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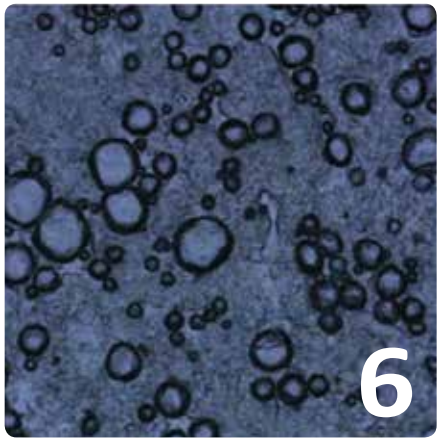

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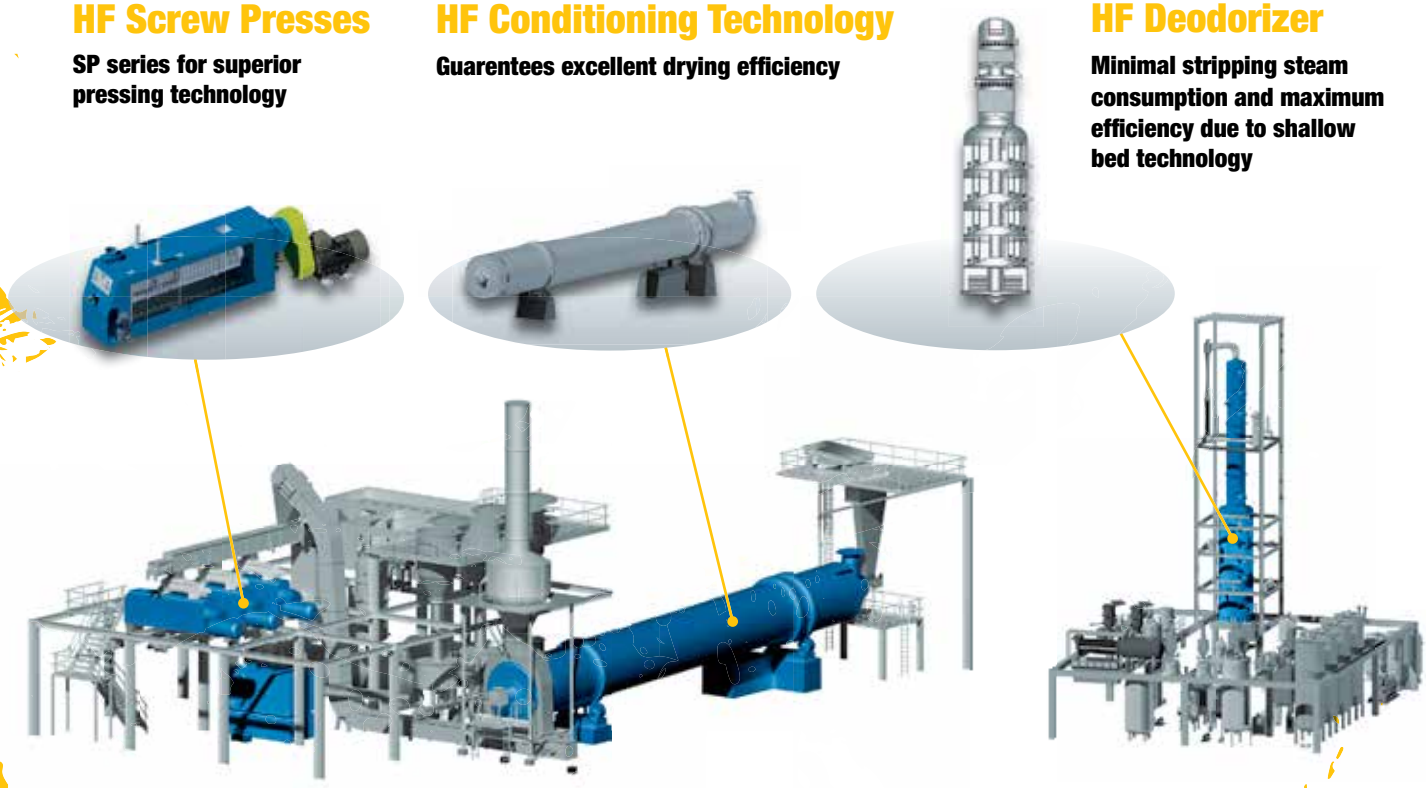

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Oleogels for drug delivery

Rebecca Guenard

As consumers began avoiding saturated fats and governments started banning trans fats, food ingredient formulators sought a healthy alternative that offered similar stabilizing and binding properties without compromising texture and rheology (https://doi.org/10.1016/B978-0-08-100596-5.21662-4). They discovered that they could retain these traits and provide structure by trapping a liquid oil in a lattice of non-fat material. At the same time, researchers acknowledged that the gelation of liquid oils, known as oleogels, had the potential to act as a drug delivery mechanism for hydrophobic active pharmaceutical ingredients (APIs). Researchers are now studying these new materials in hopes that oleogels are the key to providing targeted, long-term drug delivery for a variety of complex pharmaceutical treatments.

- **Oleogels are versatile alternatives to widely studied systems like hydrogels and organogels for applications requiring an injectable drug depot that slowly releases a pharmaceutical to a treatment site.**
- ***In vivo* studies indicate that oleogels are capable of acting as a drug delivery depot that administers treatment over a couple of weeks, but more research is needed.**
- **Bigels, the newest type of gelation system for drug delivery, capitalize on the advantages of both hydrogels and oleogels, allowing a researcher to tune its properties to best suit a given pharmaceutical treatment.**

The soft, psuedoplastic make-up of oleogels means they can be left under the skin or in a muscle as a drug depot for an extended period without the patient discomfort experienced with rigid implants. In addition, an oleogel encapsulated drug can be injected with a needle instead of by a surgical procedure as required for stiff devices.

FROM HYDROGELS TO OLEOGELS

For more than two decades, scientists have studied a drug delivery system known as hydrogels (https://doi.org/10.1016/j.polymer.2008.01.027). Hydrogels are three-dimensional, cross-linked networks of water-soluble polymers whose biocompatibility and highly porous structure make them a good candidate for releasing drugs into the body.

Though biomedical engineers have studied hydrogels extensively, they have been unable to resolve several limitations. Since hydrogels are water-filled with large pores, APIs can spill into the body within hours, contrary to their intended function as a slow-release mechanism. Hydrogels tend to be too rigid to inject through a needle and may require surgical implantation. Even then, hydrogels can float away from a treatment site. Most importantly, hydrogels are not robust enough to protect the proteins and peptides that make up the latest medicines, and they cannot house hydrophobic APIs.

Some scientists have proposed a hydrophobic, organic alternative to address hydrogels' limitations. Organogels form a drug depot *in situ* when a mixture of polymer, API, and organic solvent is injected into the body. Gelation occurs as the organic solvent leaches from the implant. Though a slow diffusion of drug is more effective with organogels, the obvious downside is that organic solvents are released into the blood stream as well.

Oleogels could provide the ideal drug delivery matrix. Much of the research on oleogels to date has been conducted with the intention of understanding their use in food applications. This is an advantage for

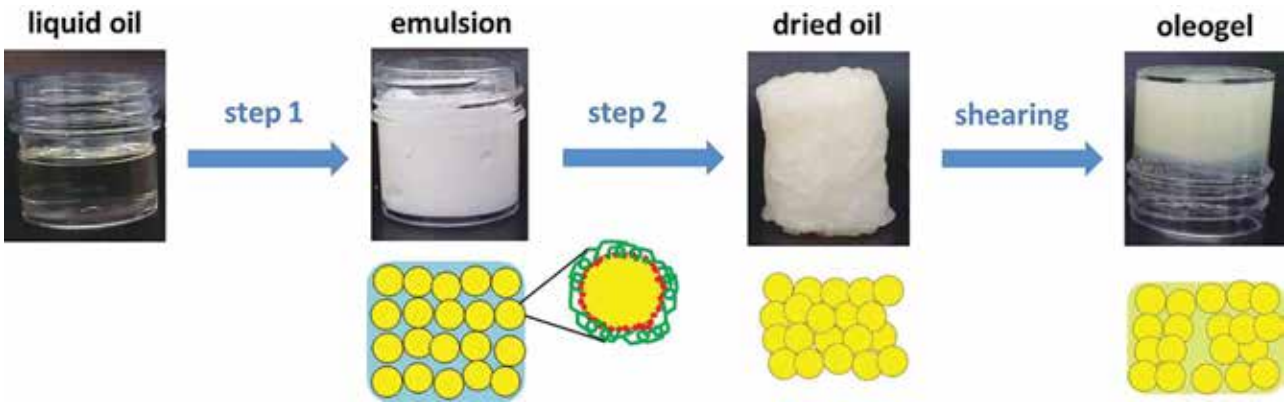

FIG. 1. An example of oleogel formation from an emulsified liquid oil that has been dried and sheared. Microstructure drawings depict emulsion droplets (yellow) with an adsorbed layer of gelatin (red) and sheets of xanthan gum (green). When water (blue) is removed with drying, the oil droplets pack tightly. The oleogel forms after shearing the dried oil, resulting in islands of packed droplets. This figure has been republished from [Patel, A.R., *Langmuir 31*: 2065–73, 2015] with permission under an ACS AuthorChoice License.

drug delivery researchers, since a wealth of research exists on how to adjust a gel's mechanical properties for a given application.

Chun Wang, associate professor of biomedical engineering at the University of Minnesota, Minneapolis, USA, says that as non-covalent, self-assembled systems oleogels exhibit a flexibility and reversibility that are ideal for a treatment requiring long-term delivery. "You can take the drug and mix it with the oleogels very easily. It can be adapted to release all sorts of drugs," says Wang. He points out a secondary advantage to using oleogels for drug delivery. "A lot of these oleogels are made of molecules that are biocompatible, like fatty acids and lipids. As a result, they have very good safety records in humans."

