading cleaning product trade associations, featuring a new compilation of industry best practices on health and safety, sustainability, and consumer issues.

INCPA is a coalition of trade associations located in various regions of the world that represent cleaning product formulators, including Australia, Brazil, Canada, Europe, Japan, and the United States. The Network coordinates and actively engages in targeted efforts to better understand and address issues of an international or a cross-regional nature that affect the cleaning products industry.

The two new member organizations of INCPA are the Mexican Personal and Home Care Industry Association (CANIPEC) and the Indian Home & Personal Care Industry Association (IHPCIA).

"Bringing the Mexican and Indian associations into the Network strengthens our ability to share information, expertise, and knowledge on the key issues affecting the global cleaning product marketplace," INCPA leaders said in a statement. "INCPA member organizations serve economies totaling over 2.7 billion people, with the market value of their member companies' products totaling $130 billion."

INCPA's members include:

- ABIPLA—Brazilian Cleaning Products Industry Association: www.abipla.org.br
- ACCORD Australasia: www.accord.asn.au
- ACI—American Cleaning Institute: www.cleaninginstitute.org
- AISE—International Association for Soaps, Detergents and Maintenance Products, Europe: www.aise.eu
- CANIPEC—Mexican Personal and Home Care Industry Association: www.canipec.org.mx

- CCSPA—Canadian Consumer Specialty Products Association: www.ccspa.org
- CSPA—Consumer Specialty Products Association (USA): www.cspa.org
- IHPCIA—Indian Home & Personal Care Industry Association: www.ihpcia.org
- JSDA—Japan Soap and Detergent Association: www.jsda.org/w/e_engls/

Sunblock may pose hazard to sea life

The sweet and salty aroma of sunscreen and seawater signals a relaxing trip to the shore. However, scientists are now reporting that idyllic beach vacations come with an environmental hitch. When certain sunblock ingredients wash off skin and into the sea, they can become toxic to some of the ocean's tiniest inhabitants, which are the main course for many other marine animals.

Researchers Antonio Tovar-Sánchez and David Sánchez-Quiles of the Mediterranean Institute for Advanced Studies (Balearic Island, Spain) point out that other than staying indoors, slathering on sunscreen is currently the best way to protect skin from the sun's harmful rays. However, when sunbathers splash into the ocean to cool off, some of their lotions and creams are rinsed into the water. The problem is that titanium dioxide and zinc oxide nanoparticles, which are common ingredients in sunblock, can react with ultraviolet light from the sun and form new compounds, such as hydrogen peroxide, that could be toxic. High amounts of hydrogen peroxide can harm phytoplankton, the microscopic algae that feed everything from small fish to shrimp to whales. The scientists wanted to figure out just how serious an impact beachgoers could be having on life in coastal waters.

To investigate the matter, the researchers went to Majorca Island's Palmira beach on the Mediterranean along with about 10,000 beachgoers, a small portion of the more than 200 million tourists that flock to Mediterranean shores every year. Based on lab tests, seawater sampling, and tourism data, the scientists concluded that titanium dioxide from sunblock was largely responsible for a dramatic summertime spike in hydrogen peroxide levels in coastal waters—with potentially dangerous consequences for aquatic life.

"Sunscreens as a source of hydrogen peroxide production in coastal waters" appeared in *Environmental Science & Technology* (http://dx.doi.org/10.1021/es5020696, 2014). ■

CLASSIFIED

ENERGY (cont. from page 24)

biorefinery will use Honeywell's UOP/Eni EcoFining process technology for the production of renewable diesel and jet fuel, according to www.BiofuelsDigest.com.

Fulcrum BioEnergy. Fulcrum will build a 10-million-gallon-per-year refinery in McCarran, Nevada, USA, using municipal solid waste as its feedstock and the Fischer-Tropsch process to create fuel. On September 4, USDA announced a $105 million Biorefinery Assistance Program loan guarantee to Fulcrum for the construction of this facility. The company, which is based in Pleasanton, California, USA, is under contract with Cathay Pacific Airways to supply 375 million gallons of fuel over 10 years—about 2% of the airline's fuel usage—according to *Biofuels Digest.*

Red Rock Biofuels. Red Rock will build a 12-million-gallon-per-year refinery in Lakeview, Oregon, USA, using woody biomass, or the by-products of forest management, as its feedstock and the Fischer-Tropsch process to create a refined product. The company, which is a subsidiary of IRI Group LLC and has headquarters in Fort Collins, Colorado, USA, will also supply Southwest Airlines with about 3 million gallons per year of renewable jet fuel in a separate deal, according to the *Denver Business Journal* newspaper.

Also in September 2014, the DOE announced up to $25 million in funding to reduce the cost of algal biofuels to less than $5 per gasoline gallon equivalent (gge) by 2019. The funding will support projects in two topic areas: Topic Area 1 awards (anticipated to fund one to three projects) will range from $5 million to $10 million and focus on the development of algal cultures that, in addition to biofuels, produce valuable bioproducts that increase the overall value of the biomass. Topic Area 2 awards (anticipated to fund three to seven projects) will range from $500,000 to $1 million and will focus on the development of crop protection or carbon dioxide utilization technologies to boost biomass productivity in ways that lead to higher yields of algae.

Algal biomass can be converted to advanced biofuels that offer promising alternatives to petroleum-based diesel and jet fuels. However, barriers related to algae cultivation, harvesting, and conversion to fuels and products need to be overcome to achieve the DOE's target of $3 per gge for advanced algal biofuels by 2030. ■

Argentina leads the oilseed industry in an expanded Mercosur region (2003-2013)

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

Hector Autino (in collaboration with Direction of Information and Economic Studies of the Rosario Stock Exchange)

During the last 10 years, the processing capacity of the oil industry in the extended Mercosur confirms the leadership this geographic area has achieved in the world oil market. The total theoretical crushing capacity of the countries that make up the extended Mercosur now surpasses that of the region's two main soybean and sunflower crushing competitors: China and the United States. As seen in Table 1, oil processing plants from some member and associate countries of the Mercosur (in particular Argentina, Brazil, Paraguay, Bolivia, and Uruguay) currently have a theoretical oilseed processing capacity of ~354,770 metric tons per day (MMT/day). Ten years ago, the combined capacity was ~239,335 MMT/day; this represents a 48% increase in processing capacity during this period.

Information regarding the oilseed processing capacity of China is conflicting. China's National Grain and Oils Information Center (CNGOC) estimated a total soybean processing capacity in 2012 of ~360,000 MMT/day (125 MMT/year), while the private sector's estimate was 115 MMT/year. Furthermore, CNGOC reported that 50% of the soybean processing capacity was unused, resulting in an active processing capacity of ~180,000 MMT/day (59.5 MMT/year). Meanwhile, the February 2014 issue

Table 1: Theoretical capacity of oilseed crushing per country (active plants) in the extended Mercosur. Comparison of second semesters during the years 2003 to 2013.

Country	2nd. Semester 2003	2nd. Semester 2013	Increment in MMT/day between 2003 and 2013	Increment in % (2003-2013)
Argentina Oilseed Industrial Complex	97,500	189,825	92,325	95%
Brazil Oilseed Industrial Complex	128,835	138,055	9,220	7%
Paraguay Oilseed Industrial Complex	6200	16,100	9,900	160%
Bolivia Oilseed Industrial Complex	6200	10,100	3,900	63%
Uruguay Oilseed Industrial Complex	600	690	90	15%
Total of Oilseed Industrial Complex from the Expanded Mercosur	**239,335**	**354,770**	**115,435**	**48%**

Source: Prepared by Bolsa de Comercio de Rosario based on data from J.J. Hinrichsen SA and experts from companies

of the US Department of Agriculture's Foreign Agricultural Service division's publication, *"Oilseeds: World Markets and Trade*," placed the theoretical processing capacity of China at ~424,000 MMT/day (140 MMT/year), which is 73–75% higher than the utilization of installed capacity reported by other sources. Although the milling capacity of the aforementioned extended Mercosur countries is slightly lower than the capacity installed in China, the milling capacity of the Mercosur countries greatly surpasses the ~350,000 MMT/day mark, and is higher than that the US crushing capacity of ~205,000 MMT/day reported by private sources (the US figure accounts for both active and inactive facilities).

ARGENTINA

A case-by-case analysis of capacity in the extended Mercosur region during the last 10 years shows that Argentina was the country with the most growth. Argentina currently has a theoretical processing capacity of ~189,825 MMT/day (see Table 1). The information in this table, which has been extracted from the "Annual Yearbook of Oilseeds Markets (2014 edition)" from the Broker company J. J. Hinrichsen's *SA*, and updated by the Latin American magazine *A&G* [produced by Asociación Argentina de Grasas y Aceites (ASAGA)], only includes data pertaining to active plants. Roughly 78% of Argentina's theoretical capacity (~190 MMT/day) can be attributed to 20 industrial plants located in the Greater Rosario area, in the province of Santa Fe, in the oilseed industrial complex, "Gran Rosario". Three of the largest include plant Terminal 6 SA, located in the city of Puerto General San Martín; Molinos Rio de la Plata SA in San Lorenzo; and Renova S.A. in Timbúes. These plants have a daily milling capacity of about 20,000 MMT, respectively. They are followed by Vicentín SA located in San Lorenzo (~16,000 MMT/day); Cargill in Villa Gobernador Galvez (~13,000 MMT/day); and Dreyfus in General Lagos (~ 12,000 MMT/day).

Although there are plants in other areas of the country, such as General Deheza SA in the Argentinean province of Córdoba (processing capacity of ~10,000 MMT/day), the "Gran Rosario oilseed industrial complex" is the most significant in terms of crushing. The large number of plants per area, and their large daily theoretical crushing capacity, leads to greater efficiency and lower production costs when compared to similar plants in other countries, such as Brazil, the United States, and China.

As seen in Table 1, Argentina's crushing capacity during the second half of the year 2003 was ~97,500 MMT/day. By 2013, Argentina had increased its national crushing capacity by ~95%. This increase was possible due to the following investments in the Gran Rosario plants:

a. Between the years 2004 and 2007: Terminal 6 SA experienced an expansion that boosted capacity from 8,000 to 17,000 MMT/day, followed by another expansion that brought the capacity to ~19,000 MMT/day. The company Molinos Río de la Plata SA in San Lorenzo increased its capacity from ~4,500 to

CONTINUED ON NEXT PAGE

~17,000 MMT/day, while the company Oleaginosa San Lorenzo SA (~10,000 MMT/day capacity) was incorporated to the complex. Dreyfus's new plant in Timbúes (~6,000 MMT/day milling capacity) and Cargill's in Villa Gobernador Galvez (~13,000 MMT/day) were also incorporated.

b. In the year 2010, the company Noble Argentina S.A incorporated its new plant in Timbúes (~ 8,000 MMT/day capacity)
c. Since the year 2013, the company Renova SA (~20,000 MMT/day crushing capacity) has been incorporated in Timbúes. Renova SA belongs to Glencore (former Oleaginosa Moreno) and Vicentín.

BRAZIL

Brazil, the second largest oilseed processor in the extended Mercosur, has an active crushing plant complex with a combined capacity of ~138,055 MMT/day (Table 1). Unlike the plants in Argentina, the crushing facilities in Brazil are smaller and geographically more dispersed (despite their general location in the central-western and south area of the country). The states of Parana, Mato Grosso, Goiás, and Rio Grande do Sul, have the largest crushing capacities, accounting for 21% 19%, 13%, and 13% of the total crushing capacity, respectively. Bunge is the multinational company with the largest processing capacity. The company's active plants throughout the country are capable of producing a total of ~24,600 MMT/day. Bunge is followed by Cargill (~13,500 MMT/day), ADM Brazil (~ 12,000 MMT/day), and Louis Dreyfus Commodities (~8,000 MMT /day). In the past ten years, the evolution of the processing capacity in Brazil has been moderate (7% growth).

PARAGUAY

Paraguay has had the largest relative growth during the past ten years with respect to the theoretical capacity of oilseed crushing (160% growth). During the second half of 2003, the total capacity of all installed plants was ~6,200 MMT/day. This grew to ~16,100 MMT/day by the end of that same year. The increase was generated by the growing presence of large international export companies, such as ADM, Bunge, Cargill, and Louis Dreyfus. Therefore, Paraguay is in a position to possibly triple the production and export of soybean oil in 2014, due to the recent presence of several new large processing plants: ADM in Villeta, and a Bunge-Louis Dreyfus and AGD plant in the same locality. Together, the total crushing capacity of these two plants is ~7,300 MMT/day. Add that to what the Paraguayan oil industrial complex is already cranking out, and Paraguay could mill 4 to 5 million MMT/day. According to the USDA, Paraguay expected to produce 8.1 million tons of soybeans in 2014.

BOLIVIA

Bolivia has grown its crushing capacity by ~60 % during the past 10 years, reaching a total of ~10,100 MMT/day. The increase is mainly due to the following investments:

a. The Gravetal SA plant, located in Puerto Quijarro, increased its capacity from ~1,500 MMT/day in 2003 to ~2,200 MMT/day in 2013.
b. Industrias de Aceites SA (IASA), located in Santa Cruz de la Sierra, increased its capacity from ~1,300 MMT/day to ~2,000 during the same period.
c. ADM-SAO SA, also in Santa Cruz de la Sierra, increased from ~1,000 to ~1,500 MMT/day in 10 years.
d. Small plants were established in Santa Cruz de la Sierra. These include Coop. Caico Ltda. (~210 MMT/day), ETASA (~500 MMT/day) y Caisy Ltda. (150 MMT/day). Moreover, in June 2013, a Nutriol Plant with a capacity of ~800 MMT/day was installed in Puerto Suarez.

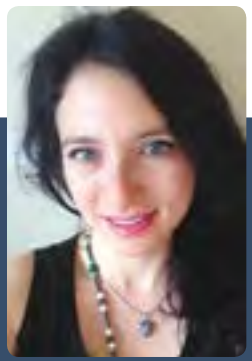
URUGUAY

The Oriental Republic of Uruguay had almost the same capacity for oilseed crushing today that it had 10 years ago (~690 MMT/day in the year 2013). Nevertheless, Uruguay has a promising future in terms of an extended crushing capacity. The company CereOil Uruguay SA (Johnson-Viana Group) plans to build an industrial plant located in Soriano, in Agraciada, on the shore of the Uruguay River. This plant would potentially mill between ~2,500 and ~3,000 MMT/day. This translates to a soybean crushing capacity of ~900K to 1 million *t* per year given 330 days of operation days/year. However, this investment project is expected to start in early 2015.

Meanwhile, Uruguay has experienced remarkable growth in the planting of soybean crops. During 2005–2006 ~ 309,000 acres of soybeans were planted. By 2012–2013, the cultivated area increased to 1,050,000 hectares (240% growth in production). From 2005–2006, Uruguay produced 631,900 tons of soybean, but from 2012–2013, ~2.8 million tons of soybeans were harvested. Soybean production in Uruguay has quadrupled in the last seven years, and if the CereOil Uruguay plant is installed, it could crush ~30% of the soybeans that Uruguay produces.

Hector Autino is a corporate industrial manager at Bunge Argentina SA, and a past president of Asociación Argentina de Grasas y Aceites (ASAGA). He can be contacted at hector.autino@bunge.com.</ab>

This article was summarized and translated from an article by Calzada and Matteo (collaborators in Rosario Stock Exchange) in 'Informativo Semanal de la Bolsa de Comercio de Rosario, año XXXI, Nº 1655, from April 11th, 2014." We thank *A&G*, a publication from ASAGA (Asociación Argentina de Grasas y Aceites), for serving as a source.



Latin America Update is produced by Leslie Kleiner, R&D Project Coordinator in Confectionery Applications at Roquette America, Geneva, Illinois, USA, and a contributing editor of *Inform*. She can be reached at LESLIE.KLEINER@roquette.com.

PATENTS

Process for purification of vegetable oils upon withdrawal of solids by centrifugation in the miscella stage

Diego Ferres Dellapiane, J., and J. Anterho Catanio Pelloso, Granol Industria Comercio e Exportacao SA, US8692004, April 8, 2004

Process for purification of vegetable oils upon withdrawal of solids by centrifugation in the miscella stage consists of the industrial extraction of vegetable oils, including the soybean oil by using a solvent, usually a mixture of hydrocarbons, in which the main constituent is hexane. The percolation extractors that operate continuously and in countercurrent provide an optimized extraction and a good performance. The replacement of the traditional miscella purification processes by a process of centrifugation in the mixture of oil and solvent (miscella) within the process with the removal of solids contained therein, returning it to the extractor results in final products as oil and lecithin of a better quality, also providing a better functioning of the process by avoiding fouling in heat exchangers and distillation of solvent, reducing downtime and lower fuel consumption resulting in lower production costs.

Catalyst for esteramine production

Nepras, M.J., *et al.*, Stepan Co., US8692005, April 8, 2014

Provided are methods for decreasing the reaction time between an alkanolamine such as triethanolamine and a fatty acid alkyl ester, such as a triglyceride, a vegetable oil, a methyl ester, an ethyl ester, and the like; a fatty acid; or a mixture thereof. The methods utilize a divalent zinc catalyst to facilitate and accelerate an esterification or transesterification reaction between the alkanolamine and the fatty acid, or fatty acid alkyl ester.

Enzyme interesterification process

Kellens, M., *et al.*, DeSmet Ballestra Engineering S.A., US8697393, April 15, 2014

A fatty material enzyme interesterification process comprising the steps of: (i) providing a fatty material that has optionally undergone at least one prior purification treatment; (ii) introducing soap into said fatty material to form a soap-containing fatty material mixture; (iii) homogenizing said soap-containing fatty material mixture by mixing, (iv) contacting said soap-containing fatty material mixture with lipase to produce a soap-containing interesterified fatty material, (v) removing soaps from said soap-containing interesterified fatty material to yield interesterified fatty material, wherein said soap is either formed in-situ by addition of aqueous alkali to said fatty material or said soap is introduced directly into said fatty material.

Solid soap

Nishina, T., *et al.*, P & PF Co., Ltd., US8697620, April 15, 2014

The object of the present invention is to improve the solidification point and the hardness of a soap of the fatty acid soap series wherein the percentage of potassium used as the counterion is large. The solid soap of the present invention to achieve the above-described object is characterized by comprising 1–5 mass percent of a betaine, and in that sodium and potassium are contained as the fatty acid counterion, and potassium is 20 mole percent or more of the counterion.

Compositions for internal application of microorganisms

Chen, C.-C., *et al.*, DSM IP Assets B.V., US8697126, April 15, 2014

The present invention describes a process for the manufacture of a solid composition comprising a microorganism, which process comprises a first step of blending and/or compacting the microorganism with a salt of a medium- or long-chain fatty acid to prepare a powderous mixture or compacted granulate, and a second step of providing said powderous mixture or compacted granulate with a coating. The microorganisms are preferably probiotics. The invention also relates to the solid composition obtained by said process and to its use in food.

Thermoplastic linoleum

Knoll, K., and M. Pepers, US8697795, April 15, 2014

The present invention relates to a thermoplastic molding composition at least comprising particles made of nonoxidatively polymerized vegetable oil that has been crosslinked by way of functional groups, as component (A), at least one thermoplastic polymer, as component (B), optionally at least one resin, as component (C), optionally at least one filler, as component (D), and optionally further additives, use thereof, and a process for production thereof.

Method for preparing fatty acyl amido carboxylic acid based surfactants

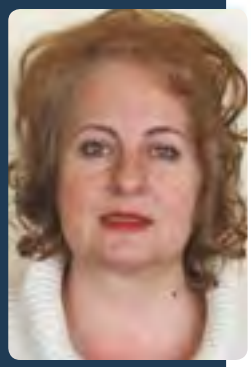
Harichian, B., *et al.*, Conopco, Inc., US8697897, April 15, 2014

A process is provided for preparing C_8–C_{22} acyl glycinate acid or salt thereof via reacting and heating a mixture of glycine or salt thereof with a C_1–C_3 alkyl ester of a C_8–C_{22} fatty acid in a medium of glycerol or propylene glycol. The reaction proceeds well where the mixture is formulated to have a pK_a ranging from 9.5 to 13.

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

Professional Pathways

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



Gabriela Sekosan is a senior project leader in the Analytical Group at Bunge North America, Bradley Illinois, USA. She started her career as an analytical chemist in 2009 at Bunge after receiving an MS in Chemistry from Long Island University in New York.

Why did you join AOCS?