In a recent review article, Wang describes some of the materials and processes food researchers have used to optimize oleogels and how they may be applied for drug delivery. These biocompatible materials are composed of small, amphiphilic molecules that trap liquid oil as they self-assemble through non-covalent interactions. In addition, they exhibit desirable properties for slow-release drugs, like cancer or mental health treatments. Under mechanical force, oleogels become less viscous, but then recover when the force is removed. This property allows oleogels to provide protection for delicate protein or peptide treatments within a drug delivery device that is flexible enough to be injected through a needle.

Researchers have determined several gelation techniques for the formation of oleogels (Fig. 1). Examples typically involve supramolecular networks of protein or polysaccharide from gelatin or xanthan gum that encapsulate mineral, safflower, or sunflower oil. Gelation in these cases is most often induced by a solvent exchange. One unique gelation method involves an oleogel composed of an insect-derived polymer resin that crystalizes to entrap a rapeseed oil.

Though few *in vivo* studies have been performed, a research group at the University of Montreal in Canada, conducted a thorough study of an oleogel for the delivery of Alzheimer's disease medication. The drug rivastigmine is a cholinesterase inhibitor that helps delay the onset of Alzheimer's by facilitating neurotransmission. However, patients with dementia are required to take the medication twice a day by mouth to slow their disease. Jean-Christophe Leroux and his team developed an oleogel by first dissolving rivastigmine in safflower oil and then adding N-stearoyl L-alanine methyl ester as a gelator. They injected the drug delivery system under the skin of rats to study how it performed. They demonstrated the feasibility of oleogel system with the successful delivery of therapeutic levels of rivastigmine for up to 11 days. A disadvantage of their gelator is that it incorporates an organic solvent that initiates gel formation as it evaporates into the body.

BIGELS

A group of researchers at National Institute of Technology in Rourkela, India, have developed a different strategy for slowly dispensing drugs. They have created a mixed matrix they call a bigel by blending a hydrogel and an oleogel. Like the researcher at the University of Montreal, this team has validated the feasibility of slow-release though not *in vivo*.

"Bigels are a new type of gel system which are bi-physical systems just like emulsions, but both the internal structure and the external structure are gel," says Kunal Pal, assistant professor of biotechology who participated in the study. Pal says the team had been working on emulsion gels, a similar bi-physical system, with a liquid internal phase. "When we were working with these emulgels in the lab, we observed that the inner phase leached out when kept for a long duration," he says.

By making the inner structure a gel, the researchers were able to prevent leaching. The team prepared the bigels by mixing an oleogel, made of rice bran oil in a stearic acid matrix,

with a hydrogel made from tamarind gum surrounding a hydroethanolic solution. Immobilizing both phases prevents leaching or coagulation of the dispersed inner phase over time.

TABLE 1. Composition of the formulations (Paul, S.R., *et al.*, 2018)

	Weight (g)		
Formulations	**Oleogel**	**Hydrogel**	**Moxifloxacin HCl**
S1	30.00	0.00	–
S2	24.00	6.00	–
S3	12.00	18.00	–
S4	0.00	30.00	–
S1D	29.85	0.00	0.15
S2D	24.00	5.85	0.15
S3D	12.00	17.85	0.15
S4D	0.00	29.85	0.15

Pal says bigels have all the advantages of emulsion systems, but with the added stability of the inner phase. When the API is dissolved in a gel and dispersed through another gel it becomes a tunable time-release system. Drugs have to pass through two network structures, each with their own degree of attraction to the molecule. "The partition coefficient of the drug within the oleogel and then within the hydrogel plays an important role in the diffusion process," says Pal. He adds that the diffusion through both networks determines when the drug is released, and changing the characteristics of either gel can regulate that rate (Table. 1).

In addition, there is versatility in whether the system is primarily hydrophobic or primarily hydrophilic, so bigels can be used for a wide variety of drugs. Pal says they can disperse an oleogel in a hydrogel and dissolve hydrophobic drugs into the dispersed space. He says they have also done the opposite and made a reservoir for a hydrophilic molecule by dispersing a hydrogel into an oleogel (Fig. 2). "For example, if you have

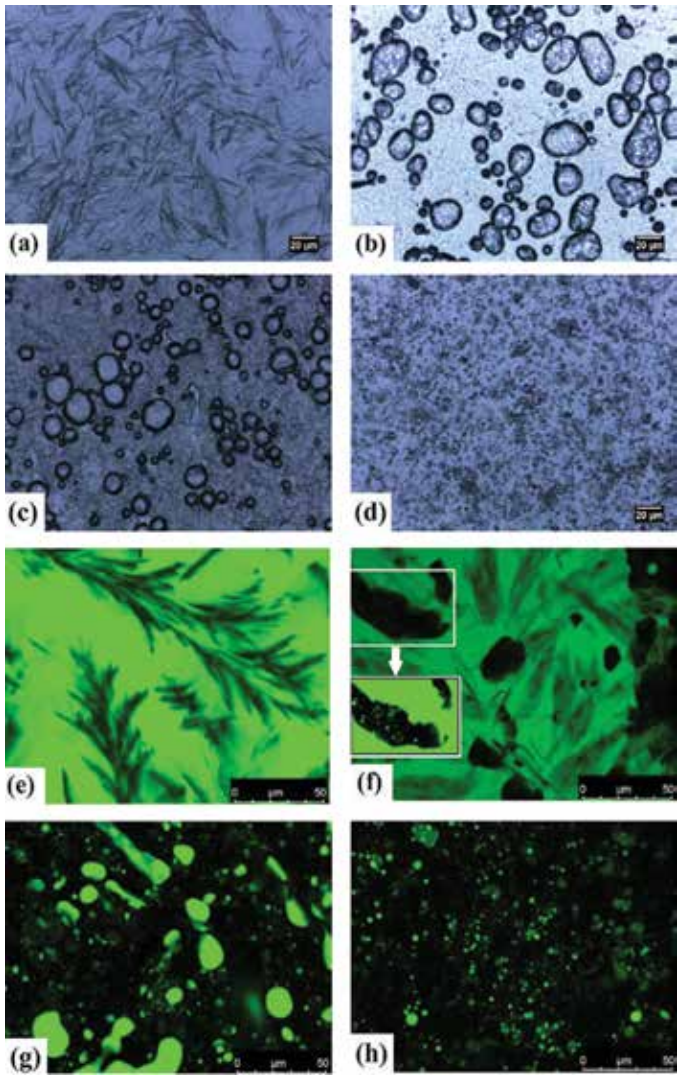

FIG. 2 Bright-field micrographs of the formulations: (a) S1, (b) S2, (c) S3, and (d) S4; and confocal micrographs of the formulations: (e) S1, (f) S2 (inset: contrast- and brightness-enhanced section showing the dispersion of the aggregates), (g) S3, and (h) S4 .
From Paul, S.R., *et al.* https://doi.org/10.1002/jsde.12022.

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Gels without vapor pressure: soft, nonaqueous, and solvent-free supramolecular biomaterials for prospective parenteral drug delivery applications, Tabet, A. and C. Wang, *Adv. Healthcare Mater.*, November 2018.

a hydrophilic drug you can put it into the aqueous phase and dump it into the oleogel and inject it into the body," he says. "You can use this for vaccines because it will release the bioactive agents very slowly."

COMBINATION DRUGS

Perhaps the biggest opportunity for oleogels to improve drug delivery is their use with combination drugs that have multiple APIs, each requiring precise release rates. Wang has studied silica nanoparticle-infused polymers for this purpose. These colloidal systems are composed of porous nanoparticles loaded with small molecules or proteins within a supramolecular network containing other drugs. The physical dynamics of this system can provide the controlled release of each treatment within a drug cocktail.

Wang says that to optimize nanoparticle systems there needs to be research efforts directed at developing materials from these gels that are biocompatible and biodegradable. He also recommends research focused on whether nanocarriers can be incorporated into polymers with bottle brush geometries for better tuning of drug release kinetics for combination drugs.

In general, Wang sees a lot of promise in the application of oleogels for drug delivery, but he says that not many people in the biomedical and drug delivery community are focusing their research on these materials. For oleogels to have any hope of being applied in the pharmaceutical industry, they have to be evaluated for safety, biocompatibility, and efficacy in cells and in animal models.

"One caveat here is that a lot of the GRAS [Generally Recognized as Safe] materials, like small molecule oils, are safe for oral consumption," says Wang. "If we use them for drug delivery in other contexts, for example, as an injection into the muscle or into the skin, then the difference in the relative delivery has to be taken into account."

Little is understood about how the API will interact with oleogel materials. And there have not been studies on the stability and retention of activity for protein drugs in a nonaqueous system. But the potential to address the challenges in the solubility of small molecule drugs and combination therapies make oleogels worth further exploration.

Rebecca Guenard is the associate editor of Inform *at AOCS. She can be contacted at rebecca.guenard@aocs.org.*

Household cleaning product formulation: a delicate balance

Catherine Watkins

Dave McCall remembers one of his formulation "fails" as if it happened just this year: "I was working on a detergent for the carwash industry," he explains. "That industry expects its formulae to possess three properties: They must generate huge quantities of foam while possessing significant viscosity and a brilliant color."

Find out from a formulation expert who holds more than 35 US patents:

- **How surfactant adsorption and monomer–micelle equilibria affect the efficacy of antibacterial wipes.**
- **Whether nonideality in mixed micelles affects products for cleaning soap scum.**
- **If the crystal structure of triglycerides has an impact on laundry detergency processes, and how to probe that phenomenon.**

McCall, a chemist at IPAX in Detroit, Michigan, USA, continues his rueful story.

"Viscosity is built into a product solely because the customer will perceive the product to have been watered down if this is not done," he notes, adding that the materials included to boost viscosity generally contribute nothing to the detergent or foaming properties of the product.

Correctly formulating the amount of dye in a product for the brilliant color buyers demand is very difficult in the lab, according to McCall. In addition, the amount of dye added to a product is typically a tiny fraction of a percent, which often is beyond the capabilities of the analytical devices available in the lab. "The dye rarely has any impact on any other property of the product," he adds. "Quite often, we do not dye lab samples for these reasons."

Carwash detergents, however, are different. "I should have known that," McCall sighs. Because buyers expect bright color in the foam and not just the detergent, such formulations require as much as a full 1 wt% of dye.