I was introduced to AOCS by my manager, Tiffanie West, in 2009. I was given copies of *JAOCS* and found them very helpful for my transition from polymer chemistry to fats and oils chemistry. AOCS has enabled me to present my research findings at the annual meetings and keeps me connected with the oils experts in the world.

Describe your career path.

When I moved to the United States, it was difficult to find a job using my engineering degree in food processing from Romania. At that point, I decided to return to school for an advanced degree. I went back to school at Long Island University in New York and got my Master's in Chemistry in 2009. My research in polymer chemistry helped me to understand that polymerization is a wonderful magic that can be used to obtain valuable materials for our lives. For more than two years, I lived my life with the synthesis and analysis of polymers. After that I made another change in my career and started as a project leader in R&D at the analytical department of Bunge North America. In this role, I apply my expertise in crystallization and develop new rapid techniques for the analysis of fats and oils. The main focus of my work involves developing and applying new methods to test raw materials, intermediates, and finished shortenings and oils produced during the development process. These can be small amounts made by chemists in the lab or samples from batches made in the pilot plant or a production facility.

What do you love about your job?

I love the fact that I am challenged every day. A day in the analytical lab requires flexibility, because things can change

quickly. Patience is necessary when things don't change quickly enough. Perseverance is valuable when not everything works the first time. It is very rewarding seeing that a method you developed helps other scientists finish their projects. I like to say that the analytical laboratory is the place where every new product starts and ends its journey before it meets the customers, and I am happy to be involved in every step of this experience.

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

The fats and oils industry is changing every day and is committed to helping people *improve* their *nutrition*, *health, and wellness*. Improving living standards in emerging economies, population growth and changing diets, and the expansion of biodiesel are new trends that will have a major impact in the future development of this sector.

Describe memorable job experiences.

It is always a memorable experience when your analysis results do not match the ones expected by the other project leaders. It is not easy to tell people they need to go back to the drawing board. As an analytical chemist, it is important to have an inquisitive mindset so that you are the first one questioning the meaning of your results, but you also need to be able to explain your work to people who may not be experts.

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

My father told me that I can learn something from every person I meet. My husband encouraged me not to give up and to continue my studies after arriving in the United States. From all my professors at Long Island University and my colleagues at Bunge North America, I learned the necessary skills to be a good professional. Professor Nadarajah Vasanthan taught me how to design and conduct a successful experiment. From my manager Tiffanie West I learned how to be a good leader and keep *calm*, cool, and in control in all situations. I am very fortunate to work with an excellent R&D team at Bunge NA. It is from these colleagues that I learned not to give up when something does not work as planned and to question all the steps in the process.

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

Engage with professional societies such as AOCS, participate in one of the divisions, and present your work at the annual meeting. Do not be afraid to ask questions and remember that you can learn something from every person you meet.

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

I would not change anything. Every step in my career helped me to be a well-rounded analytical chemist.

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

Opportunities are out there, but we need to remember to look for them because they do not knock on our doors. Every industry needs a good analytical chemist to analyze the present and future line of products. Be involved with the scientific communities, acquire a rigorous training in research methods, do not be afraid of change, and show passion. Science is an international language.

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

Analytical chemistry is undergoing a renaissance every day. We have seen extraordinary improvements in the efficiency, speed, and reliability of our instrumentation. For this we need to give proper credit to the scientists and engineers developing these instruments. Today, many of the best analytical scientists collaborate with their counterparts in other disciplines and all fields show a great interest in cooperation with analytical chemists.

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

I am sure that it can be rewarding to be a specialist, but I would have to say that a balanced, well-rounded individual is generally more flexible and adaptable. I believe that well-rounded professional can move into a specialist-type role.

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

Having a graduate degree does not hurt. But is that enough? I think more could be done to develop wider skills, including team-work, communication, leadership, critical thinking, and problem solving. All these attributes will help any professional during a challenging economy. ■

EXTRACTS & DISTILLATES

Extraction of pigment information from near-UV vis absorption spectra of extra virgin olive oils

Domenici, V., *et al.*, *J. Agric. Food Chem*. *62*: 9317–9325, 2014, http://dx.doi: 10.1021/jf503818k.

This work reports a new approach to extract the maximum chemical information from the absorption spectrum of extra virgin olive oils (EVOOs) in the 390–720 nm spectral range, where "oil pigments" dominate the light absorption. Four most important pigments, i.e., two carotenoids (lutein and β-carotene) and two chlorophylls (pheophytin-a and pheophytin-b), are chosen as reference oil pigments, being present in all the reported analytical data regarding pigments of EVOOs. The method allows the quantification of the concentration values of these four pigments directly from the deconvolution of the measured absorption spectrum of EVOOs. Advantages and limits of the method and the reliability of the pigment family quantification are discussed. The main point of this work is the description of a fast and simple method to extract of such information in less than a minute, through the mathematical analysis of the UV–vis spectrum of untreated samples of oil.

Anti-inflammatory and cholesterol-reducing properties of apolipoprotein mimetics: a review

White, C.R., *et al.*, *J. Lipid Res*. *55*: 2007–2021, 2014, http://dx.doi: 10.1194/jlr.R051367.

Reduced levels of HDL cholesterol (HDL-C) are a strong independent predictor of coronary artery disease (CAD) risk. The major anti-atherogenic function of HDL is to mediate reverse cholesterol transport. This response is highly dependent on apoA-I and apoE, protein components of HDL. Randomized clinical trials have assessed effects of several classes of drugs on plasma cholesterol levels in CAD patients. Agents including cholestyramine, fibrates, niacin, and statins significantly lower LDL cholesterol (LDL-C) and induce modest increases in HDL-C, but tolerance issues and undesirable side effects are common. Additionally, residual risk may be present in patients with persistently low HDL-C

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- Mevers, E., *et al*., A cannabinomimetic lipid from the marine cyanobacterium *Moorea bouillonii*
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- Omura, K., *et al*., Improved chemical synthesis, X-ray crystallographic analysis, and NMR characterization of (22*R*)-/(22*S*)-hydroxy epimers of bile acids

and other complications despite a reduction in LDL-C. These observations have fueled interest in the development of new pharmacotherapies that positively impact circulating lipoproteins. The goal of this review is to discuss the therapeutic potential of synthetic apolipoprotein mimetic peptides. These include apoA-I mimetic peptides that have undergone initial clinical assessment. We also discuss newer apoE mimetics that mediate the clearance of atherogenic lipids from the circulation and possess anti-inflammatory properties. One of these (AEM-28) has recently been given orphan drug status and is undergoing clinical trials.

Determination of hidden hazelnut oil proteins in extra virgin olive oil by cold acetone precipitation followed by in-solution tryptic digestion and MALDI-TOF-MS analysis

De Ceglie, C., *et al*., *J. Agric. Food Chem. 62*: 9401–9409, 2014, http://dx.doi: 10.1021/jf504007d.

Adulteration of extra-virgin olive oil (EVOO) with hazelnut oil (HO) is an illegal practice that could have severe health consequences for consumers due to the possible exposure to hidden hazelnut allergens. Here, matrix-assisted laser-desorption/ionization (MALDI) mass spectrometry (MS) was used as a rapid and sensitive technique for the detection of a low concentration of hazelnut proteins in oil samples. Different protocols were tested for protein extraction, and the most efficient (cold acetone) was applied to HO and EVOO adulterated with HO. The subsequent in-solution tryptic digestion of protein extracts and MALDI-MS analysis, using α-cyano-4-chlorocinnamic acid as matrix, allowed the detection of stable hazelnut peptide markers (i.e., the m/z ions 1002.52, 1356.71, 1394.70, 1440.81, 1453.85, 1555.76, 1629.83, 1363.73, and 1528.67) attributable to the main hazelnut proteins Cor a 9, Cor a 11, and Cor a 1. Thus, the approach might allow the direct detection of specific hazelnut allergens in EVOO at low concentration without time-consuming pretreatments.

High-resolution imaging of dietary lipids in cells and tissues by NanoSIMS analysis

Jiang, H., *et al*., *J. Lipid Res. 55*: 2156–2166, 2014, http://dx.doi: 10.1194/jlr.M053363.

Nanoscale secondary ion MS (NanoSIMS) imaging makes it possible to visualize stable isotope-labeled lipids in cells and tissues at 50 nm lateral resolution. Here we report the use of NanoSIMS imaging to visualize lipids in mouse cells and tissues. After administering stable isotope-labeled fatty acids to mice by gavage, NanoSIMS imaging allowed us to visualize neutral lipids in cytosolic lipid droplets in intestinal enterocytes, chylomicrons at the basolateral surface of enterocytes, and lipid droplets in cardiomyocytes and adipocytes. After an injection of stable isotope-enriched triglyceride-rich lipoproteins (TRLs), NanoSIMS imaging documented delivery of lipids to cytosolic lipid droplets in parenchymal cells. Using a combination of backscattered electron (BSE) and NanoSIMS imaging, it was possible to correlate the chemical data provided by NanoSIMS with high-resolution BSE images of cell morphology. This combined imaging approach allowed us to visualize stable isotope-enriched TRLs along the luminal face of heart capillaries and the lipids within heart capillary endothelial cells. We also observed examples of TRLs within the subendothelial spaces of heart capillaries. NanoSIMS imaging provided evidence of defective transport of lipids from the plasma LPs to adipocytes and cardiomyocytes in mice deficient in glycosylphosphatidylinositol-anchored HDL binding protein 1.

Rapid and highly accurate detection of steryl glycosides by ultraperformance liquid chromatography–quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS)

Oppliger , S.R., *et al*., *J. Agric. Food Chem*. 62: 9410–9419, 2014, http://dx.doi: 10.1021/jf501509m.

This study describes the development and validation of a fast, accurate, and precise UPLC-Q-TOF-MS method for the analysis of steryl glycosides (SGs). The best combination of separation and sensitivity was obtained with a methanol/water gradient and formic acid as additive, using electrospray ionization (ESI). SGs were detected almost exclusively as sodiated adducts, allowing identification of the intact molecule, including the sugar moiety. The TOF-MS system offered high mass accuracy (1.3 ppm), providing a valuable tool for SG identification. The method was used to quantify single SG species in oat bran and whole wheat, and it was demonstrated that reliable quantification requires accounting for the matrix effect, which may reduce the SG signal by up to 50% in some samples. The level of matrix effect also depends on food matrices with various SG contents, indicating that it should be individually considered for each sample.

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

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Commercial-scale biodiesel **using immobilized enzymes and waste feedstocks**

Ahmed Tafesh and Sobhi Basheer

The manufacturing of commercial-scale biodiesel can be carried out by different technologies. The main technologies include the use of homogenous alkaline- and acid-based catalysts, heterogeneous alkaline- and acid-based catalysts, high energy-aided reactions, and the use of enzyme-based catalysts. The most widely used industrial process for biodiesel production is based on using alkaline catalysts, such as sodium/potassium methylate ($K/NaOCH_3$). Despite its simplicity and industrial applicability, this process requires the use of high-grade feedstocks [free fatty acid (FFA) values lower than 1%] and is energy consuming. Glycerol recovery from the process is difficult, undesirable by-products are formed, low yields are mostly dependent on the initial FFAs and water content of feedstock, wastewater treatment is required, and government subsidies are perpetually required to reach profitability.

- **The most widely used industrial process for biodiesel production is based on the use of alkaline catalysts. This process requires the use of high-grade feedstocks.**
- **The use of enzymes cannot compete with the use of chemical catalysts for biodiesel production, because they are expensive, are deactivated by methanol, and have long reaction times.**
- **This article describes a commercial immobilized enzyme technology that can convert all types of feedstocks, including brown grease, into biodiesel.**

An acid-based esterification process is typically used as a pre-treatment to lower the FFA content of the feedstock below 1%. This process is carried out by esterification of the FFAs in feedstocks (typically of FFA values higher than 4%) with a large excess of methanol, in the presence of a strong acid catalyst, such as sulfuric acid. The formed feedstock, typically with FFAs below 1%, is neutralized, dried, and then transesterified using the conventional alkaline process. Although the acid-based process is the preferable option for using low-grade oil feedstocks, it has been practiced to a limited extent mainly because it requires high energy consumption (reaction temperature up to 110°C), large amounts of methanol (up to 1:20 on a molar ratio basis of FFAs: methanol), has a prolonged reaction time (the reaction is up to 4,000 times slower than the alkaline based one, typically 10–24 hours), and requires high capital expenditures.

ENZYMATIC PRODUCTION OF BIODIESEL

Enzymes are biologically derived compounds that are used to catalyze chemical and biochemical reactions, in many cases simplifying industrial processes that would otherwise require multiple or difficult steps. During the last decade, the use of enzymes did not compete with the use of chemical catalysts for biodiesel production due to the following drawbacks:

- **Cost**. The average price of enzymes adapted for an industrial application is too expensive for biodiesel applications. Depending on the source of microorganisms, purity, and performance, the cost of commercially available adapted enzymes are in the range of $50–$1,000/kg.
- **Enzymes deactivate at the end of the reaction**. Methanol is a potent enzyme inhibitor that causes irreversible deactivation of biocatalysts. Currently available methanol-resistant enzymes are short lived (5–10 batches), resulting in higher costs.

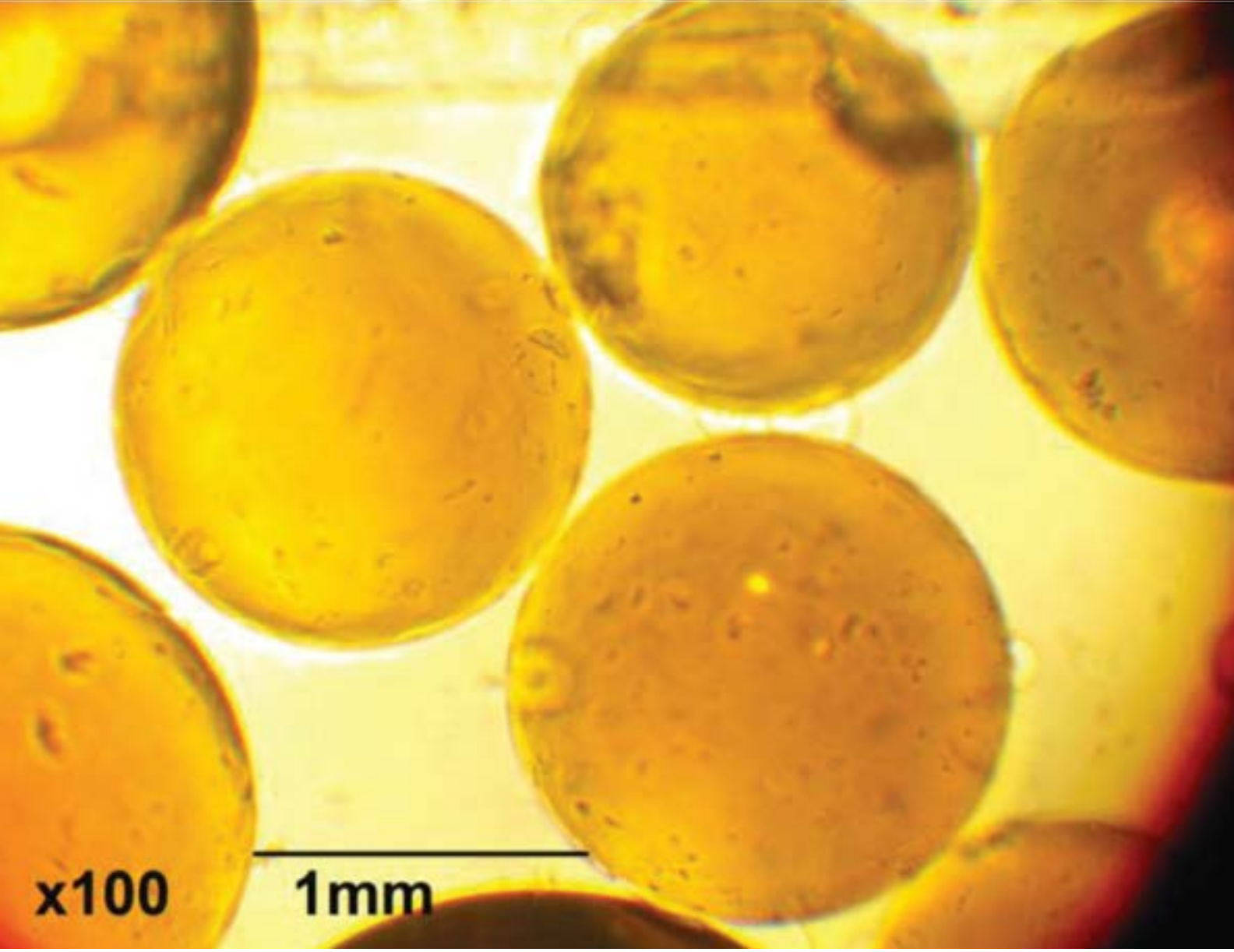

Fig. 1. A light microscope photograph of the immobilized enzyme beads.

- **Long reaction times**. Enzymatic reactions using the commercially available enzymes often require over 24 hours before reaching reasonable conversions to biodiesel, compared to just a few hours using chemical catalysts.

TransBiodiesel Ltd. has patented and commercialized a new immobilized enzyme technology for the production of biodiesel using any grade oil feedstock (FFAs 0–100%). The biocatalyst's main features are its high simultaneous esterification and transesterification activities, tolerance to high concentrations of methanol and water in the reaction medium, and its long-term operational lifetime which makes its use economically viable for biodiesel production. The biocatalyst (Fig. 1) was mainly developed as an environmentally friendly and cost-effective alternative catalyst to replace the alkaline/acid conventional catalysts typically used for the production of biodiesel.

THE IMMOBILIZED ENZYME "TRANSZYME A®

To prevent the deactivation of enzymes in the presence of methanol, TransBiodiesel has developed an innovative method of immobilization whereby commercially available enzymes are bound into a solid beaded material. This prevents them from washing out into the medium of the substance that is being processed. The enzyme molecules are mounted onto a polymer carrier support, thus strengthening the immunity of the enzymes to methanol and enabling them to catalyze the heterogeneous reaction quickly and efficiently.

The immobilized enzyme, supported on a solid organic resin, is designed to be used in batch as well as in continuous reactors. The immobilized enzyme is the company's biocatalyst with a trade name TransZyme A®. Its chief characteristic is its tolerance to high concentrations of methanol (1–30% wt./wt. of oil) and its capability to catalyze simultaneously esterification and transesterification reactions of oil components and short-chain alcohols—typically methanol and ethanol—to produce fatty acid alkyl esters (biodiesel) and glycerol/water as by-products of the process. The enzyme is capable also of transesterifying the phospholipids and wax esters typically present in crude oils and fats to form fatty acid alkyl esters and the corresponding alcohol by-product.