"I had built a good foaming product with a nice viscosity profile," McCall remembers. "The time frame I was allowed was tight—as usual—so I wrote up the formula, including a generous slug of dye, and sent it on its way to the plant. The sample that came back for quality control looked pretty good, except that the viscosity was very low—basically it was water-thin."

So, McCall made another sample of the product, only this time, "I did it the right way by including the dye." (How is it that there's never time to do it right, but there is always time to do it over, he wonders?) Sure enough, the rather large dye addition worked against the viscosity modifier. "I would never have guessed that the dye in a product would have had such a large impact on any other product property," McCall says, sheepishly.

THE DELICATE BALANCE

McCall's anecdote illustrates the delicate nature of cleaning product formulation, which involves finding the perfect balance among many factors and ingredients … and knowing how they interact. Not the least of those factors is cost-effectiveness through the judicious use of surfactants, so the product delivers what the buyer wants at a competitive cost—benefitting the consumer and the manufacturer, alike. Formulators cannot test every surfactant combination for any given formulation task, which means the careful selection of a starting point for a formulation chassis is important. That starting point is informed by experience and solid concepts like beta parameters, adsorption isotherms, and the Hydrophilic–Lipophilic Difference (HLD).

Most consumers probably do not care much about the technical complexity of cleaning product formulation and the importance of balancing the whole system of water, surfactant, oil, temperature, pH, and salts. But they *do* know what is important to them in their favorite products, be it foam or scent or antibacterial action or all of the above and more.

One person who has spent his career furthering the understanding of surfactant science as it relates to consumer product formulation is the 2018 Samuel Rosen Memorial Award (https://www.aocs.org/network-and-connect/awards-x1888#samuel-rosen-memorial) recipient, David. R. Scheuing, research director at Clorox Services Co. in Pleasanton, California, USA. Scheuing delivered his award address at the 2018 AOCS Annual Meeting & Expo, and subsequently conducted a webinar (https://www.youtube.com/watch?v=PuVJO_CKa2A&feature=youtu.be) on the same topic: "A Walk Through Your Home—Discovering the Surfactant Science for Cleaning It."

Scheuing began his walk around the typical home in the kitchen, where many consumers—particularly in the United States—are passionate about using products with antimicrobial action. In this case, Scheuing looked at factors affecting the formulation of the lotion for wipes intended to clean hard surfaces in the kitchen.

Most kitchen counters are home to both gram-negative and gram-positive bacteria. (In brief, gram-positive bacteria do not have the outer cell membrane found in gram-negative bacteria.) Cleaning wipes need to wet, spread, and clean as

well as disrupt both types of bacteria. Wipes lotions generally are mixed micelle solutions with an added antimicrobial compound. Bear in mind, however, that bacteria are found in different shapes and sizes and that shape and size affect cellular disruption. Further, the target for membrane disruption is not on the surface of the bacteria, meaning that a biocidal formulation must be able to reach the interior of the bacterial cell in addition to handling the variation in the cell wall among species.

Quaternary ammonium chloride compounds, or quats, have been used in products for their disinfectant properties ever since they were developed early in the 20th century. There are about 300 varieties, each with a varying level of antimicrobial effectiveness. Three widely used quats in wipes lotions are ADBAC (alkyl dimethyl benzyl ammonium chloride), ADEBAC (alkyl dimethyl ethylbenzene ammonium chloride), and DDAC (dialkyl dimethyl ammonium chloride).

Watch webinar

This article was adapted from an AOCS webinar (https://www.youtube.com/watch?v=PuVJO_CKa2A&feature=youtu.be) conducted by David Scheuing, research director at Clorox Services Co. (Pleasanton, California, USA). The webinar, in turn, was based on Scheuing's Samuel Rosen Memorial Award address given during the 2018 AOCS Annual Meeting & Expo.

The Rosen Award was established by the AOCS Surfactants and Detergents Division (https://www.aocs.org/network-and-connect/membership/divisions#-surfactants-and-detergents) to recognize significant accomplishments in surfactant chemistry. The award is sponsored by Professor Emeritus Milton J. Rosen of Brooklyn College, a longtime surfactant researcher and AOCS member. The award is in honor of Rosen's father, Samuel Rosen, who worked for more than 40 years as an industrial chemist investigating the formulation of printing inks. The award consists of a plaque, a $2,000 honorarium, and an award lecture given at the AOCS Annual Meeting (https://annualmeeting.aocs.org/).

Adsorption of biocides onto an organism is classified by isotherm types, which include Langmuir, high-affinity, C-shaped, and Z-shaped uptake profiles. Langmuir and high-affinity profiles are relevant to the adsorption of quats onto bacteria, Scheuing notes in his presentation.

The amount of bacteria present on a counter or other hard surface matters, he says, because both intact cells and lysed debris (disrupted cellular matter) serve as reservoirs or sinks for quats and surfactants. Even anionic surfactants such as sodium dodecyl sulfate (SDS) are affected by cell debris. The log reduction of bacteria slows as the amount of debris increases (known as self-quenching); the kill rates of quats are highly nonlinear.

Further, the quat structure affects the adsorption onto intact *Staphylococcus aureus* cells [see Ioannou, *et al.*, "Action of disinfectant quaternary ammonium compounds against *Staphylococcus aureus*" in *Antimicrobial Agents and Chemotherapy, 51*: 296–306, 2007]. In this work, Ioannou and others found that ADBAC forms approximate monolayers and DDAC forms multilayers on *Staph. aureus* when the solution is below CMC (critical micelle concentration).

"This means that wipes lotions can't just be micellar quat solutions," Scheuing emphasizes, particularly since consumers want an all-purpose product that disinfects and cleans greasy soils and soap scum, has a pleasant odor (from fragrance oils), does not leave a film or streaks, and has a good handfeel and mildness.

But wait, there's more: The lower the CMC, the less adsorption onto the microbe, making co-surfactant selection key. (CMC decreases based on the tail length of the surfactant.)

Another surfactant phenomenon that must be considered in wipes lotion formulations utilizing amine oxides is pH since monomer and micelle compositions can vary with pH. At a low pH, monomers are cationic-rich. At a high pH, monomers are cationic-poor—a fact that those with less experience in formulating might not consider.

The fragrance many consumers demand also is tricky, in that fragrance solubilization varies with mixed micelle composition. Changing the pH of formulations utilizing amine oxides changes the micelle composition, which changes the solubility of the fragrance oil. In addition, several fragrances are needed in order to satisfy multiple consumers, so the lotion formulation must be able to handle multiple fragrance oils.

"In the real world," says research chemist Dave McCall, "some fragrance oils are oilier than other fragrance oils, which means they exhibit a wide range of Effective Alkane Carbon Numbers. This affects the polarity of the oil, or its HLD."

David Scheuing elaborates on the theme. The HLD range exhibited by a group of fragrances for a given surfactant package directly affects costs. If formulators don't avoid large mismatches between the surfactant package and the fragrances, they will tend to simply increase the total surfactant concentration, and hence costs, in order to deliver the desired set of fragrances.

THE CHALLENGE OF SOAP SCUM

Moving on to the next household cleaning challenge, Scheuing discusses the case of formulating cleaners that can deal with "soap scum," or the relatively insoluble matter that can test the patience and brand loyalty of consumers if the shower or bathtub cleaning effort is anything but fast and easy.

This formulation challenge requires an understanding of mixed micelle formation, the effect of adding a chelant or builder, and both equilibrium phase behavior and interfacial kinetics. Calcium stearate, for example, is too insoluble to form micelles at any temperature; therefore, the addition of Na_2EDTA (ethylenediaminetetraacetic acid) or other chelants is necessary to modify the CMT (Critical Micellization Temperature). On the other hand, sodium stearate does form micelles—but not at room temperature. Further, too much EDTA increases the CMT of more soluble soaps.

Scheuing points out that the equilibrium solubility of calcium stearate is much greater in C12 amine oxide (DDAO) micelles than SDS at room temperature at low and high pH. Changing the pH of the formulation affects both the chelant and solubilization efficiencies. [See Soontravanich, *et al.*, "Dissolution study of salt of long chain fatty acids (soap scum) in surfactant solutions—Part 1: Equilibrium dissolution, *J. Surfact. Deterg. 13*: 367–372, 2010, https://onlinelibrary.wiley.com/doi/abs/10.1007/s11743-010-1208-5].

Rapid bathroom cleaning comes down to understanding beta parameter trends as a measure of nonideal mixing, Scheuing notes, as well as mixed micelle synergism. "As the beta parameter increases, mixed micelle synergism also increases," he says.

LAUNDRY DETERGENCY AND THE TRIGLYCERIDE CRYSTAL STRUCTURE

Next on the home tour is the laundry room, where much has changed over the past several decades. "Laundry machine wash temperatures have been decreasing over time in Europe, North America, and Japan," Scheuing notes, adding that data from 2014 showed average wash temperatures of 42°C in Europe, 29°C in North America, and 23°C in Japan.

This trend is accelerating, he stresses, which creates the challenge of dealing with solid triglycerides, or "the glue of many stains," as he puts it. Solid triglycerides exhibit polymorphism, packing into several different crystal structures, including a chair-like shape and a tuning fork shape. Therefore, it is necessary to probe how surfactants in washing baths affect these crystal structures.

Scheuing was a pioneer in applying Fourier-Transform Infrared Spectroscopy (FTIR) in studies of micelle structure. "I hope the approaches and results obtained reinforce the impact of geometry on the interactions between molecules that are, for thermodynamic reasons, self-assembling into these fascinating structures," he said in an interview published on the AOCS website (www.aocs.org).

"FTIR provides a view that is very complementary to those obtained with other techniques, like nuclear magnetic resonance spectroscopy. I also hope that the application of FTIR to studies of the interfacial interactions of micellar solutions and solid oils will reinforce the relationship between surfactant-oil-water phase diagrams and phenomena [such as] enhanced oil recovery and the detergency process in your laundry room. The spectroscopic analyses are actually fairly easy to do, and yet tell us about what is going on at the interface as a system tries to move to equilibrium."

Consumers, of course, do not have spectrometers in their laundry rooms. They only know whether their detergents have removed all the stains on their clothing. It is fortunate for them, however, that researchers like David Scheuing and Dave McCall are on the job, working to apply solid science to solving practical, everyday problems.

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Characterizing dispersions throughout the oil industry: comprehensive formulation analysis

Matt Vanden Eynden, Christelle Tisserand, Yoann Lefeuvre, Pascal Bru, and Gerard Meunier

There is no shortage of demand for new and improved consumer and industrial products that have oil of some type as a significant component in the product. Formulations, such as emulsions, now have many different types of oils that can be incorporated for a wide variety of uses, such as home and personal care creams and lotions, metalworking fluids, beverage emulsions, and coatings. The identities and types of materials used to generate and stabilize these emulsions are also extensive. With more and more viable options becoming available, previously acceptable standards are now drifting out of regulation, and some materials that are now considered to be toxic or harmful are being removed from the market all together.

- **Emulsions and suspensions throughout the oil industry have a need for simple and facile stability analysis.**
- **Full characterization of these formulations—even for new materials, novel products, and ambitious projects—can be obtained.**
- **Applications for food, beverage, home and personal care, and crude oil are validated with a single technology.**

This shift in consumer demand for new and alternative materials challenges formulators to create and compose novel blends while ensuring that they are safe for the public to use and consume throughout the shelf life of the product. In an analytical sense, this opens doors to uncharted territories where, in some cases, zero data exist for the use of certain components within a new formulation, and the rheological, biological, and sensory parameters must all be investigated from scratch. Devices and instrumentation are certainly available to generate such data, but are we sure that the data we are generating is useful? Are accelerated and extrapolation techniques acceptable now as well as in the future? Or are there other, better ways to quantify critical characteristics of these new products?

Previously in *Inform* ("Engineering enzyme stability for liquid detergents," September 2018, https://tinyurl.com/yd5jgy23), we described an analytical technique that utilizes Multiple Light Scattering (MLS) to monitor and predict the physical stability of emulsions, suspensions, and foams by monitoring with high resolution the particle movement and size-change kinetics within a native, concentrated, unmodified sample. This provided a rapid and

quantitative way to develop new home and personal care formulations without having to rely on long-term visual shelf analysis requiring days, weeks, or even months, while also feeling secure that the product will have an acceptable shelf life once it leaves the production facility. While this was a single application, the industry of oil-infused formulations spans a much broader scope. To this end, this article will outline the broad capabilities of this technology and the importance it can have in the lab. To show how MLS is implemented in these analyses, a Turbiscan™ device (Formulaction, France) is used exclusively to provide aging kinetics of both particle migration (sedimentation, creaming, clarification) and particle size change (flocculation, coalescence) as well as the calculation of mean particle size and other dispersion quality and re-dispersibility kinetics.

PERFUME STABILITY IN FRAGRANCE EMULSIONS

The hydrophobic character of many fragrances causes inherent stability problems to end-use perfume products. In addition, such formulations are in need of a minimum dosage to ensure that the aroma is strong enough for general use. Given the desired long-term stability that these emulsions demand, and the potential to screen multiple fragrances or fragrance blends, a short, quantitative analysis of the emulsions will facilitate a faster time to market, as the formulation studies will not be held up in the lab for nearly as long.

In this case, testing in the Turbiscan™ device was performed at 45°C, further accelerating the destabilization of the sample. Coupled with the high resolution of the device, this

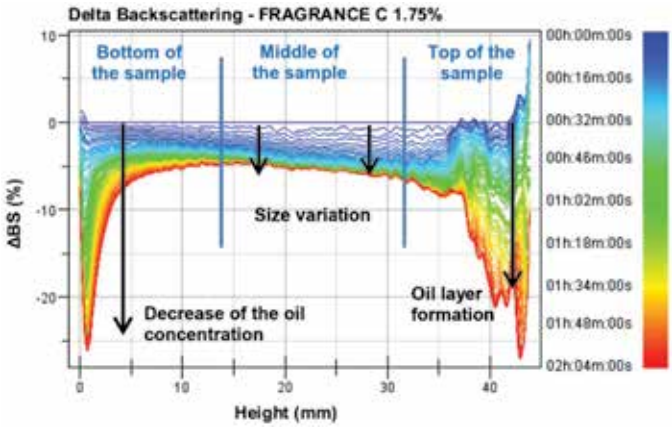

FIG. 1. A typical evolution profile

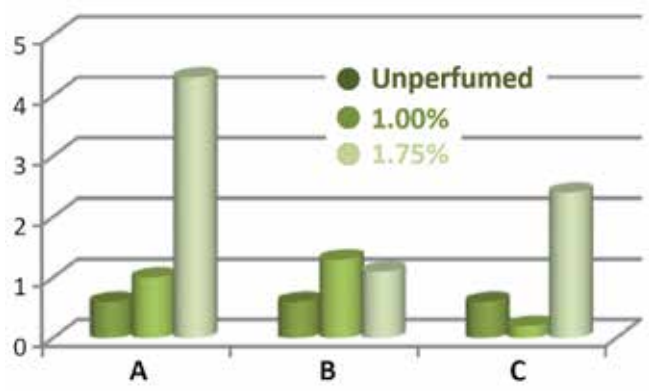

FIG. 2. Analysis of TSI values is used to determine the stability of emulsions when different fragrances are used at two different concentrations.

allows for complete formulation comparisons to be made in only 2 hours.

Common in these plots are a flocculation event that causes an eventual creaming and coalescence of the fragrance droplets to the top of the sample. Figure 1 shows a typical evolution profile of one of these formulations. The flocculation that is observed is characterized by the global vertical evolution of the sample seen in the middle of the graph. The left side of the graph, representing the bottom of the sample, sees a decrease in the backscattering (BS) signal as particle concentration is decreasing in this zone. On the right side of the graph, another negative BS signal is evolving as the particles migrating to that zone are coalescing and increasing their particle size.

The easiest way to quantify the overall stability of these samples is with an internal algorithm in the Turbiscan™ device called the Turbiscan Stability Index (TSI). This TSI assigns a value based upon the evolution intensity of the samples; more intense evolutions are assigned higher numbers and deemed unstable. This can replace the monotonous task of removing local aliquots of the sample for alternative analysis over some time period and replace it with a simple, one-click function. Shown in Figure 2, the TSI values for these samples are analyzed in order to determine the stability of these emulsions when three different fragrances are used at two different concentrations, all against a control. It is clear that the use of a higher dosing of fragrances A and C renders the emulsion unstable while a higher dosing of fragrance B has a negligible effect.

This result cannot only be used to determine the overall stability of an emulsion, but also to fine tune the amount of ingredient required to garner a certain effect. This results in better overall formulations while also optimizing costs, all at a rapid analysis rate and reducing the time-to-market for the project.

CRUDE OIL DEMULSIFICATION

In an analogous manner of determining the stability of an emulsion, the Turbiscan™ device can also be used to track the kinetics of an emulsion that is forced to break. This type of procedure is seen in the crude oil industry where kinetics of demulsification are important to optimize the types and amounts of destabilizers that are used but also to enhance the speed of the separation to maximize production throughput. Typical kinetics can be captured using a burette and multiple manual calculations but can be tedious and at times subjective. Utilizing this MLS technology, a single test is performed to determine the water volume produced in the breaking of an emulsion, the speed at which the water layer is formed, the quality of the water/oil interface, and the quality of the extruded water itself.

A representative sample is shown in Figure 3. The speed of the water phase development can be analyzed by the scan timestamp seen from the initial transmission (T) signal at the bottom of the sample (green traces, left of graph) to the last T signal in the plot (red traces, middle of graph). Final water quality is seen by the height of the last scan plateau (which is then compared to a pure water reference), while total water volume is seen by the signal evolution on the x-axis (height, in mm). Emulsions that break faster will have more rapid kinetic evolutions, while demulsifiers that provide a better water quality will have a higher scan plateau, closer to that of a pure water T signal.

Calculating the individual parameters for all five demulsifiers that were analyzed takes only several seconds, and key trends are immediately apparent. The importance of this kinetic analysis is to give the operator the ability to optimize for speed of separation or for the quality of the water pro-

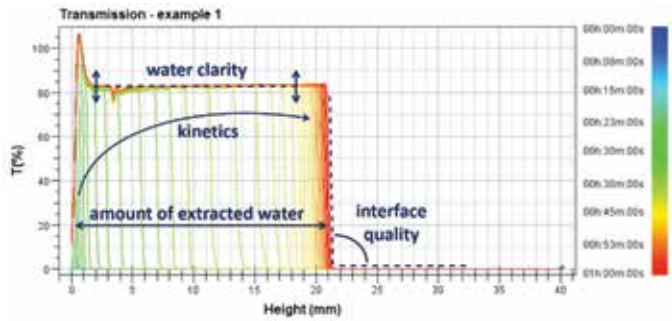

FIG. 3. Timestamp of a representative sample

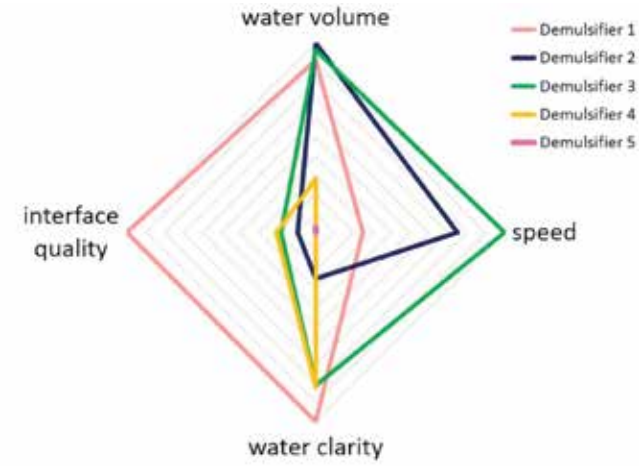

FIG. 4. A comparative radar chart

duced in the demulsification, or both. A comparative radar chart is seen in Figure 4 and shows the extent of each parameter that is observed, which also serves as a useful marketing tool to provide customers with specific details about the methods that went into the demuslification process. Whether optimizing for speed or quality, the answer can be determined with this short 1-hour test.