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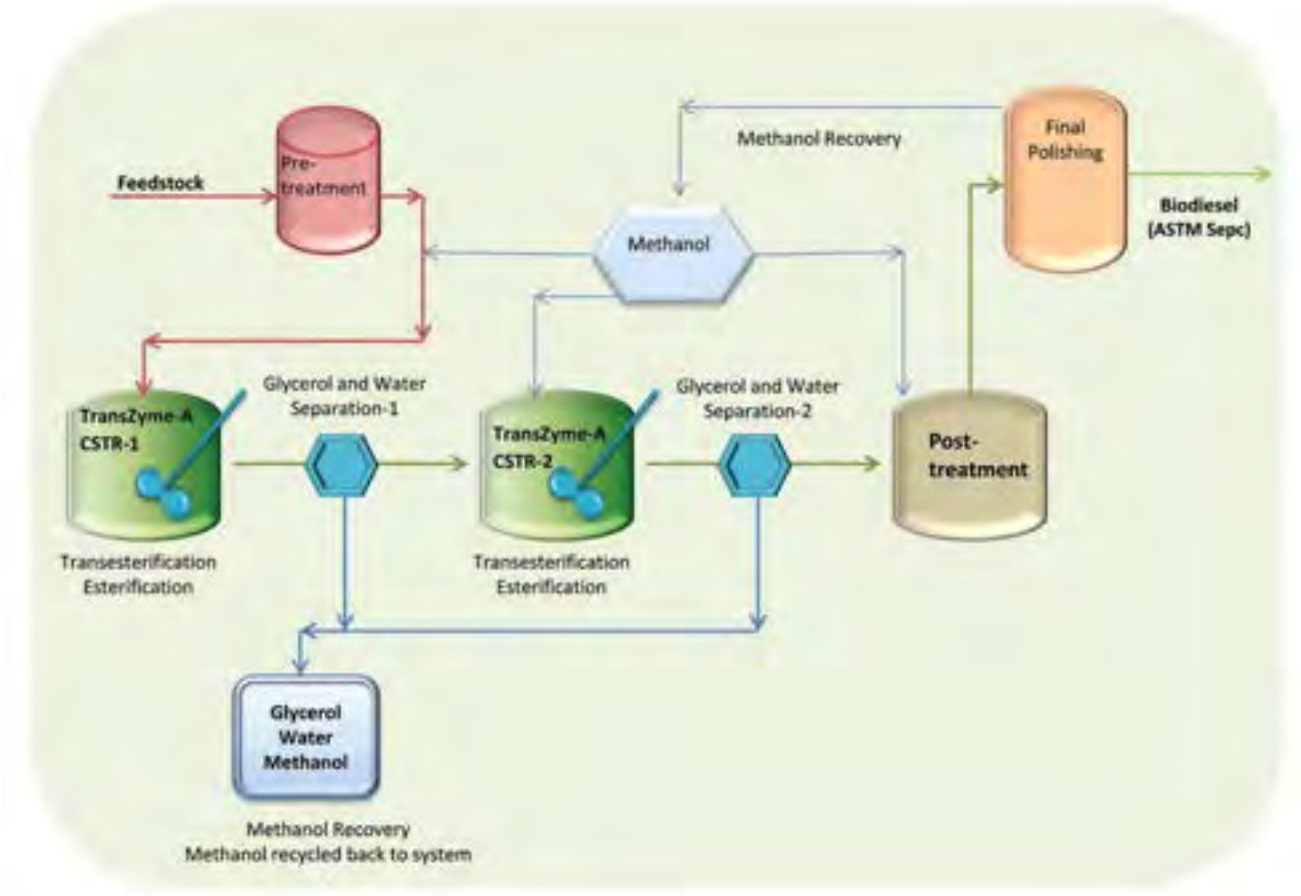

Fig. 2. Block diagram of enzymatic transesterification and esterification process by TransBiodiesel. Abbreviations: CSTR, continuous stirred-tank reactor.

THE ENZYMATIC PROCESS

Enzyme activity and stability are the main prerequisites for an industrially viable enzyme-based biodiesel manufacturing process. Water and polyols, such as glycerol, are the stabilizers which are mixed thoroughly with oil and methanol to allow good formation of slurry. The slurry is mixed in a batch- or continuous-wise reactor which contains the confined immobilized enzyme. Such slurry comes in direct contact with the surface of the beads containing the immobilized enzyme. Water which stabilizes the enzyme also converts some of the oil glycerides via hydrolysis to FFAs, which consequently are esterified with the alcohol to form fatty acid alkyl esters and water as a byproduct. Alternatively, in the presence of the immobilized enzyme glycerides can also be directly reacted with an alcohol and converted to fatty acid alkyl esters and glycerol as a by-product of the reaction.

In the proposed enzymatic process, biodiesel is produced in a two-stage batch process in which 80–90% conversion of the convertible fatty acids to fatty acid methyl esters (FAMEs) can be achieved in the first stage (Fig. 2). The reaction mixture after the first stage is evacuated from the batch reactor in to a gravity separator or centrifuge, while the immobilized enzyme is maintained in the reactor for a consecutive cycle. After phase separation, the lower phase is comprised of water, glycerol and methanol is removed, and the remaining upper organic phase is treated with the enzyme and alcohol in a second reactor to achieve conversions of higher than 96% to FAMEs. The reaction medium of the second stage is then evacuated into a gravity separator or a centrifuge for phase separation. The upper layer comprised of more than 96% FAMEs, residual unreacted fatty acid derivatives, and dissolved alcohol is removed and treated with TransBiodiesel's proprietary technology to yield biodiesel complying with the ASTM specifications for biodiesel. The reaction time in each stage is 4 hours when using 10 w% of biocatalyst by weight of oil. TransBiodiesel operates most of its biodiesel plants using continuous units comprised of two-stage stirred tank reactors (CSTR's) where the immobilized enzyme is confined in the reactors with an operational life time of more than 6 months. The typical residence time of the substrates is 2–3 hours depending on the amount of biocatalyst used in each reactor.

FEEDSTOCKS

Since the feedstock represents the largest share of the biodiesel price (75–90%), there is a need for much less expensive feedstocks with high FFAs. The higher the FFAs feedstock, the lower the feedstock's price—and therefore the better process economy achieved for the final products.

It has been demonstrated that TransZyme A is capable of esterifying/transesterifying more than 96% of the convertible fatty acid derivatives [FFAs, monoglycerides (MGs), diglycerides (DGs), triglycerides (TGs), phospholipids and wax esters) present in any type of feedstock to form fatty acid alkyl esters and the corresponding reaction by-products.

Table 1. Conversion of different waste feedstocks and refined edible plant oils to FAMEs using TransZyme A after two-stage batch reactor.

		After two-stage batch reactor					
Feedstock	**FFAs (%) (Initial)**	**FAMEs**	**FFAs**	**MGs**	**DGs**	**TGs**	**Bound glycerol (%)**
Crude corn oil	12	97.25	2.1	0.35	0.25	0.05	0.13
Used-cooking oil	3.5	96.98	2.3	0.32	0.3	0.1	0.14
Soybean acid oil*	53	96.8	2.3	0.45	0.35	0.1	0.8
PFAD	92	96.65	2.5	0.45	0.25	0.15	0.17
Tallow Fat	3	96.73	2.5	0.5	0.25	0.02	0.17
Chicken fat	5.5	97.13	2.2	0.35	0.3	0.02	0.14
Sludge palm oil	43	96	2.7	0.6	0.4	0.3	0.25
Brown Grease	87	95.85	3.1	0.55	0.5	0	0.22
Crude soybean oil	1.5	97.28	2.1	0.35	0.25	0.02	0.13
Crude rapeseed oil	1.2	97.38	2.1	0.3	0.2	0.02	0.11
Crude palm oil	2.7	97.18	2.1	0.45	0.25	0.02	0.16

*Obtained from acidulated soap stocks by-product of soybean oil refineries. Abbreviations: FFAs, free fatty acids; FAMEs, fatty acid methyl esters; MGs, monoglycerides; DGs, diglycerides; and TGs, triglycerides.

One of TransBiodiesel's unique selling propositions is the ability of its immobilized enzyme to convert all types of feedstocks, including brown grease, to biodiesel complying with the ASTM spec. Brown grease is collected from the grease traps installed in commercial, industrial, or municipal sewage facilities to separate grease and oil from wastewater. It is estimated that the annual production of brown grease processed from trapped grease in developed countries averages at about 13.37 pounds per person. It is assumed that more than 1.8 billion liters of biodiesel could be produced from brown grease generated in the United States alone. Table 1 presents data analysis for the different components of various feedstocks used with TransZyme A after a two-stage reaction process operated in batches. The results show that the post-reaction upper phase is comprised of a total bound glycerol composition less than 0.3% and an average of 2.5% FFAs for all types of tested feedstocks. The post-treatment proprietary method developed by TransBiodiesel guarantees to achieve ASTM spec for biodiesel when applied to all reaction mixtures obtained from using the different feedstocks presented in Table 1. Depending on the FFA content of the feedstock, the formed glycerol is almost salt-free and transparent, and can be brought to meet the specifications for USP-grade glycerol without distillation.

OPERATIONAL LIFETIME OF THE IMMOBILIZED ENZYME

TransBiodiesel offers a cost-competitive and highly efficient novel immobilized enzyme for the production of biodiesel using low quality feedstocks with FFA contents of 0–100%. The biocatalyst improves the efficiency of the biodiesel production process by easing its operation. It also tolerates the high content of chlorophylls, salts, water, waxes, proteins, gums, and FFAs. Overall, the enzymatic process improves the quality of the final products (biodiesel and the by-product, glycerol) and reduces waste effluents. The immobilized enzyme is maintained in the reaction vessel at all times and it is removed only after it starts showing decreased activity. The company has achieved more than 200 cycles in batch reactors without the biocatalyst losing its activity, thus implementing industrially what many companies regarded as a commercial challenge. The process is operated favorably in a continuous mode in which the enzyme would exhibit an operational lifetime of 6–8 months. Several clients , including M Energy-Korea operating a continuous enzymatic process with annual production capacity of 32,000 tons biodiesel, have demonstrated that the use of one ton of TransZyme A can produce an average of 3,000 tons of biodiesel or more.

RETROFITTING: NEVER BEEN EASIER

TransBiodiesel has also patented its new reactor configurations for the use of immobilized enzymes in the production of biodiesel. The reactor is based on using stirred-tank units in batch and continuous operations. To lower capital costs, the company has also developed low-cost methods to retrofit existing biodiesel plants for the immobilized enzyme technology. The propellers needed for appropriate mixing and the screens that confine the enzyme beads in the reactor are the main reactor components that must be properly modified to adapt existing conventional equipment. The cost of retrofitting is <3 % of biodiesel plant cost.

Table 2 (page 50) shows the features, advantages and benefits distinguished for using the immobilized enzyme as compared to use of conventional chemical catalysts.

LIQUID VS IMMOBILIZED ENZYME

Recently, liquid enzyme formulations have been introduced to the market. However, the process is limited to batch operation,

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Table 2. The features, advantages and benefits of using the immobilized enzyme technology.

Features	Advantages	Benefits
Feedstocks	Low quality feedstocks including waste oils of FFAs 0-100%, such as brown grease.	Multiple feedstocks can be used. All FFAs are used up to make biodiesel.
Plant	Batch and continuous operations.	High productivity per reactor volume. Plant robustness.
No Soap & no caustic	Easy phase separation.	No chemicals, smoother process & no clogging of soaps.
Scalability	Simple and scalable to any scale.	Reduced CAPEX and OPEX.
Low energy	Less than tenth of the energy consumed in the chemical process.	Maximizes the Biodiesel formation with low energy and without the use of chemicals.
	Easily retrofitted with a screen to confine the immobilized enzyme.	Flexibility to expand to new cheaper oils, & saves installation cost.
Glycerol	Much cleaner glycerol than the chemical process.	The quality of the glycerol is high; it is an added-value and sells much more.
Alcohol	Wet alcohols can be used including methanol, ethanol and other longer chain alcohols.	The use of cheap wet methanol. Multi-functional products.

which requires larger volumes of reactor per unit production capacity than continuous processing. Furthermore, using low-quality feedstocks, such as used cooking oil and grease, which typically contain impurities (proteins, emulsifiers, polymers, etc.) with liquid enzyme formulations imposes difficulties during phase separation between the formed biodiesel and glycerol/water phases. Improper phase separation would negatively affect the recycling of the liquid enzyme and would therefore increase the cost contribution of the enzyme in the overall process. Alternatively, the immobilized enzyme can handle waste feedstocks such as brown grease, acid oils (from soapstocks), and sludge palm oil—and be used efficiently in continuous processes. The concentration of the immobilized enzyme can also be increased to achieve high conversions to biodiesel during a reasonable reaction time that is competitive with the short reaction times of conventional chemical processes.

Furthermore, when used according to TransBiodiesel's instructions, the immobilized enzyme is methanol-resistant, less expensive, and lasts longer than the liquid form. Table 3 compares the use of the immobilized enzyme technology developed by TransBiodiesel with that of liquid enzyme formulations.

IN CONCLUSION

The use of immobilized enzyme technology for the production of biodiesel is a new game-changing technology. TransBiodiesel's cutting edge immobilized enzyme technology produces biodiesel complying with the ASTM and EN specs not only from crude and degummed vegetable oils (corn, soybean, palm and rapeseed), but also from low quality feedstocks such as, used-cooking oil, brown grease, palm fatty acid distillate, sludge palm oil, acid oils (from soap stocks), and algal oil. In particular, TransBiodiesel offers a solution to the biodiesel worldwide market that will allow it to meet government set projections and reach profitability without subsidies—by lowering production costs and providing the technological capabilities to use second generation feedstocks.

The company is expanding this technology throughout the world. The large commercial plant M Energy Korea, now in its second year of operations using TransBiodiesel's enzymatic technology for more than a year, produces 30,000 tons of EN-spec biodiesel per year using low quality waste feedstocks (FFAs > 50%). The company is now building more industrial units worldwide, which will increase its production of biodiesel from second-generation feedstocks. TransBiodiesel has developed engineering concepts for industrially viable mobile units that can produce biodiesel in remote areas, and the company has already built some initial units.

Ahmed Tafesh is VP for Business Development and Marketing at TransBiodiesel Ltd., where he is focusing on opening a new market for commercial production of biodiesel worldwide using of the developed immobilized enzyme technology. He can be contacted at: atafesh@transbiodiesel.com.

Sobhi Basheer is CEO at TransBiodiesel Ltd., where he is developing new commercial applications for his pioneered enzyme immobilization technology started it since the year 1997. He can be contacted at: sbasheer@transbiodiesel.com

Table 3. Comparison of the use of an immobilized enzyme (TransBiodiesel's Process) vs. the use of free liquid enzymes for production of biodiesel*.

Parameter	Free Liquid Enzyme Process	Immobilized Enzyme (TransBiodiesel's Process)
Enzyme formulation	– Liquid – Water soluble	– Embedded on solid beads – Insoluble
Feedstock: – FFAs – Purity – Filtration – Low quality feedstocks, such as (brown grease)	– 0 – 100% – Degummed – Down to 1-5µ – Not possible	– 0 – 100% – Unrefined (crude) – Down to 1-5 µ – Possible
Reactor type: – Operation mode – Reactor volume	– Batch (stirred) – Big (100-300m3)**	– Batch and continuous – Small (10-20m3)*
Reaction time:	– 24 hours	– Batch-wise: 6 hours – Continuous-wise: 5 h – (Residence time)
Product composition: – FAMEs – FFAs – MGs, DGs, TGs	– > 95% – 2-3% – 1.5-3%	– >96% – 2-3% – <1.5%
Separation of glycerol/water by-products:	– Depending on feedstock	– Easy
Tolerance to: – Methanol – Water – Surfactants and polymers in Feedstocks	– In steps (up to 5% of reaction medium) – Up to 3% – Low	– One stage addition (up to 20%) – Up to 10% – High
Enzyme recovery – Batch operation – Continuous operation	– Challenging – Not possible	– Easy – Easy
Enzyme life time – Batch operation – Continuous operation – Shelf life time	– 5 cycles – No use – 6 Months (5°C)	– > 200 cycles – > 6 months – > 3 year (room temp.)

*The comparison was made based on data of TransBiodiesel and data published in *Inform 25*: 143–144, March 2014 and *Inform 25*: 412–415, July/August 2014.
** For production capacity of 24,000–72,000 Ton Biodiesel/year.

Electronic storage of LC/ESI-MS total ion current profiles of total lipid extracts **for subsequent recovery of new data**

Arnis Kuksis and Waldemar Pruzanski

Plasma contains many thousands of distinct molecular species [1] that fall into six main categories of lipids: phosphatidylcholine s (PtdChos), phosphatidylethanolamines (PtdEtns), phosphatidylserines (PtdSers), phosphatidylinositols (PtdIns), triacylglycerols (TAGs) and cholesterol esters (CEs). Detailed analyses of such lipid mixtures are time consuming and require a minimum of short-time storage, which subjects samples to degradation even at-78 °C [2]. Storage of peroxidized lipid samples at 4 °C or-20°C in a laboratory freezer is totally unacceptable, as is the freezing and thawing of samples. Preparing a new set of samples is inconvenient, expensive or impossible when dealing with a large number of individual specimens, or unique material. An unlimited length of storage, however, is possible via the electronic records of total lipid extracts obtained by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/ESI-MS) in presence of internal standards and appropriate methods of data analysis [3].

This report illustrates the successful utilization of electronically stored data in several originally unintended applications, including chemical, physicochemical and enzymatic analyses of oxolipoprotein lipids.

- **Detailed analyses of plasma lipid mixtures are time-consuming, and samples degrade when stored.**
- **Freezing is unacceptable, and preparing a new set of samples is inconvenient, expensive, or impossible when dealing with large numbers of individual specimens or unique material.**
- **This report illustrates the successful utilization of electronically stored data for several originally unintended applications, including chemical, physico-chemical and enzymatic analyses of oxo- lipoprotein lipids.**

IDENTIFICATION OF PtdCho HYDROPEROXIDES AND HYDROXIDES IN HUMAN PLASMA LIPOPROTEINS

Eckey *et al.* [4] had reported that oxo-PtdCho of LDL served as an activator of group IIA sPLA$_2$, but specific oxo-PtdChos involved in the process were not identified. When Adachi et al [5] identified specific PtdCho-OOHs and PtdCho-OHs as components of oxo-PtdCho of auto-oxidized plasma, we used the electronically stored samples to demonstrate nanomolar amounts of PtdCho-OOHs and PtdCho-OHs in all lipoprotein samples that had given significant hydrolysis with group IIA sPLA$_2$ in the past [6].

Figure 1 shows the major molecular species of PtdCho-OOH (A) and PtdCho-OH (B) in human plasma LDL following a 4 h incubation at 37 °C. [7]. The absolute amounts ranged from high picomoles of PtdCho-OOHs to low nanomoles of PtdCho-OHs. Although the oxo-PtdChos could have brought about local disorganization of the lipid mono-layer of the lipoprotein particles, any lasting effect would have to be due to the PtdCho-OHs, since the PtdCho-OOH are unstable and readily converted to the more inert hydroxides.