EVALUATION AND EFFICACY OF PEA PROTEINS AS EMULSIFIERS

The replacement of gluten-, egg-, and whey-based emulsifying proteins with new, cheaper, and more sustainable materials is of great interest in a variety of fields. Optimally, any new formulation would have the same, if not better, stability and appearance as those with animal-derived emulsifiers. It would be time-consuming and costly to use standard visual analysis to predict the aging profile of these materials or to utilize multiple different instruments for the purpose of predicting how the new formulations will behave. So, rather than using extrapolation or correlation, MLS technology can be used to quantify both the mean particle size and destabilization kinetics of each sample in an experiment, and predict the variation that will exist in each sample for each modification of the formula.

Standard evolution of an oil/water (O/W) emulsion is for the oil to gradually cream to the top of the sample container, while the bottom of the sample starts to clarify to the point of transparency. The TSI plots for all eight emulsion samples are shown in Figure 5. They reflect the summation of any destabilization kinetic that is seen throughout the aging period of the samples, including creaming, clarification, and flocculation. The TSI plot of sodium caseinate samples remain low and flat for the duration of the concentration change (red plot), indicating that these emulsions are consistently stable regardless of the loading of the protein. But, the pea protein trace (blue plot) only begins to develop the sample level of stability with loadings near 0.5%, roughly 5 times that of the caseinate formulations. So, while the pea protein loading may end up being higher the overall stability of the caseinate emulsions can be reproduced.

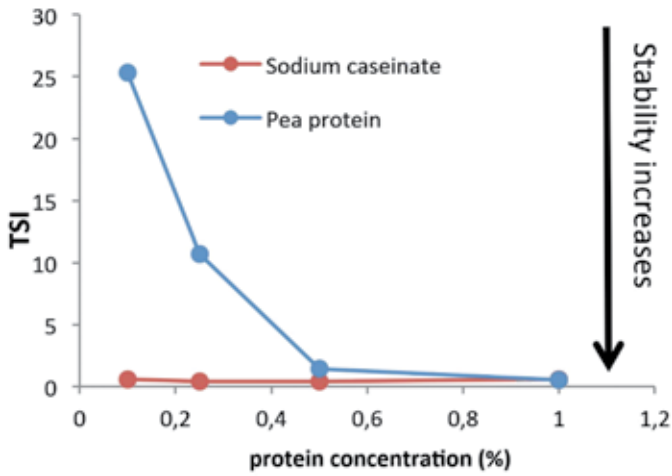

FIG. 5. TSI plots for emulsions loaded with various concentrations of pea protein or sodium caseinate

The benefits of interdisciplinary synergy

The analytical technique for monitoring and predicting the physical stability of emulsions, suspensions, and foams described in this article is just one example of how an advance in one industry segment can have a ripple effect across a broad range of industries.

In this case, the multiple-light-scattering technique can not only be used to analyze enzyme stability in liquid detergents but can also be used to analyze perfume stability in fragrance emulsions, track the kinetics of crude oil demulsification, evaluate pea proteins as emulsifiers, and predict the shelf-life of high-viscosity food emulsions such as mayonnaise, peanut butter, sauces, creams, chocolate ganache, and other fillings.

Organizations that focus on one particular industry help share knowledge within that industry, but AOCS goes one step further by spreading knowledge and technology from one industry to the next by bringing all of the knowledge and innovation that is happening in adjacent industries together, where they become stronger than any of them would be on their own—through networking with industry professionals from different fields, joint technical sessions that bring industry professionals together to focus on common problems, and the broad-based interdisciplinary content in *Inform SmartBrief*, *JAOCS*, inform|connect, and, of course, *Inform* magazine!

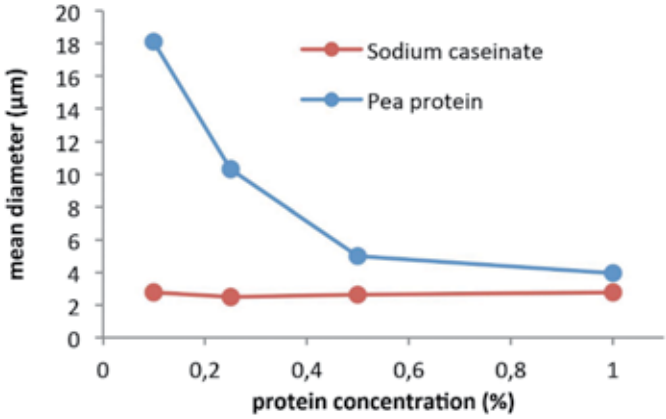

FIG. 6. Loadings of 5–10 times of pea protein are needed to generate similar particle sizes in the emulsion.

Going further, the efficiency of the protein to generate and maintain similar particle sizes to that of their caseinate reference, the mean particle size of the emulsion is also detected by MLS as determined by Mie theory (see http://www.thermopedia.com/content/956/). Seen in Figure 6, this too shows that loadings of 5–10 times of pea protein are needed to generate similar particle sizes in the emulsion. This sizing technique, along with the overall destabilization profile, can then verify the general size and stability of the particles in the sample with a single test that would have otherwise required multiple devices and methods (particle size analyzers, HPLC, visual analysis) to obtain.

HIGH VISCOSITY SAMPLES: CHOCOLATE GANACHE

Many emulsions and suspensions that are created and undergo some type of stability analysis are readily-flowing materials with low viscosities. Materials with higher viscosities are still prone to phase separation and other stability concerns, albeit at lower speeds as the inherent matrix in the medium prohibits particles from migrating quickly. The visual shelf life analysis of these materials can take weeks, months, or even years to fully characterize, even while these materials are in the consumer marketplace and casting some uncertainty on the integrity of the products.

In the case of food emulsions, products such as mayonnaise, peanut butter, sauces, creams, and fillings are also subject to physical destabilization, whether it be from the creaming and coalescence of oil separating from a medium, or from solid particulates settling to the bottom of a container, thus prompting a redispersion technique before use. However, there are cases in which a redispersion technique does not result in a homogeneous product. In this case, the material is either disposed of for regulatory reasons or customers find the product to be unattractive or of poor quality.

In the case of chocolate ganache, chocolate and warm cream are added together to make an emulsion that must reach a specific temperature. The warm emulsion is then cooled in a particular manner to generate a desired cocoa

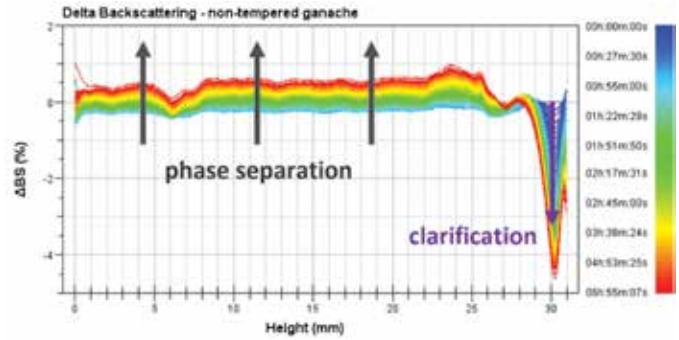

FIG. 7. Turbiscan™ output shows a clarification-type layer forming on the top of the sample, indicating that a visible phase separation is likely to occur.

butter crystal structure known as β^V. This tempering process is critical to the physical appearance, production quality, and shelf life of the material. If a crystal structure other than the β^V structure is formed, the crystal is likely to evolve and promote a blooming effect, leading to phase separation and an undesirable physical appearance. A substantial effort must be made to ensure that the mixture has crystallized properly and is stable for a duration of time. Otherwise, texture, appearance, and overall quality concern will arise in the product and present negative customer feedback.

Chocolate ganache emulsions will destabilize faster and with greater intensity if the chocolate that is used is poorly or non-tempered compared to that of a properly-tempered chocolate. Specifically, it is seen through the Turbiscan™ output in Figure 7 that there is a clarification-type layer forming on the top of the sample, indicating that an eventual visible phase separation is likely to occur, if it is not already visible.

Peer-reviewed articles detailing the Turbiscan™

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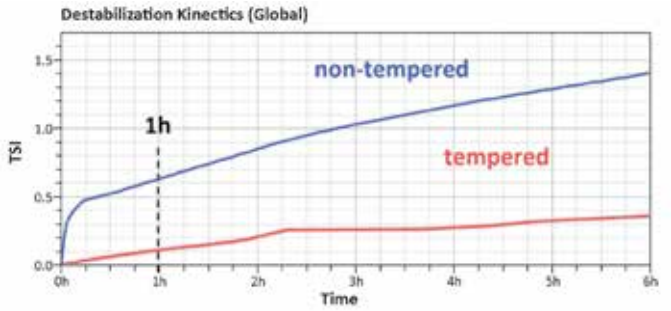

FIG. 8. Turbiscan™ output showing destabilization kinetics of non-tempered and tempered chocolate ganache emulsions

While this eventual phase separation may be true for all chocolate ganache emulsions made of poorly or well-tempered chocolate, it is clear that the non-tempered version possesses a higher rate of destabilization. Using the internal TSI function, this phase separation is resolved between the samples in as little as an hour of measurement time on the device as shown in Figure 8. This quick, facile method can act as a quality control method on products lines as well as a fast way to help R&D chemists to optimize the entire process without incorporating long analysis times and subjectivity.

Oil-based formulations and materials that contain varying types of additives, emulsifiers, viscosity profiles, and oil makeups can be analyzed for physical stability in a quantitative manner by utilizing Multiple Light Scattering (MLS) via the Turbiscan™ device. Applications in crude oil, home and personal care, food, beverages, cosmetics, coatings, and many others fields where emulsions, suspensions, or foams are in critical need of physical stability analysis can benefit from a single device and bring clarity to both R&D as well as the quality control side of production and operations.

Matt Vanden Eynden received a B.S. in chemistry from Wright State University in 2006, and a Ph.D. concentrated on catalytic method development from The Ohio State University in 2011. After completing a post-doc focusing on combating biological nerve agents, followed by teaching undergraduate organic chemistry for three years, Matt joined Formulaction, Inc. in Columbus, Ohio. He now maintains and supports a line of devices that utilize light scattering technology to help monitor and predict the stability, rheology, and end-use properties of raw materials as well as concentrated emulsions, dispersions, and foams. He can be contacted at matt@formulactionusa.com.

Coauthors Christelle Tisserand, Yoann Lefeuvre, Pascal Bru, and Gerard Meunier are with Formulaction in Toulouse, France.

"Directed evolution" steers new lipid chemistry: my experience working with a Nobel Prize Laureate in Chemistry

Zheng Guo

- **Although the Nobel Prize in Chemistry 2018 was divided, the contributions were similar in significance: The three recipients had successfully harnessed the power of evolution for purposes that benefit humankind.**

- **Half of the prize was for the directed evolution of enzymes that enable biofuels, pharmaceuticals, and other chemicals to be produced in greener ways [1]. The other half was for peptides and antibodies that were evolved through phage display to combat autoimmune diseases and, in some cases, to cure metastatic cancer [2].**

- **I was working as a visiting faculty member in the lab of one of the recipients when the Royal Swedish Academy of Sciences announced its decision. This article explains how the emerging field of directed evolution I observed in the Nobel Laureate's lab is steering the development of new chemistries and greener pathways for producing chemicals—and how this emerging technology is revolutionizing lipid science.**

"Scientists discover the world that exists. Engineers create the world that never was."
—Theodore von Kármán

Frances H. Arnold, Linus Pauling Professor of Chemical Engineering at California Institute of Technology (Caltech), Pasadena, USA, was sleeping in a hotel room in Dallas, Texas, when she got the call from Sweden informing her that she was to be a Nobel Laureate in Chemistry. "It came as a total surprise, woke me up at 4 am in my hotel room," she wrote in an email to her team back at Caltech. Her first thought on hearing the news was to share it with us in real time, and to let us know that she was returning to celebrate with us instead of attending a scheduled seminar in Dallas. "I am coming home now, and there will be a party on campus this afternoon," she announced. "Love to all of you amazing people, whose creativity and hard work made this possible." Interestingly, thanks to the speed of modern media, many of us knew about the prize and had already begun to spread the good news.

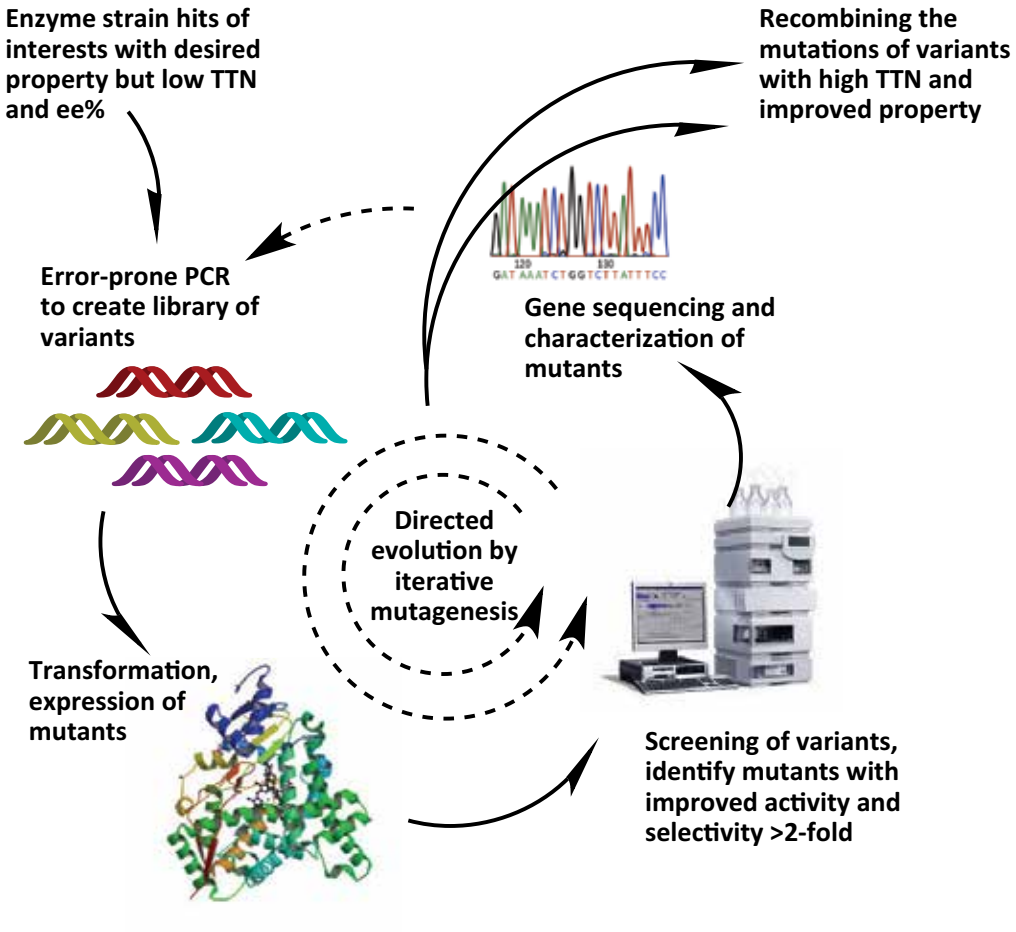

FIG. 1. Iterative technical cycles for "directed evolution" of enzymes

WHY IS EVOLUTION IMPORTANT TO CHEMISTRY?

In her speech at the Nobel Banquet, Dr. Arnold said that evolution "has led to the finest chemistry of all time, and to all living things on this planet." Evolution is fuelled by diversity, she explained, "with natural selection leading to continuous adaptations and improvements in Nature's handiwork."

This force of nature that has been operating over eons offers modern chemists an important opportunity because, "Nature has explored only a tiny fraction of the life and life's molecules that are possible. With evolution in our hands, with the ability to set genetic diversity and to tailor the forces of selection, we can now explore paths that Nature has left unexplored. We can also explore paths that Nature will never explore: We can select living organisms and their chemistries for our benefit—to create new sources of energy, to fix the carbon in our atmosphere, to cure disease, or to make us younger."

MIMICKING NATURAL SELECTION

In the laboratory, directed evolution mimics natural selection very quickly in a way that guides the evolutionary development of new proteins with desired characteristics. Because enzymes are protein catalysts that drive millions of different reactions, steering their evolutionary development provides new and highly effective chemical tools that lead to new and greener ways of making materials and chemicals.

Toward this end, Dr. Arnold has not only established a general universal procedure for directing the evolution of enzymes, but she has also continually demonstrated the directed evolution concept in a variety of applications, such as new-to-nature chemistry.

The first experimental study of directed evolution conducted in her laboratory focused on improving the activity of subtilisin E in the highly polar solvent (dimethylformamide, DMF) [3]. A new enzyme variant with 256 times the activity of the wild-type (WT) was developed through three cycles of evolution. In her first seminal paper [3], Arnold set up a general work flow for directed evolution of enzymes (Fig. 1) that typically includes: 1) identifying a starting enyzme and a preferred property for it, with essential gene-sequence and protein structure information, if possible; 2) defining a criteria for selecting enzyme variants; 3) re-diversifying the gene-sequences,; 4) putting mutated genes into an expression frame and selecting the mutant enzymes that meet improved stringent performance; 5) analyzing the genes of new mutants and subjecting them to a new directed evolution cycle, and iterating the evolution until the set target is reached.

Error-prone polymerase chain reaction (PCR) is initially used to create mutants and gene diversity, and later as a tool for protein engineering. ”DNA shuffling” is another important technique used to propagate mutations by random fragmentation and re-assembly of genes [4].

Because Caltech is a world leading institute in many fields of chemistry, Dr. Arnold has been able to apply the directed evolution concept to some frontier areas of chemistry, such as carbene chemistry, in combination with metalloenzymes. This has greatly expanded the directed evolution concept to new-to-nature chemistry; which can be considered the ”second spring” of directed evolution. Through site-mutation C400S in cytochrome $P450_{BM3}$, Arnold´s group has designed and developed a unique serine-heme ligated cytochrome “P411” [5]. More importantly, they were the first to demonstrate P450’s ability to catalyse the carbine insertion of double bond for olefin cyclopropanation, which initiated a flourishing interest in new-to-nature chemistry (i.e., forge C–C, C–S, C–Si, C–N and C–B bonds, and so on) [6–9].

APPLICATIONS IN LIPID CHEMISTRY

As Arnold explained in an interview with National Public Radio, “I wanted to rewrite the code of life, to make new molecular machines that would solve human problems.” She succeeded in doing just what she set out to do, as her contribution in directed evolution has set the stage for new types of chemistries and greener pathways for chemicals.

Conventional lipid chemistry relies heavily on chemical processes. However, more and more technologies are coming into use in modern lipid chemistry. Of these, enzyme-based processes must be the most important technology applied in modern lipid processing industry. What’s more, directed evolution is at the very core of enzyme technology, as it has established a powerful platform to engineer enzymes for different applications.

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The Swedish ingredient company Aarhuskarlshamn (AAK) has industrialized a packed bed reactor with immobilized lipase for production of structured lipids. Enzymatic degumming with phospholipase C has been implemented in many plants. A liquid lipase developed by Novozymes has also been used for production of biodiesels with used or low-quality oils. Although there has been no detailed disclosure, but it is firmly believed by many that the directed evolution concept has played a central role in engineering that enzyme for industrial oil applications.

Directed evolution has additionally offered some unique solutions beyond applications that improve enzyme thermostability, activity, regioselectivity, and other properties. Using *Candida antarctica* (CAL-A) as starting point, Bornscheuer et al., [10] created a highly trans-selective variant for the efficient removal of trans-fatty acids from partially hydrogenated vegetable oils. Through rational design and site-target mutations, they achieved trans-over-cis selectivity (elaidic to oleic acid) from 2.5 for wild-type (WT) to 15 for the best mutant; by narrowing down the fatty-acid-binding tunnel, the releasing rate for cis-fatty acid binding is significantly decreased. In another example, directed evolution of a cyanobacterial D9 fatty-acid desaturase (DSG) from *Synechococcus elongates* and transformation in yeast and Arabidopsis seed, Bai et al., [11] demonstrated that a single amino acid change, Q240R, yielded a nearly 25-fold increase in total desaturation in *S. cerevisiae*. Several other variants of the protein sequence with multiple amino acid changes increased total desaturation more than 60-fold! Natural lipases are often rapidly inactivated by the high methanol concentrations used for biodiesel synthesis, but Korman et al., [12] used directed evolution to develop a new enzyme called Dieselzyme 4. Directed evolution resulted in a *Proteus mirabilis* lipase variant with 13 mutations after 4 generations of directed evolution. Dieselzyme 4 has greatly improved thermal stability, with a 30-fold increase in the half-inactivation time at 50°C relative to the wild-type enzyme. The enzyme has a high methanol tolerance, showing a 50-fold longer half-inactivation time in 50% aqueous methanol.