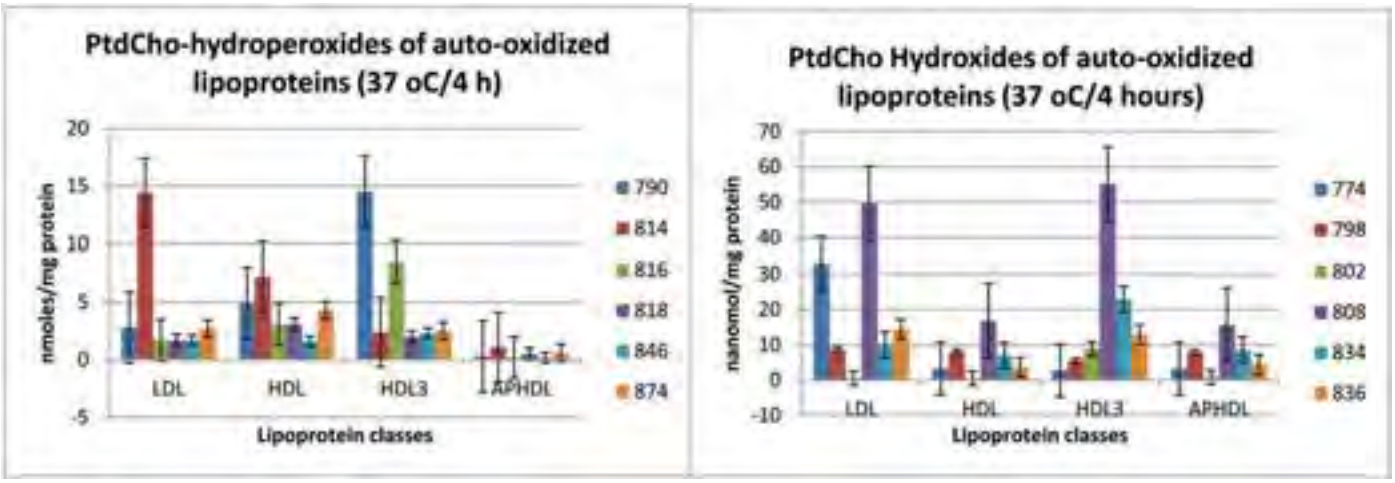

FIG. 1. PtdCho-OOH (A) And PtdCho-OH (B) of auto-oxidized human plasma lipoproteins [7] Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; HDL3, a subfraction of HDL; APHDL, acute phase HDL. Reproduced with permission from *Lipid Technology* (3)

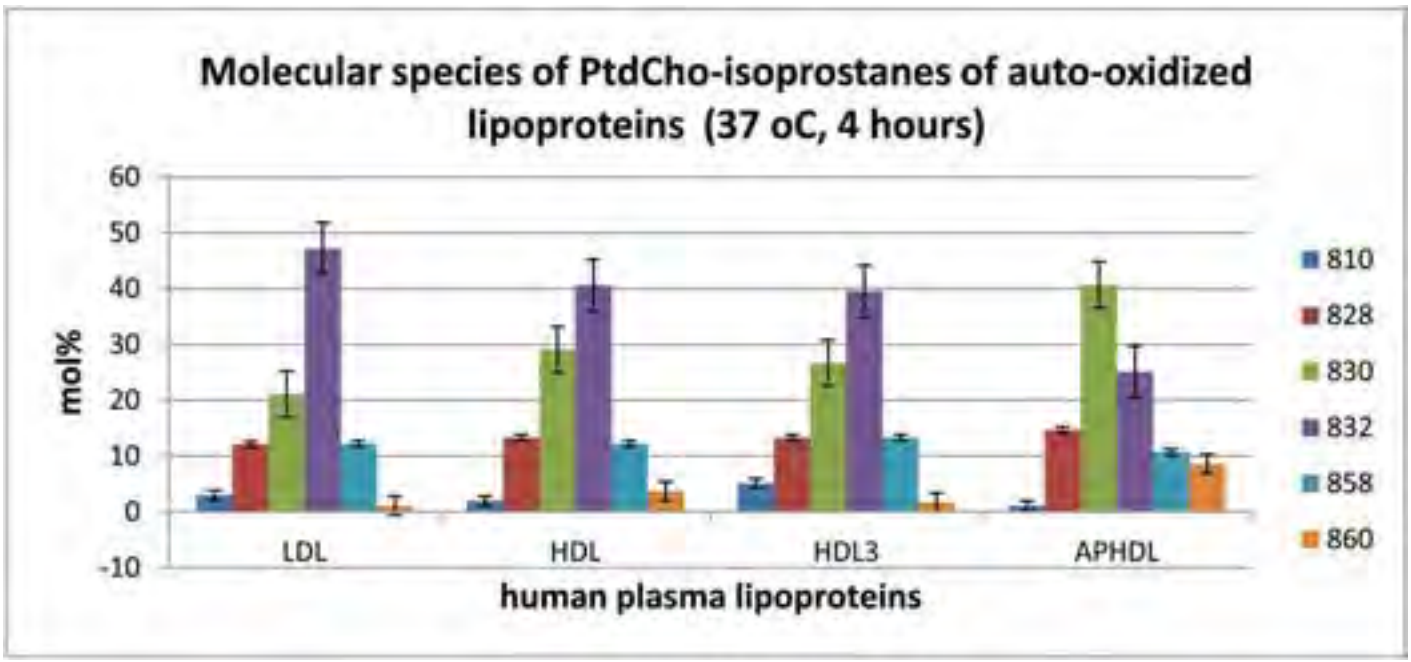

FIG. 2. Molecular species of PtdCho-IPs of auto-oxidized human plasma lipoproteins [9].Abbreviations as in Fig. 1 Reproduced with permission from *Lipid Technology* (3)

IDENTIFICATION OF PtdCho ISOPROSTANES IN HUMAN PLASMA LIPOPROTEINS

Morrow *et al.* [8] had discovered that PtdCho-IPs arise from PtdCho auto-oxidation and had suggested that their kinked configuration would affect cell membrane structure and function. Figure 2 shows the major molecular species of PtdCho-IPs of various plasma lipoproteins following a 4 h incubation at 37 oC. [9]. The total concentrations of the PtdCho-IPs in the original samples ranged from high picomoles to low nanomoles, but were increased up to 100 fold during the incubation.

HYDROLYSIS OF PtdCho ISOPROSTANES BY GROUP IIA, V AND X sPLA$_2$s

Stafforini *et al.* [10] had previously reported that PAF acetylhydrolases were solely responsible for hydrolysis of plasma

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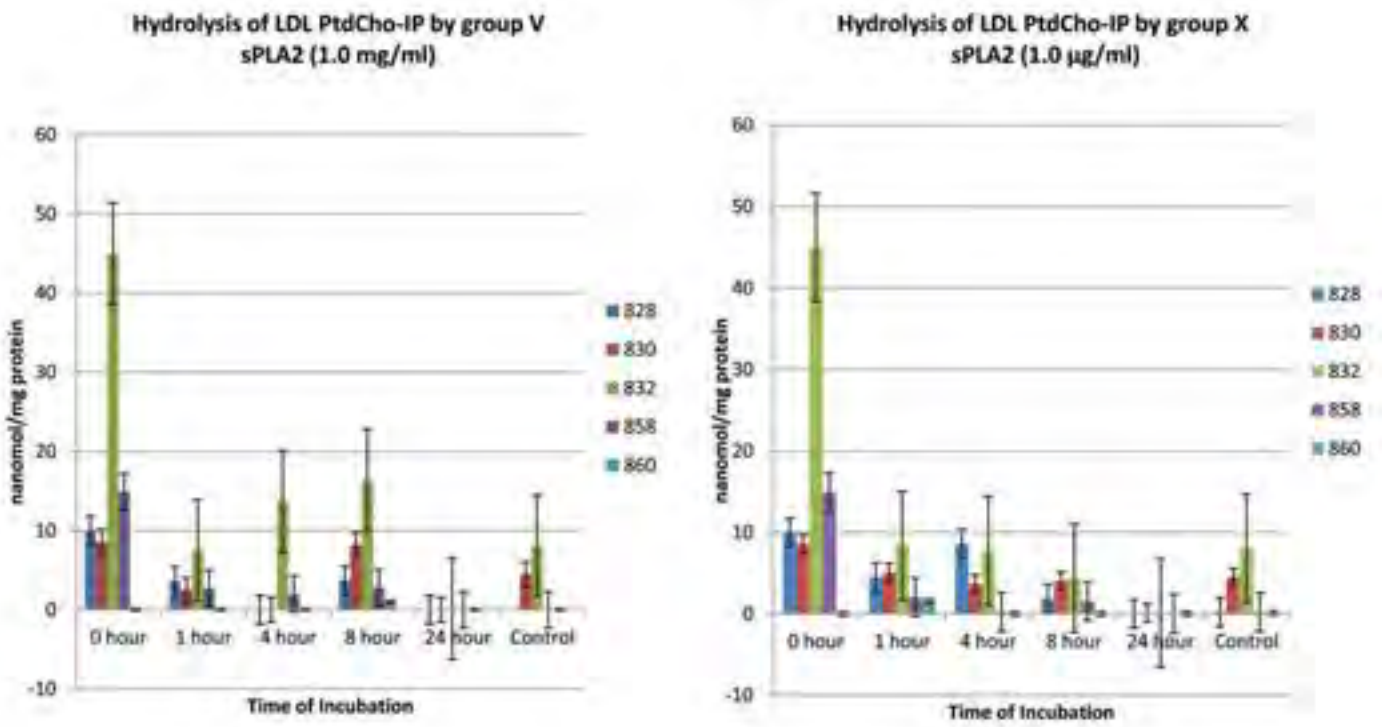

FIG. 3. Hydrolysis of LDL PtdCho-IPs by group V (A) and group (X) sPLA2s (1.0 µg/ml) [9].Mean±S.E.M. Reproduced with permission from *Lipid Technology* (3)

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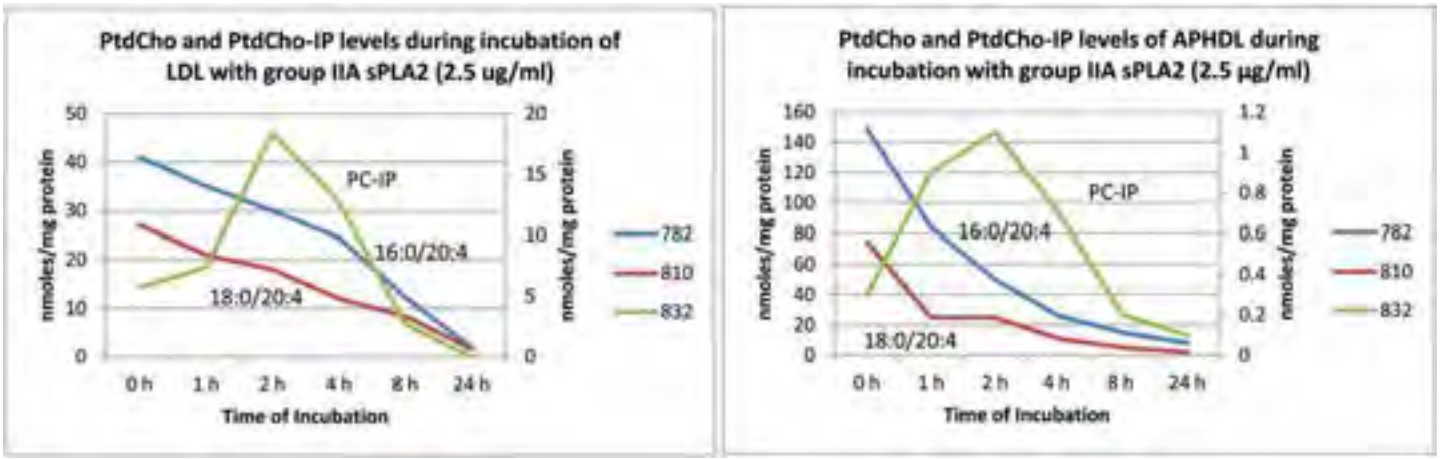

FIG. 4. Correlation of hydrolysis of 16:0/20:4 and 18:0/20:4 GroPCho with iso-PGF2 ester of 16:0(18:0) GroPCho of LDL (A) and APHDL (B) by group IIA sPLA2 (2.5 µg/ml) [11].Abbreviations: PC-IP, PtdCho-IP; 16:0/20:4, 16:0/20:4GroPCho; 18:0/20:4, 18:0/20:4GroPCho

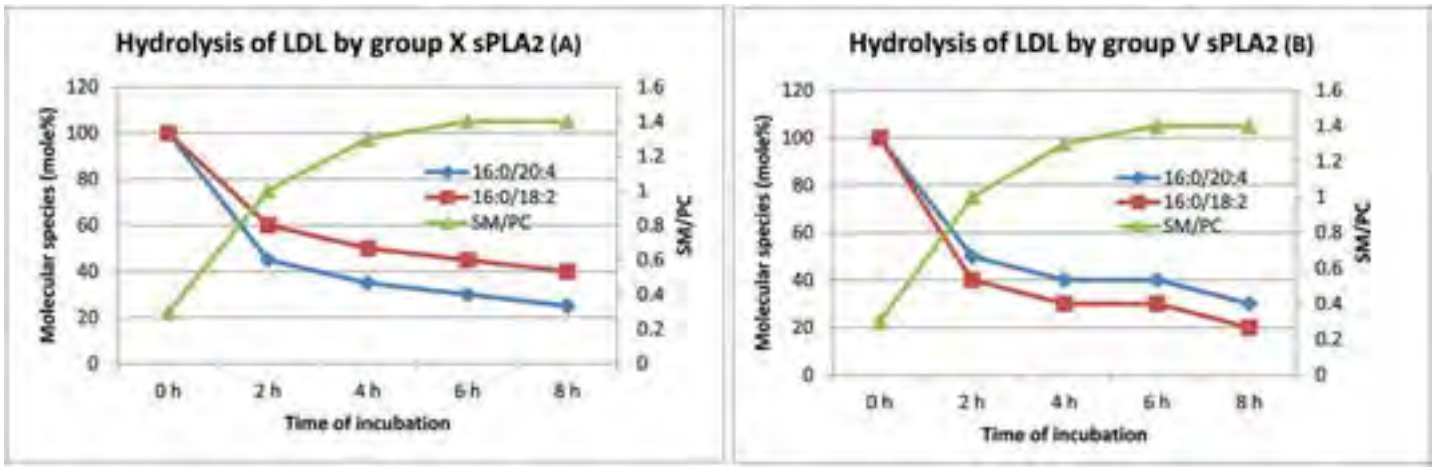

FIG. 5. Differential hydrolysis of 16:0/18:2 and 16:0/20:4 GroPCho in relation to SM/PtdCho ratio during incubation of LDL with group X (A) and group V (B) sPLA2s (1 µg/ml). [13].Abbreviations: 16:0/20:4, 16:0/20:4GroPCho; 18:0/18:2, 18:0/18:2GroP-Cho; SM/PC, sphingomyelin/PtdCho

PtdCho-IPs. We used the electronically stored LC/ESI-MS profiles of the lipoprotein total lipid extracts to demonstrate the hydrolysis of PtdCho-isoprostanes by sPLA2s, which had not been previously reported. Figure 3 shows the hydrolysis of PtdCho-IPs by group V and X sPLA2s. [11]). There appears to be significant variation in the rates of hydrolysis of the different molecular species of the oxo-PtdChos, which is difficult to evaluate in view of a continued biosynthesis especially during the early times of digestion, for which we had no controls.

CORRELATION OF GROUP IIA $sPLA_2$ ACTIVITY AND PTDCHO-IP CONTENT.

On the basis of model building, Morrow *et al.* [9] had suggested that the kinked structure of isoprostanes would affect cell membrane structure and function. Figure 4 shows the time course of hydrolysis of the 16:0/20:4 and 18:0/20:4 GroPCho, the precursors of PtdCho-IPs, and of the iso-PGF2 ester of 16:0(18:0) GroPCho of LDL (A) and APHDL (B) by group IIA sPLA2 (2.5 ug/ml over a period of 1-24 hours [11]. There are about 7 nanomoles/mg protein of isoPGF2 ester in the LDL sample at 0 time, which increases to 18 nmoles/mg protein after 2 hours of incubation, after which it decreases to 10, 5 and 0 nmoles/mg protein at 4,8, and 24 hours. In B, a closely similar trend of hydrolysis for the group IIA sPLA2 is seen for the APHDL, except that the effect is reproduced at a nearly 10 times lower concentration of PtdCho-IPs. In the mean time the hydrolysis of the PtdCho-IP precursors (native PtdCho)

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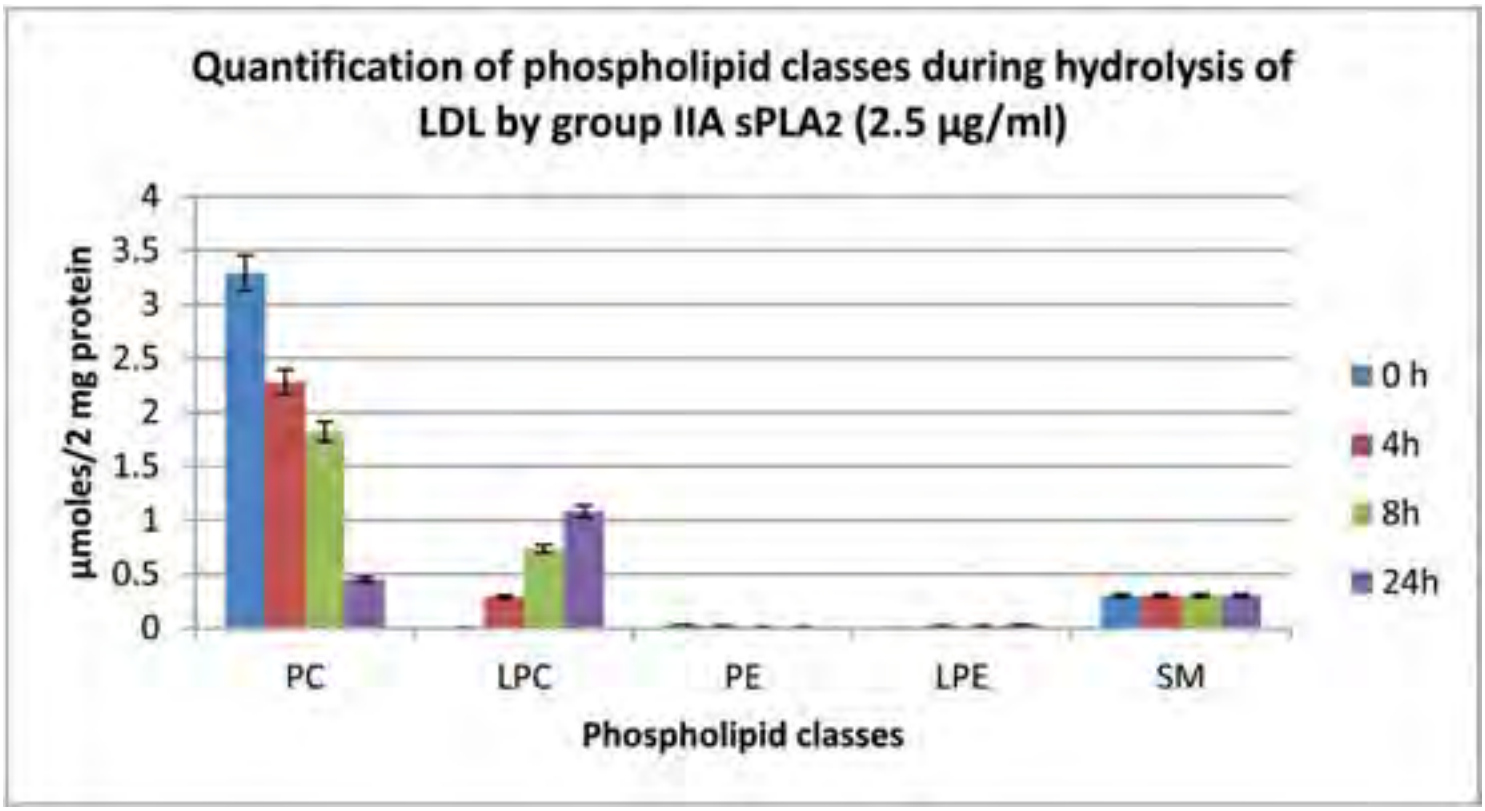

FIG. 6. Quantification of phospholipid classes during a 0-24 h hydrolysis of LDL by group IIA sPLA2 (2.5 µg/ml). [Kuksis & Pruzanski, 2008. Unpublished).Abbreviations:PC, PtdCho; LPC, LysoPtdCho; PE, PtdEtn; LPE, LysoPtdEtn; SM, sphingomyelin

proceeds at nearly linear rate, not responding to the changes in the isoprostane production or hydrolysis.

LIPOPROTEIN PHASE COMPOSITION AFFECTS FATTY ACID SPECIFICITY OF $sPLA_2$s

Singh and Subbaiah [12] had shown that SM regulates the activity of group V and group X sPLA2s and contributes to selective arachidonic acid release. Using the stored data we were able to attribute the change in fatty acid specificity to segregation of molecular species of PtdCho and of sPLA2s between disordered and ordered SM/FC/PtdCho lipid phases. The significance of SM and FC/SM ratio during sPLA2 hydrolysis of lipoprotein PtdCho had been previously overlooked. Fig. 5 (page 55) shows time course of differential disappearance of 16:0/18:2 and 16:0/20:4 GroPCho in relation to SM/PtdCho ratio during hydrolysis of LDL by group X (A) and group V (B) sPLA2s over a period of 1-8 h at 1 µg/ml enzyme [13].

ABSENCE OF SPHINGOMYELINASE ACTIVITY IN PLASMA LIPOPROTEINS.

Holopainen *et al.* [14] had reported that plasma LDL, but not VLDL or HDL were associated with sphingomyelinase activity, which degrades SM to ceramide and PCho. A quantitative assay of SM during LDL and HDL digestion with group IIA, V and X sPLA2s failed to demonstrate any loss of SM over a period of 1-24 hours. Oxidation of LDL abolished the sphingomyelinase activity. Figure 6 shows that the content and composition of lipoprotein SM remain constant during incubation of plasma lipoproteins in the absence or presence of exogenously added sPLA2s [Kuksis and Pruzanski, 2008. Unpublished].

MOLECULAR SPECIES OF PtdChos AND ANTECEDENT MEMORY IMPAIRMENT.

Mapstone *et al.* [15] recently reported the discovery of a set of ten lipids from peripheral blood that predicted phenoconversion to either amnestic mild cognitive impairment or Alzheimer's disease within a 2-3 year time frame with over 90% accuracy in a group of cognitive normal older adults. The lipids were made up mostly of minor species of PtdCho identified by carbon and double bond number, and alkyl/acyl pairing, where appropriate. Figure 7 shows readily detectable amounts of PtdCho species of interest to Alzheimer's in the stored HDL and LDL samples [Kuksis and Pruzanski, 2014. Unpublished].