Evolution is based on linkages between genotype and phenotype that arise due to the surrounding of the genetic materials with a barrier. Molecular breeding applies biological tools to change an organism's genome to a preferable phenotype. By applying the principles of breeding and *in vitro* evolution, Arnold et al., [13, 14] has used lab evolution to diversify carotenoid biosynthetic pathways, create new synthetic pathways, and produce more than 30 carotenoids never before seen in nature.

MOONLIGHTING ENZYMES AND PROMISCUITY

More than 2,000 classes of enzymes have been discovered in organisms that inhabit ecological niches across extreme environmental conditions. These have provided a great wealth of capability and diversity in catalytic functions. In addition to their main activity, many enzymes also have other weak "moonlighting" catalytic functions, called promiscuty [15], and this property can constitute the basis for enzyme engineers to develop new activites.

Since proteins are composed of 20 natural concanical amino acids, the sequence-space for a protein of 100 amino acids (most proteins consisted of 200–400 amino acids), is an astronomical number, up to 10^{130} possible structures (combinations) [16]. Nature solves this problem in a time span of billions of years. For an effective lab evolution, a smart strategy must be adopted.

People have compared "directed evolution" in a lab to an "adaptive walk" on rugged lanscapes. Figure 2 depicts the NK model Stuart Kauffman has developed for local/global optima selection on a fitness landscape [17]. As indicated in the figure, any forward walk step (selected variant) is based on an improved property better than its parent variant. In contrast with this fitness model, the selections from random mutations may lead to different paths and will not necessarily reach a local optima if an incorrect strategy is taken. How, then, has Dr. Arnold achieved such a great track record in directing the evolution of enzymes for new properties and new-to-nature chemistry?

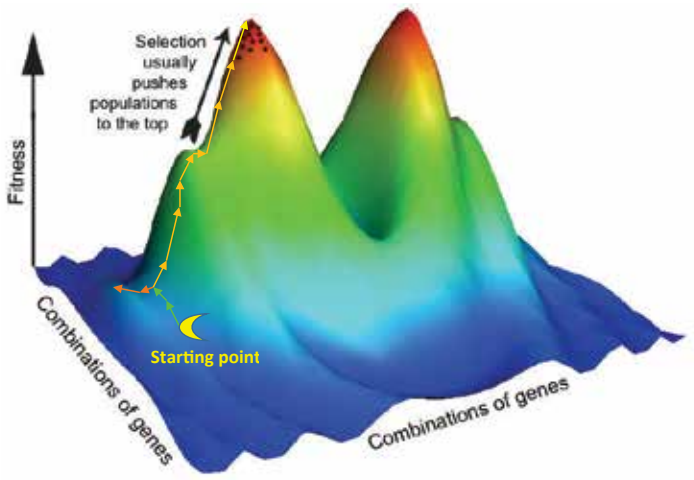


FIG. 2. Schematic of an enzyme "directed evolution" pathway based on a "adaptive walk" uphill toward the top of a local peak on fitness landscape

MY EXPERIENCE WORKING WITH A NOBEL LAUREATE

There is no doubt that Dr. Arnold's well-established reputation in the field of enzyme engineering has given her an advantage in building a strong team. Yet, it is her philosophy of choosing people who are strongly motivated to challenge the capabilities in their research area that drives success in her lab. As she repeatedly mentioned after she won the Nobel Prize, fearlessness and courage were important considerations when she chose a freshman who knew nothing about protein engineering to be on her team—because the young woman fearlessly chose the most challenging project so they would know whether it would work or not. Fearlessness and courage are what give the people on her team the motivation to take on scientific challenges and learn what they need to solve new problems.

Another hiring strategy that has helped her build an exceptional team is to choose candidates from diverse research backgrounds that complement competencies the group already has. This allows the team to easily expand its research areas and to support one another when questions of an interdisciplinary nature arise.

Nobel Prize Laureate Frances H. Arnold (left) with author Zheng Guo.

In the words of Caltech President Thomas F. Rosenbaum, Caltech is a community that values "excellence, fearlessness, reinvention, seizing the big idea, trampling disciplinary boundaries, connecting fundamental understanding of nature to technological innovation." At Caltech, research excellence in each discipline makes it possible to explore big ideas at the interdisciplinary interface. This has allowed Dr. Arnold to successfully explore a completely new area at the interface between chemistry and biochemistry, while building her own excellency in enzyme engineering. In just one example, close connections with the chemistry community facilitated her development of the P450-carbnene complex, and supported the development of a series of new-to-nature reactions implemented in cell system [5–9].

Two things that Dr. Arnold emphasizes repeatedly is that innovation is from the ground, and that hard work is always the cornerstone of success. This is particularly true at Caltech, where an attitude of hard work pushes research from one success to another.

Thanks to the ground-breaking work of Dr. Arnold and many others, the scientific tools of directed evolution have been widely used in enzyme engineering. The Nobel Prize in Chemistry 2018 is a re-recognition of this technology. Unfortunately, the current exploration is still very limited—particularly when it comes to enzymes used in lipids, food, and molecular breeding in oil plants, where applications are still very limited due to concerns about genetically modified organisms (GMO). However, the future of directed evolution with respect to engineering enzymes for green process, new-to-nature chemistry, and industrial biocatalysis and biotransformation is brighter than ever.

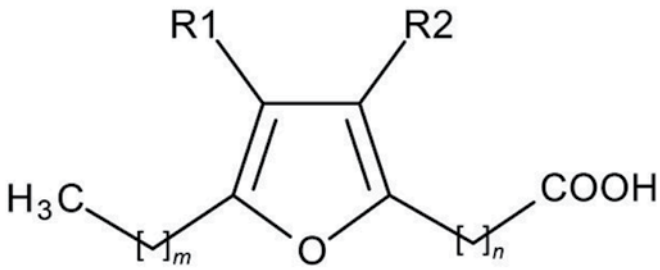
Zheng Guo is an associate professor and group leader in the Department of Engineering, Aarhus University in Denmark. He has authored more than 150 peer-reviewed papers, book chapters, and international patents. His research areas involve lipid processing and refining, design and synthesis of novel lipids, and enzyme engineering for biochemicals and new chemistry.

Furan fatty acids— a new approach

Matt Miller, Donato Romanazzi, Masashi Hosokawa, and Hajime Uchida

Furan fatty acids (F-acids) are minor lipid components in organisms, plants, and mammals. F-acids have a number of different species according to chain length on the alpha-positions of the furan ring and methylation of the ring, as outlined in Figure 1. F-acids have been identified in a number of fish species, particularly in the liver and gonads. The concentration of F-acids in liver oil from Northern pike can be as high as 5%, but the F-acids in oil from the pike's testes can be as high as 65%.

- **Furan fatty acids (F-acids) are heterocyclic fatty acids with a furan moiety in the acyl chain, and show potential as therapeutics for the treatment of inflammatory-disorders.**
- **Through research collaboration between Cawthron Institute (New Zealand), Hokkaido University (Japan), and the National Research Institute of Fisheries Science (NRIFS) of Japan, new rapid liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI MS/MS) with multiple-reaction monitoring (MRM) and time-of-flight (TOF) methods were developed for un-equivocal identification and quantitation of F-acids in marine oils.**
- **Initial *in-vitro* studies indicate that specific F-acids down-regulated mRNA expression of pro-inflammatory cytokines and may be involved in regulation of FA metabolism.**



F-acids	m	n	R1	R2	Molecular Formula	Exact mass
F1	2	8	CH_3	CH_3	$C_{18}H_{30}O_3$	294.2195
F2	4	8	H	CH_3	$C_{19}H_{32}O_3$	308.2351
F3	4	8	CH_3	CH_3	$C_{20}H_{34}O_3$	322.2508
F4	2	10	CH_3	CH_3	$C_{20}H_{34}O_3$	322.2508
F5	4	10	H	CH_3	$C_{21}H_{36}O_3$	336.2664
F6	4	10	CH_3	CH_3	$C_{22}H_{38}O_3$	350.2821
F6'	2	12	CH_3	CH_3	$C_{22}H_{38}O_3$	350.2821
F7	4	12	H	CH_3	$C_{23}H_{40}O_3$	364.2977
F8	4	12	CH_3	CH_3	$C_{24}H_{42}O_3$	378.3134

FIG.1. Structures and exact mass of the most common furan fatty acids (F-acids) found in marine oils

F-acids are considered by many to be potential marine drugs, as they have been shown to enhance anti-inflammatory action to a greater extent than omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) such as EPA and DHA (Wakimoto et al., 2011). F-acids are potent radical scavengers that, after consumption, are incorporated into human phospholipid bilayers where

they have the potential to quench radicals at the site of oxidation (Ishii et al., 1989). Consequently, there is a growing interest on the part of the scientific community and fish oil market to better understand the biological role and beneficial properties of these unique fatty acids. However, the therapeutic benefits of dietary F-Acid acids have yet to be fully explored, as such exploration requires better methods for their identification and quantitation.

F-acids have traditionally been analyzed by gas chromatography-mass spectroscopy (GC-MS) or, more recently, multi-dimensional GC-MS. The GC-MS methodology has some disadvantages, including: 1) large quantities (>100mg) of sample are needed, 2) the sample preparation/concentrations steps are labor/time-intensive, 3) co-elution with monounsaturated fatty acids complicates the GC analysis, and 4) the GC analysis requires 30–60 minutes. One of the biggest hurdles in the analysis of F-acids is the lack of commercial reference materials or standards. The F-acids are highly sensitive to acidic conditions, which causes the furan ring to open/break. So, while abundant, these marine lipids are often not detected and/or reported due to their poor stability.