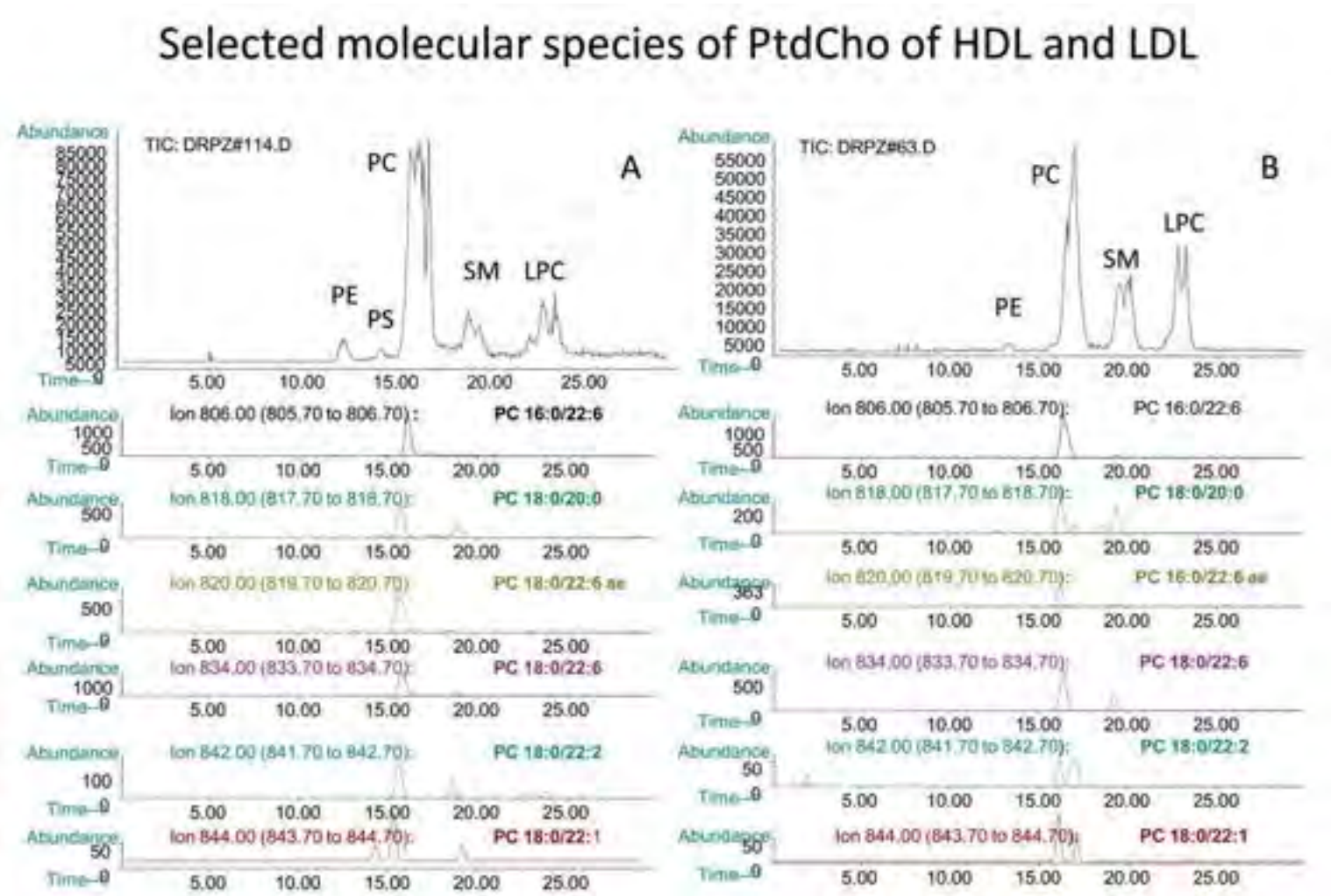

FIG. 7. Identification of PtdCho species of interest to antecedent memory impairment in older adults in stored plasma HDL (A) and LDL (Kuksis & Pruzanski, 2014. Unpublished). Abbreviations as in Fig. 6

THINKING AHEAD

The present report shows that follow-up studies using electronically stored analytical data can provide extensive new data at little additional expenditure as it avoids the expense of new sample collection and work-up. It also avoids sample storage and associated sample degradation. By inclusion of time course of auto-oxidation and enzymatic hydrolysis, the LC/ESI-MS lipid profiling becomes transformed into a dynamic multidimensional lipidomic analysis. The method is widely applicable because the conditions of sample collection and lipid isolation are sufficiently general to permit extensive subsequent work-up, despite limited original analytical objectives.

Thinking ahead, the present approach to electronic data storage and utilization could be improved further first, by deliberately designing the original experiment to provide data for application beyond the immediate needs of the analysis, which could be done with little extra expenditure of time or money. Second, the data could be collected using chromatographic columns of higher resolution (e. g. reversed instead of normal phase columns). Third, the original data could be collected using more advanced MS/MS equipment permitting selected ion fragmentation.

In addition to facilitating data storage in research laboratories, the above method could be used in control and clinical laboratories for reference purposes. Finally, the electronically stored data could be made available to other researchers for confirmation or new investigations.

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3 reasons to attend AOCS Annual Meeting

Registration is now open for the 106th AOCS Annual Meeting and Industry Showcases, which will be held May 3–6, 2015, at Rosen Shingle Creek in Orlando, Florida, USA. The premier international science and business forum on fats, oils, surfactants, lipids, and related materials is expected to draw more than 1,600 professionals from more than 60 countries to "The City Beautiful," where they will experience educational sessions and courses, participate in a variety of networking opportunities, and connect with Industry Showcase partners from around the world. Don't miss this unique opportunity to:

- **expand your knowledge;**
- **network with colleagues;**
- **experience Orlando.**

Katie Walden

1. Expand your knowledge.

Engage in one of the best educational programs in the industry.

AOCS is known for its extensive technical program, which features more than 600 presentations covering 11 interest areas. Leading experts will present the latest information in such fields as health and nutrition, biotechnology, lipid oxidation and quality, surfactants and detergents, and processing.

Discuss what's hot.

Hot Topics Symposium sessions highlighting industry innovations and emerging issues will promote global discussions critical to the future of the fats and oils industry.

Attend a short course.

Short courses in the days prior to the AOCS Annual Meeting and Industry Showcases offer classroom-style training that will build the critical technical skills you need to be successful in the fats, oils, and related materials industry. This year's short courses include:

- Analytical Methods for R&D, Product Quality Control, and On-line Process Control/Refinery Optimizations;
- Fundamentals of Edible Oil Processing;
- Update on New Technologies and Processes in Oils and Fats;
- Novel Cosmetic Surfactant Systems and their Design

Taste and learn.

Bring your taste buds to "Understanding Olive Oil: The Romance and the Reality, a three-course luncheon workshop in which you will examine the history, cultural significance, and culinary functionality of this highly sought-after oil. Experts from industry and academia will tantalize your senses and help you become a true connoisseur of the savory science behind olive oil. Explore pairings of olive oil with foods; learn the chemical science behind flavor profiles, taste preferences, and quality assessment; and discuss market trends, truths and misgivings. This workshop is appropriate for: olive growers, producers and purveyors of olive oil, chefs, restaurateurs, culinary experts, and anyone looking to further their knowledge of olive oil.

2. Network with colleagues

Eat, drink, and connect.

Several networking activities will help you connect with other industry professionals. Start your week on Sunday evening by attending the President's Welcome Reception on Rosen Shingle Creek's beautiful outdoor Gatlin Terrace. On Tuesday evening, join your colleagues for a causal outdoor lawn and patio party. Begin each morning with an Early Riser Coffee reception before sessions begin and refill your cup and list of contacts during the morning and afternoon technical sessions. All networking events are included in the conference registration fee.

Find people with similar interests.

The new, innovative conference program debuting in 2015 will give attendees with similar interests more opportunities to network, collaborate, and learn.

The eleven AOCS interest areas will be grouped into three campuses, each featuring concurrent technical sessions, an Industry Showcase, poster presentations, and networking breaks. Campuses are conveniently located near one another and will follow the same schedule, creating an easy transition between sessions.

Aside from the technical sessions, attending a Division or Section event is the best way to meet colleagues in your interest area or from your part of the world. Seven geographic AOCS sections provide a local forum for fats and oils professionals, enhancing networking opportunities in your region. Eleven AOCS divisions (also known as interest areas) allow attendees the opportunity to collaborate with like-minded professionals. Division and section events take place throughout the Annual Meeting, and all registrants are welcome to attend.

Enjoy the new Industry Showcases.

The AOCS Industry Showcases, a new and an integral part of the campus environment, will feature business solu-

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tions specific to the interests of each campus. The Industry Showcases will feature more than 50 international companies, showcasing the industry's most prestigious corporate, government, and academic institutions as well as the most current products and services.

3. Experience Orlando

Over 50 million visitors came to Orlando last year—and it's no wonder. With its wide array of resorts, world-renowned attractions and comfortable weather year-round, Orlando is unlike any other destination.

Recognized as the theme park capital of the world, Orlando is home to Walt Disney World, with its famous Magic Kingdom and Epcot Center, as well as Universal Orlando Resort, where you can enjoy Universal Studios Florida and Islands of Adventure, SeaWorld, Gatorland, and Wet 'n Wild Water Park. Located near Rosen Shingle Creek, both Universal CityWalk and Pointe Orlando feature plenty of shopping, dining, and entertainment options.

Underlying the theme park magic is Central Florida's rich cultural heritage. Join us Sunday for a tour of the Orange County Regional History Center. Set in the historic 1927 Orange County Courthouse, this state-of-the-art venue, located in the "Heart of the Community," brings history to life through audio and video presentations, hands-on exhibits, motion-sensitive figures, and costumed docents who tell the story of the state that spans from 12,000 years ago through tomorrow. Space is limited! Reserve your spot on the tour when you register for the Annual Meeting.

"The AOCS Annual Meeting is once again the place to be for those in the international fats and oils community, according to AOCS Vice President Manfred Trautmann. Join us to participate, learn, and network during the only international event that expands the depth and breadth of our diverse and collective knowledge. I look forward to welcoming you to Orlando to experience it for yourself! For more AOCS Annual Meeting information visit: AnnualMeeting.aocs.org. ■

BIOTECHNOLOGY (cont. from page 32)

benefits, according to a new report from the US Department of Agriculture (USDA). More than 90% of all soybeans, cotton, and corn grown in 2014 were a genetically engineered variety.

"Scientific innovation and seed technology allow growers to produce the most reliable and abundant yields with less tilling of the soil and fewer applications of insecticides," said Cathleen Enright of the Biotechnology Industry Organization (Washington, DC, USA), a trade association representing biotech companies, centers, and related organizations in more than 30 countries, in a statement. "These practices promote environmental sustainability, reduce on-farm fuel use, increase profit margins for US farming families, and keep food costs affordable for US consumers." Of the 18 million farmers growing biotech crops worldwide, 90% are resource-poor farmers in developing countries, Enright said. See http://tinyurl.com/USDA-2014-Biotech-Crops for the full report.

EFSA: New GM soybean, cotton, and oilseed rape safe

The European Food Safety Authority (EFSA) Panel on Genetically Modified Organisms has issued scientific opinions stating that new strains of soybean and cotton are as safe as their conventional counterparts and do not raise safety issues or concerns about human health and nutrition or environmental risks.

The two crops under evaluation were Monsanto's (St. Louis, MO, USA) soybean MON 87769, which has been modified to express elevated levels of stearidonic acid (C18; 4n-3), and Bayer CropScience's herbicide-tolerant genetically modified (GM) cotton GHB614 x LLCotton25, which contains stacked genes

rendering the crop resistant to both glyphosate and glufosinate-ammonium herbicides. For MON 87769, EFSA recommends a post-market monitoring plan "to confirm the exposure assessment using realistic consumption data for the European population." Read the full reports at http://tinyurl.com/EFSA-MON87769 (pdf) and http://tinyurl.com/EFSA-Bayer-Cotton (pdf).

EFSA has also issued a scientific opinion on MON 88302, an herbicide-tolerant GM oilseed rape produced by Monsanto. EFSA concluded MON 88302 is "as safe as its conventional counterpart and non-GM commercial oilseed rape varieties with respect to potential effects on human and animal health and the environment." Field tests revealed no biologically relevant differences compared to its conventional counterpart except for days-to-first flowering, and raised no concerns regarding the potential toxicity and allergenicity of the newly expressed CP4 EPSPS protein, which renders the crop resistant to glyphosate. Read the full report at http://tinyurl.com/MON88302-EFSA (pdf). ■

Stanol fingerprint identifies source of fecal contamination **in shellfish**

- **For more than two decades, fecal stanols have been used to track environmental fecal contaminations. This article describes the development of a simple method that can be used to identify and quantify eight stanols in oysters.**
- **The method was used in oysters from a shellfish harvesting area in France that is frequently subjected to fecal contaminations.**
- **The "stanol fingerprint" of the contaminated oysters from this area was specific to bovines—a critical insight that could lead to better watershed management and reduce health risks linked to the consumption of contaminated shellfish.**

Loïc Harrault, Emilie Jardé, Laurent Jeanneau, and Patrice Petitjean

Contamination of coastal and shellfish harvesting areas from human and animal waste poses sanitary risks and leads to economic losses. Mussels, oysters (mainly *Crassostrea gigas*), and similar suspension filter-feeders are particularly sensitive to microbial pathogens, because they accumulate environmental contaminants in their tissues.

Today, the European Shellfish Directive on shellfish harvesting (854/2004/EC) requires that sources of fecal contamination be identified via microbial source tracking (MST) methods that combine microbial and chemical markers.

Fecal stanols are direct chemical markers that can be used to identify the various sources of fecal contamination in environmental samples. This is typically done by comparing stanol ratios or through multivariate analyses (Shah *et al.*, 2007; Derrien *et al.*, 2012).

In Brittany (France), the main sources of fecal contamination in water are human wastewater treatment effluent and animal (mainly porcine and bovine) manure. The specificity of each source's stanol fingerprint can be transferred from inland waters to seawater and used to identify the main sources of fecal contamination in such areas. However, it was previously unknown whether species-specific stanol fingerprints could be

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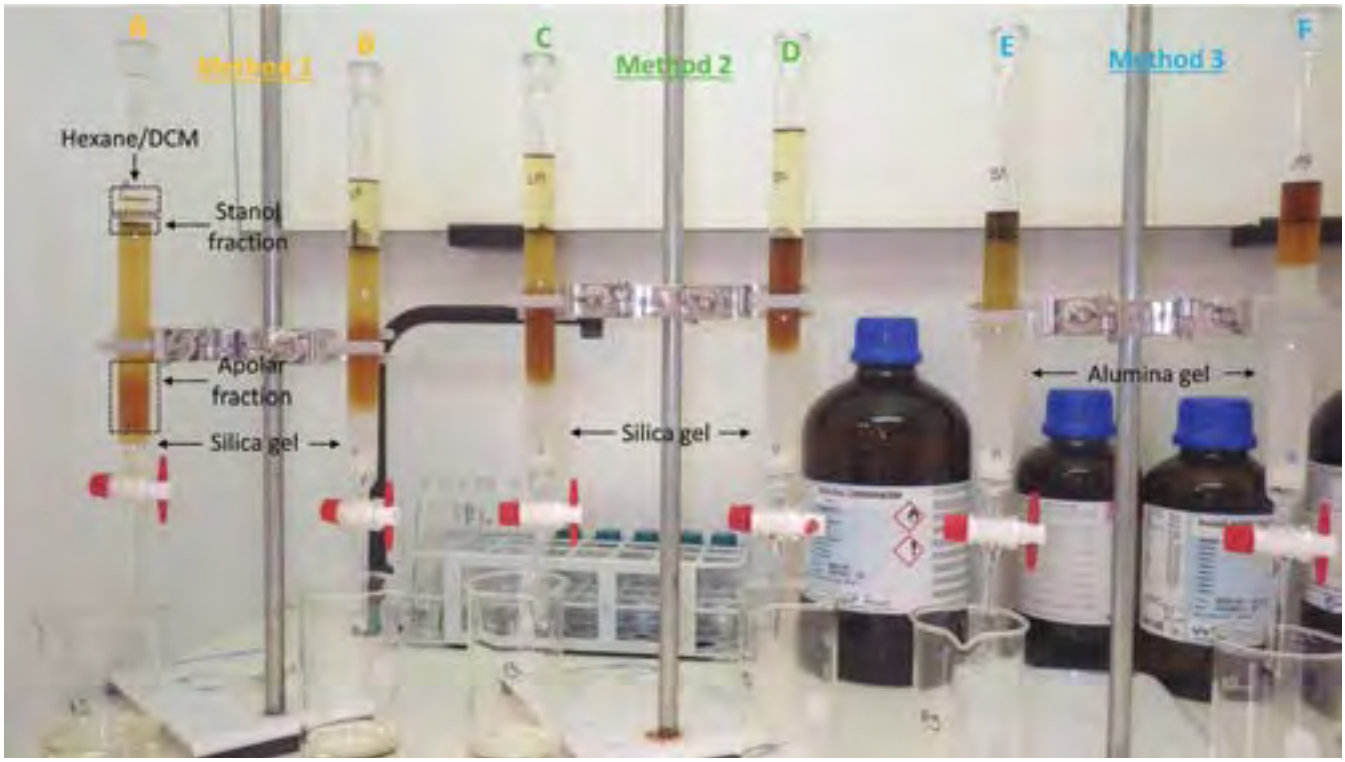

FIG. 1. Elution of apolar lipids with a mixture of hexane/dichloromethane (DCM) by chromatographic columns. The remaining stanol fraction will be then eluted with a mixture of DCM/methanol.

transferred from surrounding waters to shellfish, and if those specific fingerprints could similarly be used to identify the main sources of fecal contamination in oysters.

To date, stanol analysis in shellfish has focused only on coprostanol, but the identification of fecal sources via stanol fingerprints requires the accurate analysis of several compounds. Therefore, it is necessary to develop an analytical pathway that allows the quantification of a variety of fecal stanols in shellfish.

STANOL ANALYSIS OF OYSTERS

In fall 2013 our group compared the efficiencies and repeatabilities of three analytical pathways for stanol extraction from the oyster *Crassostrea gigas*. Two recovery standards were used.

Method one consisted of three steps: i) extracting lipids from the oyster matrix with dichloromethane, ii) purifying these lipids using a chromatographic column loaded with silica gel with different solvent mixtures of hexane, methanol, and dichloromethane (Fig. 1, columns A and B) and iii) analyzing the stanol fraction by gas chromatography-mass spectrometry (GC-MS).

Method two included a step in which the oyster tissue was washed with water prior to lipid extraction and chromatographic separation (Fig 1, columns C and D). The water wash was introduced to prevent interactions between glycogen or proteins and lipids that could decrease their recoveries.

Method three included an second round of purification with aminopropyl-bonded silica gel following that with the regular silica gel (Fig. 1, columns E and F). The lipid fraction

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Keywords : Stanol analysis; Gas chromatography; Mass spectrometry; Oysters, Fecal contamination, Microbial Source Tracking.

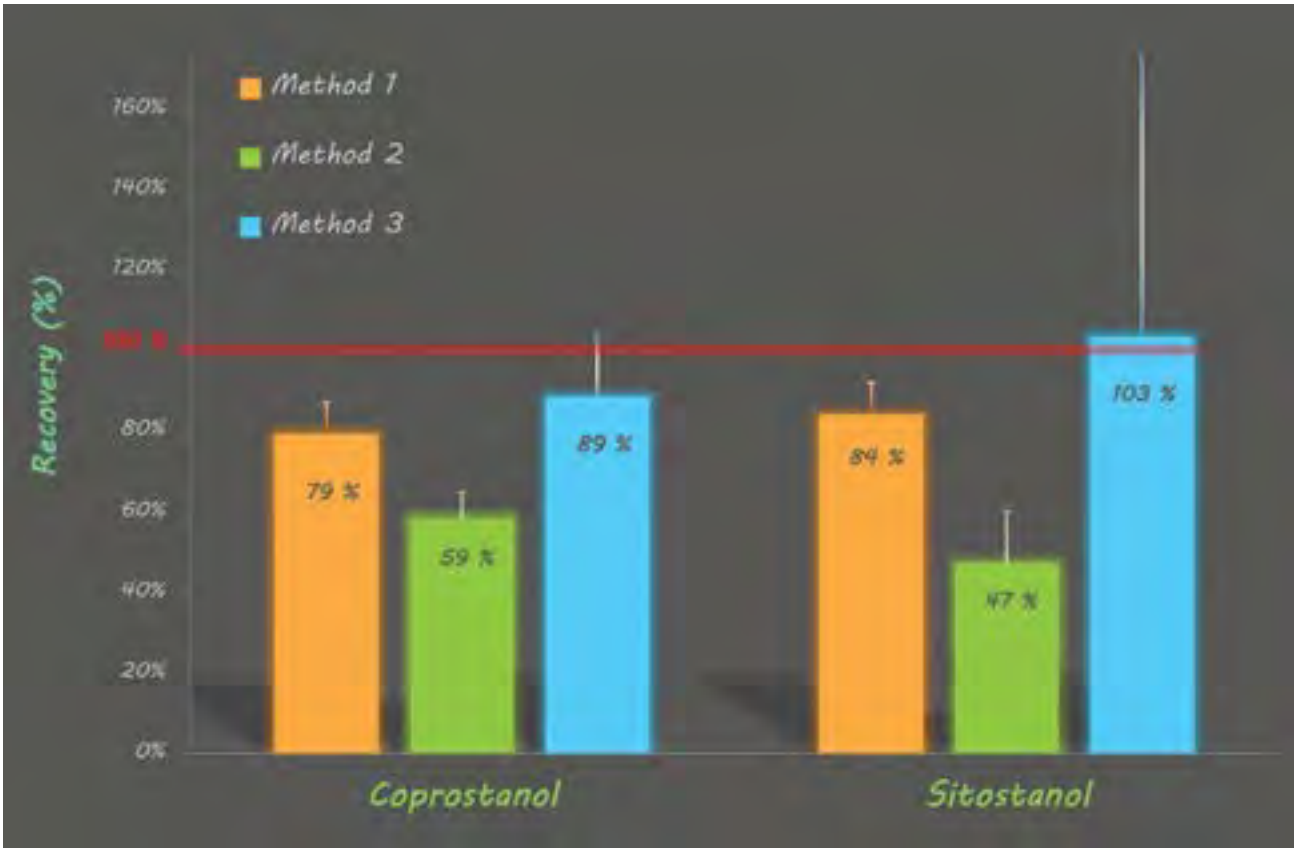

FIG. 2. Comparison of coprostanol and sitostanol recoveries in oysters for the three extraction methods. The dotted line represents the 100% recovery threshold.

of oysters is a complex mixture containing several lipid classes such as phospholipids, triacylglycerols, free fatty acids, sterols, and stanols. Therefore, the addition of a purification step could be particularly helpful in removing as many lipids of non-interest as possible.

The second method, which included a water extraction, led to opposing trends of coprostanol and sitostanol recoveries and increased their respective standard deviation (Fig. 2). Thus, the addition of this step on the extraction pathway did not increase the recovery of both recovery standards and decreased their repeatabilities. Consequently, we determined that water extraction prior to organic extraction is not reliable for the analysis of fecal stanols in oysters.

The second purification round using aminopropyl-bonded silica gel (method 3) increased the recoveries of coprostanol and sitostanol. Nevertheless, this step strongly decreased the repeatabilities of the methods tested, especially for sitostanol (Fig. 2). Therefore, this step does not appear to be reliable for the analysis of fecal stanols in oysters.

Finally, of the three methods tested, the first one led to i) statistically similar recoveries (compared to the other two methods), ii) higher repeatability, and iii) similar recoveries for both recovery standards (Fig. 2). Moreover, the recovery of coprostanol with method one (79%) was higher than that of a previous study (48%, Cathum and Sabik, 2001).

STANOL FINGERPRINT OF OYSTERS

Method one was used to analyze the concentration of fecal stanols in natural oysters sampled at Fresnaye Bay (Brittany, France), an intensive shellfish harvesting area with an annual production of about 550 tons of *Crassostrea gigas* intended for human consumption. The potential sources of fecal contaminations include the domestic wastewater treatment plant and an adjacent agricultural area in which 70% of the watershed area is devoted to the intensive livestock farming of pigs (approximately 235,000 head in 2010) and cows (approximately. 5,300 head in 2010). In February, March, and August 2013, oysters were sampled at two locations on the bay and analyzed to determine their *Escherichia coli* contents and stanol compositions. To investigate the ability of oysters to record species-specific fecal contamination via bioaccumulation, we injected the relative proportions of fecal stanols from these oysters into a principal component analysis (PCA) model previously developed (Derrien et al., 2012) to identify the main source of fecal contamination in environmental samples from this area based on their stanol fingerprint.

The concentration of *Escherichia coli* in oysters sampled during February and March 2013 was respectively 67 and 220 Most Probable Number 100 g^{-1} (MPN 100 g^{-1}). According to the

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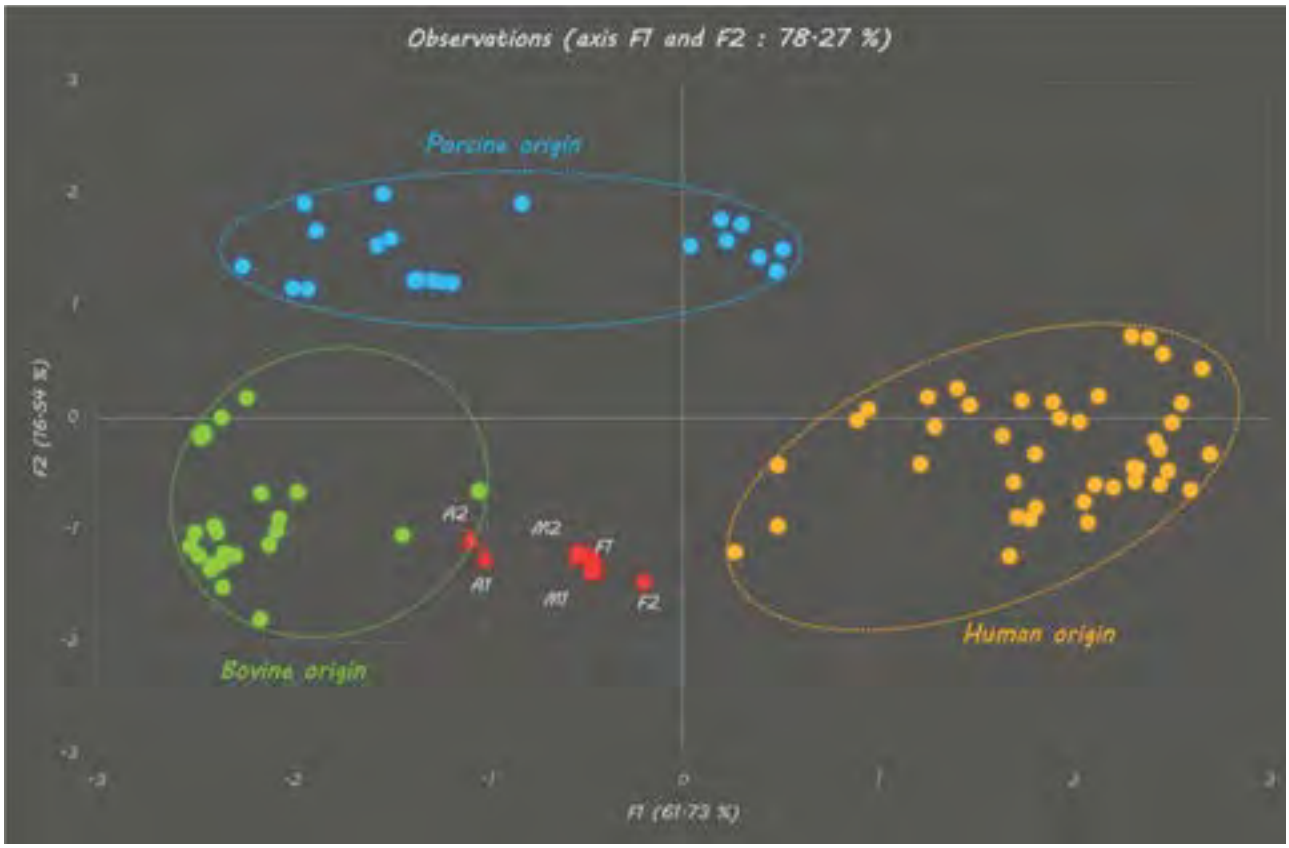

FIG. 3. Plot of the principal component analysis comparing 88 source-specific samples from humans, pigs, and cows and the six oyster samples collected in the Fresnaye Bay using the six most discriminant stanol compounds proposed by Derrien, *et al.* (2012).

European Shellfish Directive on shellfish harvesting (854/2004/EC), the oysters collected during those two months were in the A class considered to be free of feces contamination. Those sampled during August, with an *Escherichia coli* concentration of ca. 9150 Most Probable Number 100 g^{-1}, were in the B class considered to be contaminated

The stanol fingerprints of oysters sampled in February (F1 and F2) and March (M1 and M2) were located between the bovine and the human clusters (see Fig. 3). This absence of a specific fingerprint is consistent with the absence of fecal contamination in these samples as measured by *Escherichia coli* concentration. In contrast, the contaminated oysters sampled in August (A1 and A2), revealed specific stanol fingerprints located in the bovine cluster (Fig. 3). This suggests that cow manure was the main source of the contaminations during this period—a conclusion that makes sense given the significant number of cows in the watershed.

The contamination of oysters by bovine sources can be explained by local agricultural practices and the manure spreading calendar. During the summer, when the cows are grazing in the fields, even low amounts of soil runoff and erosion could transport their feces into streams that flow into the bay. In August, pig slurry spreading is forbidden, so the large quantities of pig slurry produced by pig farming in the watershed (about 235.000 pigs in 2010) remain stored, limiting the contamination of soils, streams, and, finally, shellfish by this source.

Loïc Harrault is a young researcher interested in organic biogeochemistry, ecology, and nutrient cycles of aquatic ecosystems. After completing a postdoctoral position in the 'Géosciences Rennes' laboratory (Rennes, France), he is looking for new, stimulating positions. Loïc can be contacted at loic.harrault@gmail.com.

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SUPPLEMENT

Commentary on trans fats as cause of premature deaths

Statistical analysis from Mintec

AOCS Word Detergent Conference recap

More Extracts & Distillates

Trans fats have caused premature deaths in thousands of people since 1910

Biochemist Fred Kummerow, who turned 100 in October 2014, continues to conduct research on heart disease and trans fats. In 2009, he petitioned the US Food and Drug Administration (FDA) to ban trans fats in foods and followed up by filing a lawsuit in 2013. In November 2013, the FDA made a preliminary determination that partially hydrogenated vegetable oils (the primary source of industrially produced trans fats) are not generally recognized as safe in foods. Here is Kummerow's latest commentary on the topic.

- **It took years to realize that all trans isomers did not have the same properties. Artificial trans fatty acids and ruminant trans fatty acids have entirely different properties *in vivo* as well as *in vitro*.**
- **When the amount of artificial trans fat in margarines was lowered, the sudden cardiac death rate also showed a decrease.**
- **When trans fats are out of the food supply, there will be 325,000 fewer sudden cardiac deaths per year, according to data from the US Centers for Disease Control.**

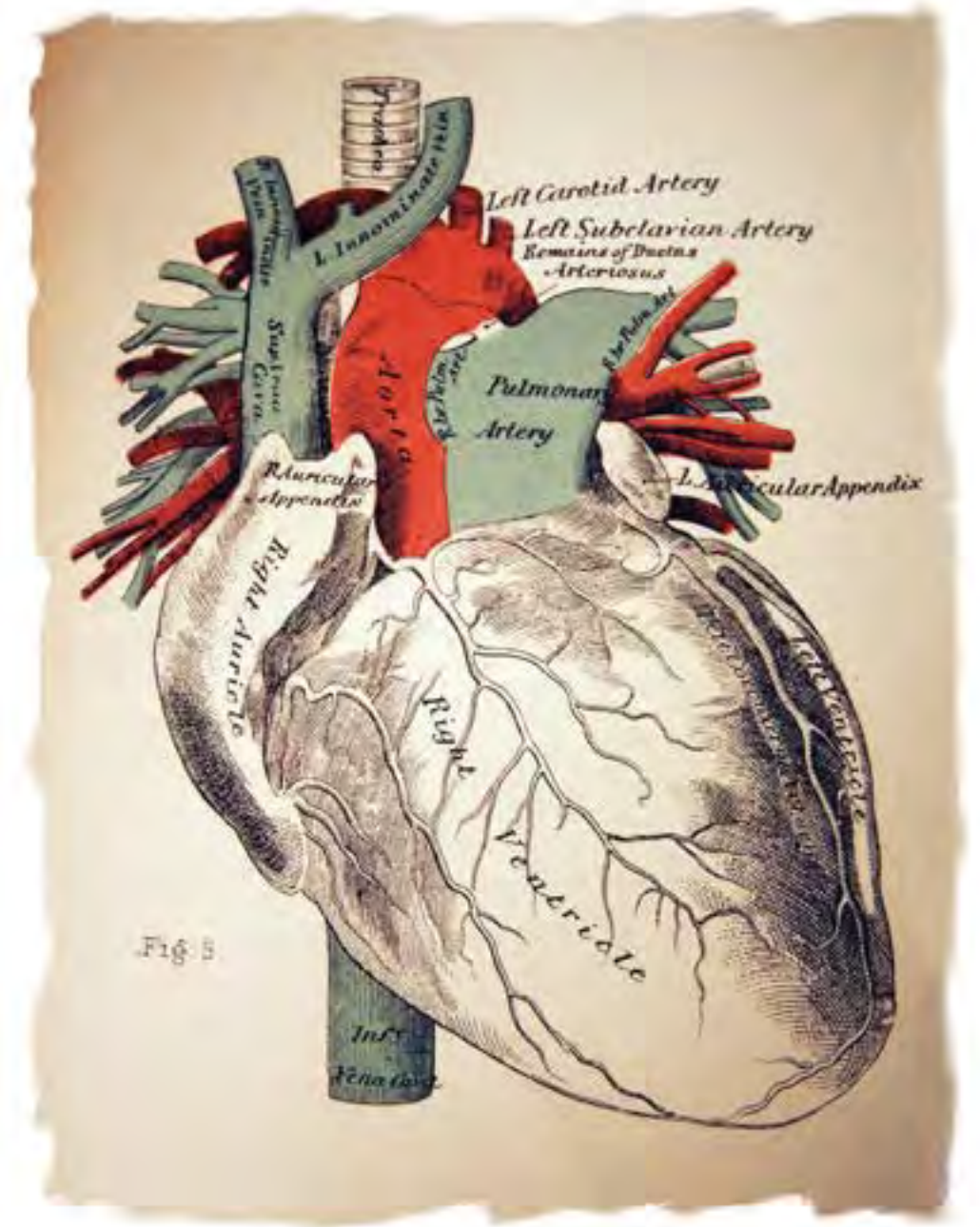
FRED KUMMEROW

In 1901, a German chemist named Wilhelm Normann showed that liquid oils could be hydrogenated, and patented the process in 1902. Production of partially hydrogenated oils (PHOs) commenced in 1909. The Procter & Gamble Co. acquired the US rights to the Normann patent and in 1911 it began marketing the first PHOs. The hydrogenated fat replaced lard and butter. PHOs had desirable culinary properties as they had melting points close to body temperatures and became liquid in the mouth like butter. In 1910, no one knew what effect PHOs would have on health.

The present mix of dietary fat in the marketplace results in less prostacyclin synthesis, which is an important factor in cardiovascular health (1). Prostacyclin is a dominant prostaglandin produced by endothelial cells in arteries and is a potent vasodilator and inhibitor of platelet aggregation and leukocyte adhesion. It limits the response to thromboxane, which is a powerful inducer of vasoconstriction and platelet adhesion on the arterial wall and is partially responsible for the interruption of blood flow. The rise of artificial *trans* fats in the diet correlates to the rate of age-adjusted heart disease-related deaths in the United States since 1910.

COMPOSITION OF TRANS FATS

It took nearly five decades before the biochemical structure of trans fatty acids was understood. The partial hydrogenation of



soybean oil adds atoms of hydrogen to 50% of the bonds 9,12 in linoleic acid (n-6) and to 50% of the bonds 9,12,15 in linolenic acid (n-3), converting them to 50% stearic acid(2). Forty to fifty percent of the double bonds of fatty acids in n-6 and n-3 are shifted to different positions on the carbon chain, making nine different synthetic trans fatty acids and five different *cis* fatty acids. These are cis and trans isomers of octadecenoic and octadecadienoic acids that are not present in animal fats or plant oils.

Both the cis and trans isomers interfere with the action of two isoforms of constitutive COX-1 and an inducible COX-2 enzyme. COX-2 is the enzyme that recognizes the isomers produced during hydrogenation as a foreign substrate and reacts to them by causing inflammation and inhibition of prostacyclin. The 14 synthetic fatty acids are a source of energy but interfere with the conversion of n-6 to arachidonic acid and n-3 to eicosapentaenoic acid (2).

Several studies, in my laboratory, have called attention to the trans fatty acids (TFAs) present in margarines and shortenings. Samples of tissue obtained from human autopsies were shown to contain up to 14% TFAs(3). Samples of fat from human placental, maternal, fetal, and baby tissue were also examined for the presence of TFAs(4). While the maternal tissue contained considerable amounts of TFAs, these lipids were not found to any measurable extent in placental, fetal, or baby fat(5)

This was also shown in rats that were fed trans fat. When the trans fat was removed from the diet, their tissue metabolized the trans fat and no longer contained trans fat(6). The results of these studies indicated that the TFAs present in human tissue apparently arise solely from dietary fat, and they do not normally appear in the tissues unless a source of TFAs is included in the diet. (This work needs a reference here.)

DIFFERENCE BETWEEN PHOs AND RUMINANT FATS

It was believed by the FDA that trans fat in partially hydrogenated soybean oil (PHO) had the same chemical structure and worked the same way in our bodies as natural vaccinic acid. However, these two *trans* fat sources have entirely different properties *in vitro* as well as *in vivo* ((1)). The elaidic acid in PHO has a double bond at position 9, while the vac-

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Table 1. Average composition of shortening and margarines before 1968 (old; before composition of fat was known) and after 1968 (new; formulation based on agreement between American Heart Association and the Institution of Shortening and Edible Oils).

Fatty acid composition (%)	Shortening Old	New	Margarines Old	New
Saturated	26	25	21	19
Monounsaturated	54	47	63	50
Polyunsaturated	20	28	17	31
cis-cis linoleic	16	24	8	27
Trans fatty acids	25	20	44	27

Source: Data taken from personal communication from WH Meyer, manager, professional regulatory relations at The Procter & Gamble Co. (1968)

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cinic acid in ruminant fats is at position 11. The enzymes in the body recognize vaccinic acid (butterfat and beef fat) as the fatty acid that has been in the diet for untold generations(7).

IN VITRO AND *IN VIVO* STUDY

An *in vitro* study showed that the fatty acids in partially hydrogenated fat had different properties than fatty acids in animal fat or vegetable oil. Trans acids increased the incorporation of $^{45}Ca^{2+}$ into the cells, whereas cis acids did not incorporate $^{45}Ca^{2+}$ into the coronary artery cells(8). An *in vivo* study showed that the TFAs inhibited the synthesis of arachidonic acid, a polyunsaturated fatty acid, in the phospholipid membrane of arterial cells (8). It was concluded that dietary trans fat perturbed essential fatty acid metabolism, which led to changes in the phospholipid fatty acid composition in the arterial wall, the target tissue of atherogenesis. Partially hydrogenated fat is a risk factor in the development of coronary heart disease because arachidonic acid is needed to synthesize prostacyclin.

DATA FROM THE CDC

Data obtained from the Centers for Disease Control (CDC) show the rate of death from heart disease started increasing in 1910 and continued until 1968, at which time the industry lowered the percentage of trans fat in shortenings and edible oils from 44% to 27% and increased the amount of linoleic acid from 8% to 25% (Table 1) (1). In 1968, the age-adjusted rate of heart disease-related deaths began to decrease. Data from the CDC state that almost 600,000 Americans died of heart disease in 2011, with 325,000 of those from sudden cardiac death. The other 275,000 deaths were due to calcification of the coronary arteries to 100% occlusion((1)).

TENTATIVE DETERMINATION TO BAN PHOs

On November 7, 2013, FDA released a tentative determination regarding PHOs (9). It stated that PHOs, which are a primary source of industrially produced TFAs, are not generally recognized as safe (GRAS) for any use in food. The FDA

requested comments of scientific data and information on this determination giving 60 days for responses. Before the 60 days were over, the agency extended the comment period by another 60 days until March 8, 2014. When the determination is finalized, it will mean that food manufacturers would no longer be permitted to sell PHOs without prior FDA approval.

The FDA released this information in the *Federal Register* on November 8, 2013 (9): "Trans fats are an integral component of PHOs and are purposely produced in these oils to affect the properties of the oil and the characteristics of the food to which they are added." At zero percent of trans fat content in the body, the prostacyclin release from vascular endothelial cells is 38.7 ng/mg of cell protein (1).

Data released in the *Federal Register* states that in 2012 the average American consumed 2.1 grams of trans fats per day, with the 90% percentile consuming 4.2 grams per day (9). While consuming 2.1 grams of trans fat per day, the arterial cells will release 25 ng/mg cell protein, which is a significant drop from 38.7 ng/mg at zero percent. Consuming 4.2 grams/day of trans fat the cells will only release 15.5 ng/mg cell protein (1). As more grams per day of trans fat are consumed, prostacyclin release from vascular endothelial cells to cell protein will decrease, proving an inverse relationship between the two processes.

CONCLUSION

The partial hydrogenation of vegetable oils has been shown to have an adverse effect on health. The FDA believed that artificial trans fats had the same chemical structure and worked the same way in the human body as natural trans fats. It has been shown that this is not true. The sooner the FDA finalizes its decision to make artificial trans fat non-GRAS, the sooner more lives will be saved.

I believe that heart disease is not a disease but a somatic response to a simple error involving the effect of *trans* fat in partially hydrogenated oil on prostacyclin synthesis. Therefore, the present mix of dietary fat in the marketplace results in less prostacyclin synthesis and more sudden cardiac death.

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Mintec

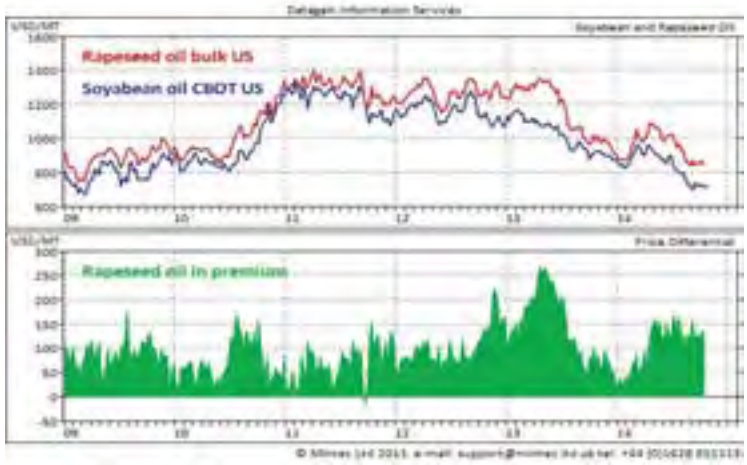
STATISTICAL ANALYSIS FROM MINTEC

Yuliya Nam-Wright

World soybean production in 2014/15 is expected to reach a record 311.1 million metric tons (MMT), up 10% year-on-year. U.S. production is projected to reach 106.5 MMT, up 19% year-on-year, driven by favourable weather conditions. Exports will also rise, but at a slower pace than production, up 3% year-on-year to 46.3 MMT. Soybean oil production is expected to rise 6% to 9.7 MMT, slower than the growth in soybeans, as cold conditions this summer have delayed crop development and resulted in a smaller than usual oil content. Ending stocks of soybean oil are forecast to rise by 21% to 0.8 MMT, driven by higher production and lower domestic consumption, down 3% to 8.3 MMT.

Rapeseed (canola) oil's premium over soybean oil increased significantly in 2014 due to a decline in rapeseed production. World production of rapeseed is forecast to fall 1% year-on-year to 70.7 MMT in 2014/15, due to lower production in Canada, where late plantings as well as cold and wet weather conditions in summer slowed crop development. Canada's production is expected to reach 14.7 MMT, down 18% from the record crop last year.

Prices for soybean oil traded below palm oil prices throughout most of 2014. Despite expectations of another record global production in 2014/15, palm oil prices have been more resistant to downward pressure, largely due to increasing domestic consumption in major producing countries, driven by increases in the biodiesel mandate. World palm oil production will reach a record high of 63.3 MMT, up 9% year-on-year.



The search for sustainability

Sustainability is everyone's and every company's business. That much was evident at the recent World Detergent Conference in Montreux, Switzerland. The event, developed by the American Oil Chemists Society (AOCS), attracted nearly 700 executives from around the world who heard presentations from leading voices from a variety of fields, including detergents, petro and oleochemicals, technology, and humanitarian aid.

- **The global detergent industry continues to make inroads on creating a more sustainable future.**
- **Past efforts, present problems, and future concerns were front and center during the AOCS World Detergent Conference.**
- **This article provides a summary.**

"We made a promise to deliver a more exciting program and we have delivered," said conference chairman Manfred Trautman, in his opening remarks. The conference theme, he explained, "Creating Value in the New Reality," can be segmented into three subgroups with three missions:

- Sustainability—face it;
- Growth—find it; and
- Interaction—fuel it.

Sustainability has been at the forefront of industry's collective mind for years; so it was no surprise to hear Kaspar Rorsted, CEO, Henkel, tackle the subject in his opening keynote address.

"We have made progress (on sustainability) in the past four years," noted Rorsted, who addressed the same conference in 2010 in Montreux. "We have taken tremendous steps in the right direction. Sustainability is front and center.

"Economic growth and resource consumption must be decoupled," he continued. "This is where innovation will be key."

OTHER ISSUES

But sustainability is not the only issue facing the global household cleaning industry, Rorsted noted, calling the consolidation of suppliers and buyers, the shift to emerging markets, and the rapid transfer of information, as other megatrends impacting the industry.

"Global volatility is increasing, but the economic powerbase is moving to emerging markets," he explained, noting that, emerging markets now account for a larger percentage of the global economy than established markets.

Meanwhile, companies and their legal staffs must grapple with transparency issues like never before, said Rorsted.

Social media such as Facebook, Twitter, and YouTube, provide unfiltered news feeds in real time that can damage a company's reputation in hours, days and weeks. Similarly, there are an increasing number of regulations and defacto standards to get a handle on.

"We need to do a better job," Rorsted insisted. "Governments are upset with how large companies behave and they are frustrated."

At the same time, NGOs and pseudoscientists are calling for more regulations of ingredients and formulas. And even when industry does the right thing, there is scant praise, and sometimes, damnation, noted Rorsted.

"The best citizen is the one who reduces the size of their product packages," he explained. "But if you are the first mover in the market, you often get punished for it."

For example, nearly every consumer product company executive laments the fact that while consumers are aware of sustainability issues, they refuse to pay more for more sustainable products and they refuse to compromise on performance.

"They want sustainable products, but at the same performance and price," said Rorsted.

And, too often, great leaps forward are often viewed as providing less value to consumers. To help get the word out about the economic, health and sustainable benefits that industry brings, Rorsted called for more cooperation between industry associations, pointing out that a recent Accenture study found that 83% of respondents that progress on environmental issues can only be achieved through regulations.

"The more that we can coordinate, the more that we can get done," he insisted. "Let's get the associations aligned—that will help us with regulators. Each body can't have its own agenda!"

Getting going and going further on sustainability issues is even more critical when one realizes experts expect the global population to reach 9 billion by 2050. The goal therefore, should be for all people to live well within the limits of the planet, noted Rorsted. But how to get there?

At Henkel, the goal is to become three times more efficient in six focus areas: performance; safety and health; social progress; energy and climate; materials and waste; and water and wastewater. To achieve that goal requires effective products, partners and people, according to Rorsted. Products must deliver more value at a reduced ecological footprint. Partners are key to driving sustainability across the value chain; and people are critical to make the company better.

One example of how novel materials can help lead to better products is the use of enzymes in Henkel's Pur Power. Incorporating high performance enzymes can reduce material and energy usage, he explained. Rorsted also called for a reasonable discussion on product and material assessment within the supply chain.

"Not all of us need to audit the same points on the same supplier," he insisted. "At the same time, we should work with retailers to encourage sustainable consumption."

To get there, he encouraged the use of sustainability elearning methods to simplify knowledge transfer.

"As an industry, it takes too long to react from supplier to marketer to retailer. The company that uses IT best has an incredible advantage," Rorsted claimed. "Working together, industry can create more value across the entire value chain."

THE CITY OF THE FUTURE

As the global population expands, it's no surprise to learn that cities are growing around the globe, bringing their own unique sets of challenges and opportunities to sustainability, said Patrick Donnelly, CEO of AOCS, who presented a paper on behalf of Héléne Lebedeff of Veolia Environment, France.

The global urban population was 3.5 billion in 2010 and is expected to reach 7.0 billion by 2030, with many living in cities of at least 500,000.

That kind of centralization puts a strain on pollution control and water supplies. In order to overcome those strains requires effective systems such as the Zero Waste House, a concept designed by a UK firm, which move from an input-output model to a systemic cyclical model.

By 2050, these homes will create organic waste that can be used to power entire districts. Waste will be sorted by something called MAGPIE technology and nanobots and move via an underground vacuum network, according to Lebedeff's predicition. Waste reductions will be aided by intelligent packaging that requires less material and breaks down easily and quickly after use. Similarly, Zero Waste Homes will feature low wateruse bathrooms and watertreatment will take place, in part, in homes that feature plantbased technology.

WHAT'S HAPPENING NOW?

While many speakers at the conference looked toward the future, Jim Collins of DuPont was focused on the here and now. That's because his company has just signed a deal to supply cellulosic ethanol to Procter & Gamble's Cold Water Tide product in the US. According to Collins, BioPDO requires 40% less energy to produce than petrolbased products, while producing 40% less greenhouse gas emissions. To make it happen, DuPont is building a biorefinery in Iowa to produce ethanol from corn stover.

This move by Dupont underscores four trends that Collins maintains are driving the market: a new value equation, convenience is king, inspiring experience, and clean and green.

The alliance between P&G and Dupont was reiterated by Procter's Gianni Ciserani, who relayed the Aesop fable

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about the grasshopper and the ant. While the grasshopper spent the warm summer days lollygagging, the ant was busy storing food for the winter. When the cold weather arrived, the ant was well prepared for hard times, while the grasshopper starved.

"Ants work together and they never stop working," observed Ciserani. "I don't see ants sitting at roundtables."

Similarly, P&G is working constantly and preparing for the future; forming effective alliances with its suppliers and making major investments, such as the construction of a new plant in Taicang, China. When completed, it will be P&G's biggest and the most environmentally friendly plant in Asia.

"The water going out will be cleaner than the water coming in (to the plant)," boasted Ciserani.

China, as well as India, are focal points for P&G, and Ciserani said his company will continue to invest in these regions with new facilities that are environmentally friendly and sustainable.

"Environmental issues don't have a passport," he reminded the audience. "What happens in one place, like our efforts in China, can have spectacular results."

Kao has been recording pretty spectacular results of its own thanks, in part to its corporate philosophy called Yoki-Monozukuri, which translates to "a strong commitment by all members to provide products and brands of excellent value for consumer satisfaction."

That philosophy was the keynote of Michitaka Sawada, president and CEO of Kao Corp., who reviewed some of the key issues impacting the global detergent industry, including climate change, resource scarcity, aging and hygiene.

The fabric and home care industry can help resolve these issues via three points:

One, engage the consumer at the point of use; Two, easily provide recognized values to the consumer that surprise and delight; and Three, make products that are more relevant to the consumer and enable her to live better.

Noble, but certainly attainable goals for Kao and other manufacturers, as long as suppliers and manufacturers remain "a halfstep ahead of the consumer's needs," asserted Sawada. "We must be appropriately disruptive!"

Examples of appropriate disruption include twistable PET bottles for easy recycling which also reduces raw materials and waste volume; hybrid automobiles and compact laundry detergent powders and, more recently, compact liquids.

Sawada urged industry to work together to develop these sustainable solutions even going so far as to urge companies with patentprotected innovations to open them up to an industrywide sustainability movement.

"No one company has all the answers," observed Sawada. "Sustainable change requires all of us."

SERVING SOCIETY

A presence in emerging markets is a must for any company searching for growth in a slowgrowth industry.

According to Nitin Paranjpe, president, home care, Unilever, by 2020, 900 million people will enter the consuming class across Asia, Latin America, and Africa. But formulators can't make the mistake of "dumbingdown" their products to suit this emerging consumer group.

"Just because they don't have large incomes doesn't mean that they don't have large aspirations," asserted Paranjpe. "You have to understand the people and create brands that mean something to them!"

He explained that the Digital Revolution has given emerging market consumers more information than ever, but they can feel helpless with all the choices available to them and that's where brands come in; especially brands that make a strong connection and make a difference.

Examples of these brands include Dove, which is working to improve the selfesteem of women around the world, LifBuoy, which is focused on infantmortality issues, and Omo, which devotes resources to child development. They're all part of the Unilever Sustainability Plan.

"You can't have a thriving business in a failing society," Paranjpe noted.

"Business must never forget that its purpose is to serve society. Profit is just a portion of the outcome."

Sustainability means not just doing good; it may mean saving the species. According to Peter White of the World Business Council for Sustainable Development, by 2050, 70% of the world's 9.2 billion people will be living in cities and putting enormous stress on resources. The goal of his group's plan, called Action 2020, is to limit temperature change to +2°C and keep carbon emissions below one trillion tons. To do that, industry must rethink the practice of deforestation to grow palm.

"We need to improve the business case for sustainable development," said White. "We have to change the game from financial capital to natural and societal capital. We are here to make more sustainable companies more successful."

Walmart doesn't have all the answers when it comes to sustainability, asserted Alberto Luis Dominguez, senior VPdivisional merchandise manager, household paper goods and chemicals, Walmart. The company's Sustainability 360 program incorporates input from associates, suppliers and customers to uncover ways to improve sustainability. The company went one step further earlier this year, by initiating its Policy on Sustainable Chemistry in Consumables, which provides a description of what it calls "priority chemicals"—substances with certain hazardous properties that can affect human health, and/or the environment. The policy defines these as chemicals that meets the criteria for classification as a carcinogen, mutagen, reproductive toxicant, or is persistent, bioaccumulative, and toxic; or any chemical for which there is "scientific evidence of probable serious effects to human health or the environment." The policy covers consumer products such as health and beauty aids, pet supplies, cosmetics and skin care, baby care products and household laundry and cleaning products.

"We launched Sustainable Chemistry, in part, to reinforce consumer trust," explained Dominguez. "Customers expect us to know great products."

Now Walmart is going further. In October, at its Global Sustainability Milestone Meeting, the retailer announced its commitment to create a more sustainable food system. The company will reach this goal through four key pillars: improving the affordability of food for both customers and the environment, increasing access to food, making healthier eating easier, and improving the safety and transparency of the food chain.

"We ask you to always put the customer first and to innovate to achieve the policy," concluded Dominguez. "Let's commit to voluntary leadership (on sustainability)."

Peder Holk Nielsen, president and CEO of Novozymes, noted that fewer people live in poverty around the world since he attended his first World Conference on Fabric and Home Care 16 years ago.

"But we have lots of challenges; we must accelerate what nature does to make real progress in 10 years."

One way to do that is through the use of enzymes, which not only replace chemicals, but also save water and energy, according to the speaker.

"Biotechnology holds the key to solving many problems," Nielsen said and insisted that less than 20% of the world's plant waste can deliver 50% of the world's fuel needs by 2050.

"But the fossil fuel business is huge, and doesn't want the world carbon neutral by 2050," according to Nielsen. "We need a government mandate." Good Chemistry

BASF chairman Kurt Bock, the Day 2 keynote, promoted the concept of sustainability as a driver of growth, but he warned that much work still needs to be done. For example, in order to grow enough feedstocks for plantbased surfactants in Europe would require farmland a third the size of Switzerland.12/9/2014 The Search For Sustainability HAPPI Household and Personal Products Industry http://www.happi.com/issues/122014/view_features/thesearchforsustainability/?email_uid=ebe9f959f8/list_id=972e8e7a65/5/8

"There is a lack of land to fuel Ludwigshafen (BASF's primary manufacturing site)," observed Bock. "You can't replace fossil fuels with renewable plants."

He added that sugar as a feedstock is not cost competitive—even in Brazil.

And he doesn't expect the situation to change any time soon. Today, 94% of the industry's feedstocks are tied to fossil fuels; 20 years from now, they will still account for 80% of feedstocks, Bock predicted.

At the same time, while consumers insist they want green products, they are unwilling to pay for them. So, what's a multinational supplier to do? For one, drop the term "green;" BASF doesn't use it. For another, rethink the issue completely.

"Renewable isn't necessarily sustainable," observed Bock. "BASF creates chemistry for a sustainable future. Our goal is increasing the sustainability contribution of our solutions."

To that end, BASF has purchased two enzyme businesses and spending more on R&D in this segment. At the same time, the company's Trilon M is an effective replacement for phosphates in automatic dishwash detergent formulas. The company has also created a biobased acrylic acid for its superabsorbent polymer and is a player in sustainable palm oil production.

"We have a wellpositioned portfolio," Bock concluded.

A NEW, OLD CENTURY

While many speakers noted that industry is moving toward China, Ian Bell, head of home care research, Euromonitor International, suggested that we are not facing an Asian Century, but rather an Aging Century.

"Real population growth is slowing," observed Bell. "The higher your income, the fewer children you have."

As a result, while it may not seem apparent just yet, by 2030, purchasers and users of fast moving consumer goods like laundry detergent and hard surface cleaners will be older and have special needs that marketers must address, such as product handling.

"Universal Design is key," he asserted. "Products must be affordable, recognizable, and easy to handle."

At the same time, women "are taking over the world," said Bell (just somewhat jokingly). They are living longer and their incomes are rising. As a result, formulators must ask themselves, "are our products helping women?"

Interestingly Bell predicted that global washing machine penetration will plateau at 70% in 2030; therefore, marketers must not forsake handwashing formulas. Bell noted that while Indian consumers are moving into the middle class at a rapid rate (by 2030, there will be 400 million of them), they will not reach the income level to afford washing machines. Finally, Bell suggested marketers prepare for a new wave of fabric blends—for the simple reason that the world is running out of room to grow cotton.

In a provocative presentation, Nabil Sakkab, managing director, Sakkab LLC, suggested that the industry, indeed much of the industrialized world, has become bogged down by incremental innovation and needs radical innovation to transform itself. He recalled a decadesold McKinsey study that predicted the cellphone market would only reach one million. The consulting group was a bit off in its prediction, as cell phone sales today exceed two billion.

"Radical innovation forms a new dominant design that transforms a market or an industry," he noted. In contrast, the automatic washing machine that debuted in 1937 has not had a radical design change even after all of these years.

Sakkab went on to take a shot at the leading tech companies of the day, noting "I love Facebook, but I wouldn't give up air conditioning, TV or my car for it. The top 10 value companies in the world, like Facebook and Google, produce nothing!"

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Similarly, since Tide debuted in 1946, laundry aisle shelves have become cluttered with incremental, not radical, change. As a result, consumers have little interest in the category or its products.

"How many of you can't wait to get home to do laundry?" he asked. "The linear model is not effective for creating radical design."12/9/2014 The Search For Sustainability HAPPI Household and Personal Products Industry http://www.happi.com/issues/122014/view_features/thesearchforsustainability/?email_uid=ebe9f959f8/list_id=972e8e7a65/6/8

Radical things are happening in Asia, as a growing middle class aspires for a wide range of products, according to Itsuo Hama, president and CEO, Lion. He urged the industry to apply global lessons learned for better cleaning products, offer innovative green solutions and spread them throughout the world. Some of the innovations that Lion has developed include a cleaning "cloud" to attack bathroom mold and a toilet bowl cleaning foam that can be used with toilet paper.

"Innovation must be shared within our industry, with other industries and with our customers," he urged attendees.

Tom Branna is editorial director of HAPPI *magazine. Reprinted from the December 2014 issue of* HAPPI*. For more information, visit www.happi.com.*

EXTRACTS & DISTILLATES

Influence of ultra-high-pressure homogenization treatment on the phytosterols, tocopherols, and polyamines of almond beverage

Toro-Funes , N., *et al*., *J. Agric. Food Chem. 62*: 9539–9543, http://dx.doi: 10.1021/jf503324f.

Ultra-high-pressure homogenization (UHPH) is an emerging technology based on the dynamic application of high pressure to obtain safe and high-quality liquid foods. The effect of six UHPH treatments at 200 and 300 MPa with different inlet temperatures (T_{in}) (55, 65, and 75 °C) on the content of tocopherols, polyamines, and phytosterols of almond beverage was studied in comparison with the base product. Total tocopherol contents decreased about 80–90% as temperature and pressure increased, and whereas both parameters affected the tocopherol content, especially the effect of temperature was noticeable. α-Tocopherol was the most predominant type of tocopherol present and was also the most affected by UHPH treatments. Spermidine was the only polyamine found not to be affected by UHPH treatments. UHPH treatments resulted in an increase of 20–40% in the total phytosterol extractability. The highest extractability was obtained at the most severe conditions (300 MPa, 75 °C T_{in}).

The role of traditional varieties of tomato as sources of functional compounds

Cortés-Olmos, C., *et al*., *J. Sci. Food Agric. 94*: 2888–2904, 2014, http://dx.doi: 10.1002/jsfa.6629.

Traditional varieties of tomato, usually associated with excellent organoleptic quality, are increasingly appreciated in European quality markets. A collection of 126 populations of 16 traditional varieties from the east of Spain (a secondary diversity center for tomato) have been evaluated over 2 years in order to determine their potential value as sources of functional compounds, including ascorbic acid, lycopene, β-carotene and total phenolic content. Population and population × year interaction significantly affected lycopene and ascorbic acid contents, while year effect was also significant for β-carotene. Despite finding some global trends in certain varieties concerning their functional value, high levels of variation have been found at the intra-varietal level. Populations with high levels of the compounds analysed have been found, as well as different levels of intra-population and inter-year variation. Maximum mean contents for both years have reached 308 mg kg^{-1} ascorbic acid, 130 mg kg^{-1} lycopene, 30 mg kg^{-1} β-carotene and 89 mg caffeic acid 100 g^{-1} total phenolic contents, though it is difficult to identify accessions with joint high values of the three compounds. These results open the possibility to promote traditional materials as sources of functional compounds, thus strengthening their quality niches and consolidating their price premium. Additionally, these materials could also be used in breeding programs for quality.

Esterification of fatty acids by *Penicillium crustosum* lipase in a membrane reactor

Gessica Possebom, *et al*., *J. Sci. Food Agric. 94*: 2905–2911, 2014, http://dx.doi: 10.1002/jsfa.6630.

This study investigated the performance of a membrane reactor system for esterification of oleic acid and butyric acid with ethanol by Penicillium crustosum lipase using polyethersulfone membranes with molecular weight cut-offs of 30, 60 and 100 kDa at pressures up to 200 kPa. The confinement of lipase with 60 and 100 kDa membranes showed the best results. The esterification of butyric acid in the membrane reactor and with free lipase showed higher conversions than those obtained with oleic acid, since the system operated with oleic acid was more subject to fouling and thus could not be run for repeated cycles. The confinement of lipase from P. crustosum in a membrane reactor was possible, resulting in the satisfactory conversion of butyric acid to ethyl butyrate with the possibility of reuse of the immobilized enzyme.

Effect of lycopene-enriched olive and argan oils upon lipid serum parameters in Wistar rats

Aidoud, A., *et al*., *J. Sci. Food Agric. 94*: 2943–2950, 2014, http://dx.doi: 10.1002/jsfa.6638.

Lycopene has the highest antioxidant activity within carotenoids and is an effective free radical scavenger. Virgin olive oil (VOO) and argan oil (AO) contain trace amounts of a wide variety of phytochemicals which have desirable nutritional properties. The present study intended to assess the effect of various dietary VOO and AO in combination with lycopene consumption on serum biochemical parameters, including total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C),

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high-density lipoprotein-cholesterol (HDL-C), triglycerides (TGs) and phospholipids, as well as on hepatosomatic index (HSI) of rats. Results showed that ingestion of VOO and AO diminished TC, LDL-C, TGs and phospholipid levels, whereas the HDL-C levels augmented in all the groups assayed. The enrichment of VOO and AO with lycopene improved the beneficial effects derived from the consumption of both oils on serum biochemical parameters. A decrease in body weight gain and HSI was detected after the consumption of lycopene-enriched oils. These findings suggest that the inclusion of lycopene in VOO and AO may be used as a natural tool to fight against hyperlipidaemic and hypercholesterolaemic-derived disorders.

Inspired by lipids (the Chevreul Award Lecture 2014)

Harwood, J.L., *Eur. J. Lipid Sci.Technol. 116*: 1259–1267, 2014, http://dx.doi: 10.1002/ejlt.201400021.

As an undergraduate student of Medical Biochemistry, I was unsure what I wanted to do with my life after graduating. Then, one day, I had a lecture about membrane lipids. It turned out that not only were they far from the inert structural compounds that they were often portrayed to be but they turned over quite rapidly. But there was one class of lipids—the higher inositides (PtdIns4P, PtdIns (4, 5) P_2)—that had a T½ of only a few minutes! I found this fascinating and was inspired not only to do my post-graduate work with J. N. (Tim) Hawthorne on inositides but to continue work on lipids ever since. I have no regrets for it has been a fascinating journey. The great French scientist Chevreul made contributions to several different areas of science so, in humble appreciation of his seminal discoveries, I will discuss some diverse lipid topics that I have worked on.

Very long chain omega-3 (*n*-3) fatty acids and human health

Calder, P.C., *Eur. J. Lipid Sci.Technol. 116*: 1280–1300, 2014, http://dx.doi: 10.1002/ejlt.201400025.

Omega-3 (*n*-3) fatty acids are a family of polyunsaturated fatty acids that contribute to human health and well-being. Functionally the most important *n*-3 fatty acids appear to be eicosapentaenoic acid (EPA) and docosahexaenoioc acid (DHA), but roles for *n*-3 docosapentaenoic acid (DPA) are now emerging. Intakes of EPA and DHA are usually low, typically below recommended intakes. Increased intakes are reflected in greater incorporation into blood lipid, cell and tissue pools. Increased content of EPA and DHA modifies the structure of cell membranes and the function of membrane proteins involved as receptors, signaling proteins, transporters, and enzymes. EPA and DHA modify the production of lipid mediators and through effects on cell signaling can alter patterns of gene expression. Through these actions EPA and DHA alter cell and tissue responsiveness in a manner that seems to result in more optimal conditions for growth, development, and maintenance of health. The effects of *n*-3 fatty acids are evident right through the life course, meaning that there is a need for all sectors of the population to have a sufficient intake of these important nutrients. EPA and DHA have a wide range of physiological roles which are linked to certain health or clinical benefits.

Enzymes in lipid modification: Past achievements and current trends

Bornscheuer, U.T., *Eur. J. Lipid Sci.Technol. 116*: 1322–1331, 2014, http://dx.doi: 10.1002/ejlt.201400020.

In this minireview, the application of enzymes for the modification of fats and oils is covered with special emphasis on lipase-catalyzed synthesis of structured triglycerides. Furthermore, protein engineering methods to alter the fatty acid chain-length or the *trans*-fatty acid selectivity of lipase A from *Candida antarctica* and an enzymatic two-step method to remove the contaminant 3-monochloro-1,2-propane diol are presented. Also the combination of several enzymes enables cascade reactions to obtain products such as ω-hydroxycarboxylic acids useful for oleochemistry and recently published achievements to produce valuable compounds by metabolic engineering of microorganisms as exemplified for PUFA or fatty acid esters starting from sugars as renewable resources are covered. Special emphasis is given on achievements made in the authors group.

Lipidomics is going great guns: Interview with Kai Simons about the power of shotgun lipidomics

Kalvodova, L. and K. Simons, *Eur. J. Lipid Sci.Technol. 116*: 1344–1346, 2014, http://dx.doi: 10.1002/ejlt.201400405.

There are thousands of lipid species in living cells but we do not fully understand this diversity as it is not possible to pin down a specific function to each lipid species. Lipids have structural, metabolic and signaling functions. While signaling lipids can be understood and functionally characterized in a way similar to proteins, membrane lipids act collectively. This may have been one of the reasons for lipid neglect in the past. In order to understand the complexity of lipids in membranes, lipoproteins, body fluids, and organisms in general, we need to know the big picture which can only be delivered by modern lipidomic analyses. This has been increasingly appreciated mainly in the biomedical area. It is likely that interpretation of blood cell lipidomes will lead to the discovery of new biomarkers and lipid signatures of pathological states and cardiovascular disease risk, and so the era of HDL/LDL, TAG and

cholesterol quantification may soon be over. But that is just one example. The motto of this year's Euro Fed Lipid Congress reads "From lipidomics to industrial innovation". Indeed with the advancement of mass-spectrometry and computational approaches to analyze and understand big data, lipidomics is becoming more interesting for clinical applications and industry.The shotgun methodology is so robust, comprehensive, fast and quantitative that it can be taken to the clinic, food R&D and into other applications.

Value-added potential of expeller-pressed canola oil refining: characterization of sinapic acid derivatives and tocopherols from byproducts

Chen, Y., *et* al., *J. Agric. Food Chem*. *60*: 9800–9807, http://dx.doi: 10.1021/jf502428z .

Valuable phenolic antioxidants are lost during oil refining, but evaluation of their occurrence in refining byproducts is lacking. Rapeseed and canola oil are both rich sources of sinapic acid derivatives and tocopherols. The retention and loss of sinapic acid derivatives and tocopherols in commercially produced expeller-pressed canola oils subjected to various refining steps and the respective byproducts were investigated. Loss of canolol (**3**) and tocopherols were observed during bleaching (84.9%) and deodorization (37.6%), respectively. Sinapic acid (**2**) (42.9 µg/g), sinapine (**1**) (199 µg/g), and canolol (344 µg/g) were found in the refining byproducts, namely, soap stock, spent bleaching clay, and wash water, for the first time. Tocopherols (3.75 mg/g) and other nonidentified phenolic compounds (2.7 mg sinapic acid equivalent/g) were found in deodistillates, a byproduct of deodorization. DPPH radical scavenging confirmed the antioxidant potential of the byproducts. This study confirms the value-added potential of byproducts of refining as sources of endogenous phenolics.

Protein profile of mature soybean seeds and prepared soybean milk

Capriotti , A. L., *et al*., *J. Agric. Food Chem*. *62*: 9893–9899, 2014, http://dx.doi:10.1021/jf5034152.

The soybean (*Glycine max* (L.) Merrill) is economically the most important bean in the world, providing a wide range of vegetable proteins. Soybean milk is a colloidal solution obtained as water extract from swelled and ground soybean seeds. Soybean proteins represent about 35–40% on a dry weight basis and they are receiving increasing attention with respect to their health effects. However, the soybean is a well-recognized allergenic food, and therefore, it is urgent to define its protein components responsible for the allergenicity in order to develop hypoallergenic soybean products for sensitive people. The main aim of this work was the characterization of seed and milk soybean proteome and their comparison in terms of protein content and specific proteins. Using a shotgun proteomics approach, 243 nonredundant proteins were identified in mature soybean seeds.

Thyme oil nanoemulsions coemulsified by sodium caseinate and lecithin

Xue, J. and Q. Zhong , *J. Agric. Food Chem*. *62*: 9900–9907, 2014, http://dx.doi:10.1021/jf5034366.

Many nanoemulsions are currently formulated with synthetic surfactants. The objective of the present work was to study the possibility of blending sodium caseinate (NaCas) and lecithin to prepare transparent thyme oil nanoemulsions. Thyme oil was emulsified using NaCas and soy lecithin individually or in combination at neutral pH by shear homogenization. The surfactant combination improved the oil content in transparent/translucent nanoemulsions, from 1.0% to 2.5% w/v for 5% NaCas with and without 1% lecithin, respectively. Nanoemulsions prepared with the NaCas–lecithin blend had hydrodynamic diameters smaller than 100 nm and had significantly smaller and more narrowly distributed droplets than those prepared with NaCas or lecithin alone. Particle dimension and protein surface load data suggested the coadsorption of both surfactants on oil droplets. These characteristics of nanoemulsions minimized destabilization mechanisms of creaming, coalescence, and Ostwald ripening, as evidenced by no significant changes in appearance and particle dimension after 120-day storage at 21 °C.

Vitamin E content and estimated need in German infant and follow-on formulas with and without long-chain polyunsaturated fatty acids (LC-PUFA) enrichment

Stimming, M., *et al*., *J. Agric. Food Chem*. *62*: 10153–10161, 2014, http://dx.doi:10.1021/jf502469b.

Many formulas (FM) for infants are nowadays supplemented with LC-PUFA. Due to the susceptibility of LC-PUFA to peroxidation, a potential risk of oxidative stress must be considered. We analyzed the concentration of unsaturated fatty acids to calculate an estimated vitamin E need and the concentration of vitamin E as an important lipophilic antiox-

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idant in LC-PUFA enriched (FM(+), *n* = 31) and not enriched (FM(–), *n* = 29) formulas as well as in breast milk (BM, *n* = 118). No differences were observed in the ratios of vitamin E content to estimated vitamin E need between FM(+) and FM(–). Ratios were consistently above the minimum value according to European law. FM showed similar or higher ratios than BM, except in a worst-case scenario where BM showed higher ratios than FM(+). Our results indicate adequate vitamin E content relative to unsaturated fatty acid content in present-day commercial formulas. Given that breast milk tends to contain higher ratios of vitamin E content to estimated vitamin E need than LC-PUFA enriched formulas (at least in a worst-case scenario), the potential effects of increasing vitamin E content in LC-PUFA enriched formulas should be explored.

Effect of high-intensity ultrasound and cooling rate on the crystallization behavior of beeswax in edible oils

Jana, S. and S. Martini , *J. Agric. Food Chem*. *62*: 10192–10202, 2014, http://dx.doi: 10.1021/jf503393h.

The objective of this study was to evaluate the effect of wax concentration (0.5 and 1%), cooling rate (0.1, 1, and 10 °C/min), and high-intensity ultrasound (HIU) on the crystallization behavior of beeswax (BW) in six different edible oils. Samples were crystallized at 25 °C with and without HIU. Crystal sizes and morphologies and melting profiles were measured by microscopy and differential scanning calorimetry, respectively, after 7 days of incubation. Higher wax concentrations resulted in faster crystallization and more turbidity. Phase separation was observed due to crystals' sedimentation when samples were crystallized at slow cooling rates. Results showed that HIU induced the crystallization of 0.5% BW samples and delayed phase separation in sunflower, olive, soybean, and corn oils. Similar effects were observed in 1% samples where HIU delayed phase separation in canola, soybean, olive, and safflower oils.

Baseline patterns of adipose tissue fatty acids and long-term risk of breast cancer: a case-cohort study in the Danish cohort Diet, Cancer and Health

Schmidt, J.A., *et al*., *Eur. J. Clin. Nutr. 68*: 1088–1094, 2014, http://dx.doi: 10.1038/ejcn.

The evidence regarding fatty acids and breast cancer risk is inconclusive. Adipose tissue fatty acids can be used as biomarkers of fatty acid intake and of endogenous fatty acid exposure. Fatty acids in adipose tissue are correlated owing to common dietary sources and shared metabolic pathways, which group fatty acids into naturally occurring patterns. We aimed to prospectively investigate associations between adipose tissue fatty acid patterns and long-term risk of total breast cancer and breast cancer subtypes characterized by estrogen and progesterone receptor status (ER and PR). This case-cohort study was based on data from the Danish cohort Diet, Cancer and Health. At baseline, a fat biopsy and information on lifestyle and reproductive factors were collected. From the 31 original fatty acids measured, patterns of fatty acids were identified using the treelet transform. During a median follow-up of 5.3 years, 474 breast cancer cases were identified. Hazard ratios and 95% confidence intervals of risk of total breast cancer and of subtypes according to quintiles of factor score were determined by weighted Cox proportional hazards regression. After adjustment for potential confounders, factor scores for the seven patterns identified by the treelet transform were not associated with risk of total breast cancer, nor with risk of ER+, ER–, PR+ or PR– tumors. No clear associations between the patterns of fatty acids at baseline and long-term risk of total breast cancer or ER+, ER–, PR+ or PR– tumors were observed.

Impact of medium- and long-chain triglycerides consumption on appetite and food intake in overweight men

St-Onge, M.-P., *et al*., Eur. *J. Clin. Nutr. 68*: 1134–1140, 2014, http://dx.doi: 10.1038/ejcn.2014.145.

Medium-chain triglycerides (MCT) enhance thermogenesis and may reduce food intake relative to long-chain triglycerides (LCT). The goal of this study was to establish the effects of MCT on appetite and food intake and determine whether differences were due to differences in hormone concentrations. Two randomized, crossover studies were conducted in which overweight men consumed 20 g of MCT or corn oil (LCT) at breakfast. Blood samples were obtained over 3 h. In Study 1 (*n*=10), an ad lib lunch was served after 3 h. In Study 2 (*n*=7), a preload containing 10 g of test oil was given at 3 h and lunch was served 1 h later. Linear mixed model analyses were performed to determine the effects of MCT and LCT oil on change in hormones and metabolites from fasting, adjusting for body weight. Correlations were computed between differences in hormones just before the test meals and differences in intakes after the two oils for Study 1 only. Food intake at the lunch test meal after the MCT preload (Study 2) was (mean±s.e.m.) 532±389 kcal vs 804±486 kcal after LCT (P<0.05). MCT consumption resulted in a lower rise in triglycerides (P=0.014) and glucose (P=0.066) and a higher rise in

peptide YY (PYY, *P*=0.017) and leptin (*P*=0.036) compared with LCT (combined data). Correlations between differences in hormone levels (glucagon-like peptide (GLP-1), PYY) and differences in food intake were in the opposite direction to expectations. MCT consumption reduced food intake acutely but this does not seem to be mediated by changes in GLP-1, PYY and insulin.

Oil rich in carotenoids instead of vitamins C and E as a better option to reduce doxorubicin-induced damage to normal cells of Ehrlich tumor-bearing mice: hematological, toxicological and histopathological evaluations

Miranda-Vilela, A.L*., et al*., *J. Nutr. Biochem*. *25*: 1161–1176, 2014, http://dx.doi.org/10.1016/j.jnutbio.2014.06.005.

The development of therapeutic strategies to attenuate chemotherapy toxicity represents an area of great interest in cancer research, and among them is nutritional therapy based on antioxidants. As research on this topic is still controversial and scarce, we aim to investigate the effects of antioxidant supplementation with vitamin C, vitamin E or pequi oil, a carotenoid-rich oil extracted from pequi (*Caryocar brasiliense*), on doxorubicin (DX)-induced oxidative damage to normal cells in Ehrlich solid tumor-bearing mice. Tumor weight and volume, histopathology, morphometry and immunohistochemistry were used to assess the treatments' efficacy in containing tumor aggressiveness and regression, while possible toxicity of treatments was assessed by animals' weight, morphological analysis of the heart, liver and kidneys, hemogram, and serum levels of total bilirubin, direct bilirubin, indirect bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase, creatinine and urea. Although all the chemotherapeutic treatments increased internal necrosis area and reduced the positive Ki-67 cells compared to non-treated tumors, the treatments with pequi oil provided before tumor inoculation (PTDX) or in continuous and concurrent administration with doxorubicin (PTPDX) were more effective in containing tumor growth, besides increasing lymphocyte-dependent immunity and reducing the adverse side effects associated with DX-induced oxidative damage to normal cells, mainly the PTDX treatment. Vitamins C and E given before tumor inoculation and chemotherapy were not successful against doxorubicin-induced cardiotoxicity, besides increasing doxorubicin-induced nephrotoxicity, indicating that, at least for doxorubicin, pequi oil instead of vitamins C and E would be the best option to reduce its adverse effects.

Monitoring of quality and stability characteristics and fatty acid compositions of refined olive and seed oils during repeated pan- and deep-frying using GC, FT-NIRS, and chemometrics

Zribi, A., *et al*., *J. Agric. Food Chem*. *62*: 10357–10367, http://dx.doi: 10.1021/jf503146f.

Refined olive, corn, soybean, and sunflower oils were used as cooking oils for deep-frying at two different temperatures, 160 and 190 °C, and for pan-frying of potatoes at 180 °C for 10 successive sessions under the usual domestic practice. Several chemical parameters were assayed during frying operations to evaluate the status of the frying oils. Refined olive oil, as frying oil, was found to be more stable than the refined seed oils. In fact, this oil has proven the greatest resistance to oxidative deterioration, and its *trans*-fatty acid contents and percentages of total polar compounds were found to be lower at 160 °C during deep-frying. Finally, chemometric analysis has demonstrated that the lowest deterioration of the quality of all refined oils occurred in the refined olive oil during deep-frying at 160 °C and the highest deterioration occurred in the refined sunflower oil during pan-frying at 180 °C.