"THE LC/MS-MS METHOD PROVIDES SELECTIVE DETECTION OF FURAN FATTY ACIDS IN MARINE OILS WITHOUT THE NEED FOR LENGTHY SAMPLE PROCESSING BY REMOVING THE PRECIPITATION STEP AND REDUCING THE RUN TIME FROM OVER AN HOUR TO 8 MINUTES."

For the past two years, the Cawthron Institute (New Zealand) has been engaged in an international research collaboration with the Hokkaido University (Japan) and the National Research Institute of Fisheries Science (NRIFS) of Japan. Through this research program, we have developed novel liquid chromatography-multiple reaction monitoring (MRM) and time-of-flight (TOF) tandem mass spectrometry (LC-MS/MS) methods that have allowed un-equivocal identification and quantitation of F-acids in marine oils (Uchida et al., 2018). A total ion chromatogram (TIC) of the total fatty acids prepared from chum salmon testicular lipids by HPLC/negative ESI-Q-TOF-MS and the extracted ion chromatograms for each deprotonated molecule ([M – H]– ± 0.01 m/z) of furan fatty acids (F2–F8) can be seen in Figure 2. The collaboration has also yielded a higher throughput LC- electrospray ionization (ESI) MS/MS method which is routinely used at Cawthron Institute, and which will be fully described in an upcoming publication.

The LC/MS-MS method provides selective detection of F-acids in marine oils without the need for lengthy sample processing by removing the precipitation step and reducing the run time from over an hour to 8 minutes. This increases the number of samples that can be run per day and, due to the high resolution of the two LC-MSMS methods, a limit of quantitation (LoQ) of around 5 ng/mL can be achieved. We have shown that the short-term precision of this method was 4–10% relative standard deviation (RSD) for individual F-acids with an overall RSD of 7% for total F-acids.

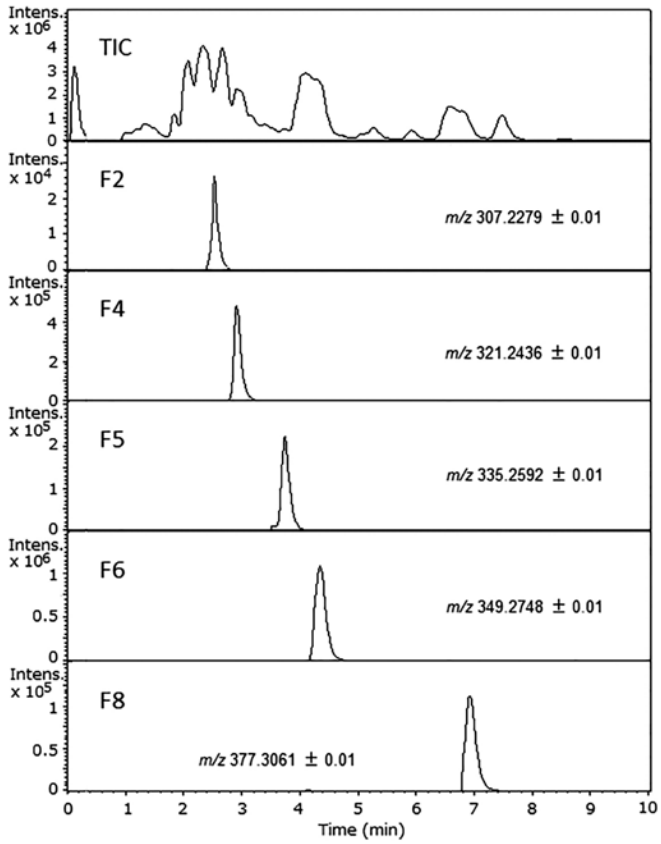


FIG. 2. Total ion chromatogram (TIC) of the total fatty acids prepared from the chum salmon testis lipids by HPLC/negative ESI-Q-TOF-MS and the extracted ion chromatograms for each deprotonated molecule ([M – H]– ± 0.01 m/z) of furan fatty acids (F2–F8).
Reprinted with permission from Uchida *et al.*, *Food Chem. 252*: 84–91, 2018.

Further reading

Ishii, K., H. Okajima, Y. Okada, and H. Watanabe, Effects of phosphatidylcholines containing furan fatty-acid on oxidation in multilamellar liposomes, *Chem. Pharma. Bull. 37*: 1396–1398, 1989.

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Teixeira, A., R.C. Cox, and M.R. Egmond, Furan fatty acids efficiently rescue brain cells from cell death induced by oxidative stress, *Food Funct*. *4*: 1209–1215, 2013.

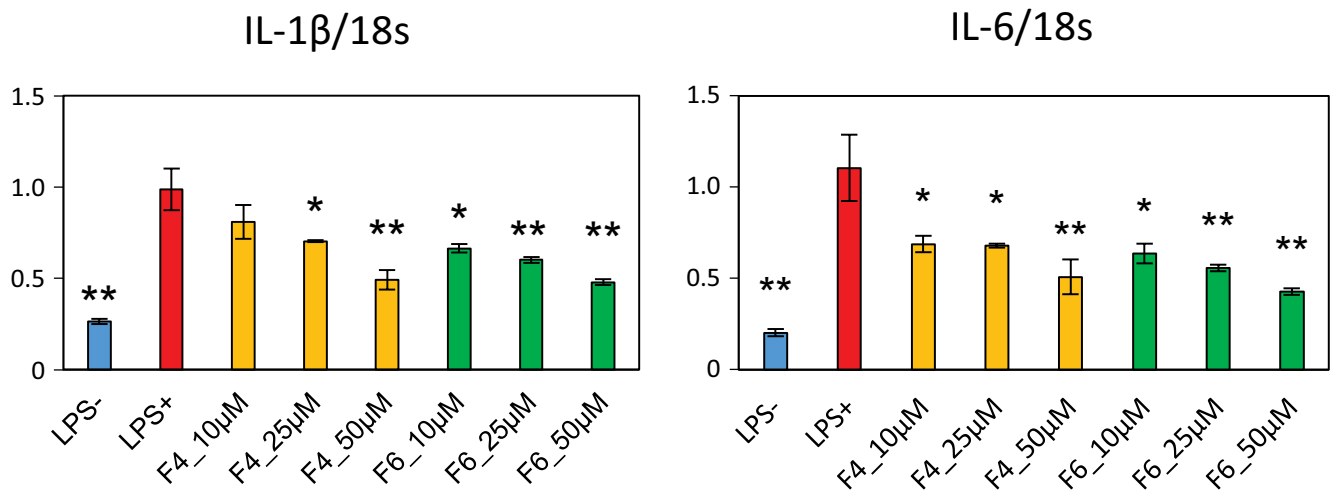

FIG. 3. The mRNA expression of pro-inflammatory cytokines IL-1β/18s and IL-6/18s in lipopolysaccharide (LPS) stimulated RAW264.7 macrophage-like cells in the presence of the F-Acid F4 and F6 at three doses. (P < 0.01, *P < 0.05 vs LPS+)**

We further investigated the anti-inflammatory effects of F-acids. We made a big point of measuring the mRNA expression of pro-inflammatory cytokines in activated RAW264.7 macrophage-like cells treated with F-acids, because macrophages play a crucial role in inflammation, and excessive production of pro-inflammatory mediators cause cell and tissue damage. Purified 10 microM F-6 acid methyl ester significantly down-regulated interleukine (IL)-6 and IL-1beta mRNA expressions in lipopolysaccharide-stimulated RAW264.7 cells (Fig. 3). Our results indicated for the first time that F-4 acid also down-regulated IL-6 and Il-1beta mRNA expression.

The anti-inflammatory effect of F-6 ethyl ester had been previously reported in an *in vivo* model of adjuvant-induced arthritis (Wakimoto et al., 2011). The protective effect of F-6 acid methyl ester against oxidative stress was also reported in C6 astroglioma cells (Teixeira et al., 2013). These findings demonstrate the high potential of F-acids in the suppression of inflammation and protection of oxidative stress as health beneficial functions.

"FURAN FATTY ACIDS ARE HIGHLY SENSITIVE TO ACIDIC CONDITIONS, WHICH CAUSES THE FURAN RING TO OPEN/BREAK. SO, WHILE ABUNDANT, THESE MARINE LIPIDS ARE OFTEN NOT DETECTED AND/OR REPORTED DUE TO THEIR POOR STABILITY."

Did you know?

Furan fatty acids can also be found in the lipids of land-based foodstuffs and plants, with grass being the main source for their presence in milk fat and butter. They are also present in human lipids and blood. Animals cannot synthesize F-acids, so their presence in animals is based on uptake and accumulation from plants.

Overall, F-acids show potential as bioactive minor components of marine oils. The new LC-MSMS methods we have developed will aid in their identification and quantification. Further work is needed to determine the efficacy of these compounds in *in vivo* and *in vitro* inflammation models to help understand F-acids role and their effects on nutrition and health. Finally, the development of certified analytical standards is greatly needed for accurate quantitation.

Matt Miller is a marine lipid chemist, president of the Australasian section of the AOCS, and is hosting the World Congress on Oils and Fats in Sydney, Australia, February 2020 (wcofsydney2020.com). He can be contacted at Matt.Miller@cawthron.org.nz.

Donato Romanazzi is the Industry Research Liaison for Cawthron's Analytical Sciences in Nelson, New Zealand. He works with industries and research organisations to develop new science projects. He can be contacted at Donato.Romanazzi@cawthron.org.nz.

Masashi Hosokawa is a professor at Hokkaido University, Faculty of Fisheries Sciences, Japan. His fields of research are molecular nutrition of marine lipids and carotenoids and production of highly functional lipids by biocatalysis. He can be contacted at hoso@fish.hokudai.ac.jp.

Hajime Uchida is an analytical chemist of marine toxins who works at the National Research Institute of Fisheries Science (NRIFS), Japan Fisheries Research and Education Agency (FRA). He can be contacted at huchida@affrc.go.jp

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—LEN SIDISKY, R & D MANAGER, MILLIPORESIGMA, PENNSYLVANIA, USA

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Are insects likely to become a significant source of proteins and oils?

Olio is an Inform *column that highlights research, issues, trends, and technologies of interest to the oils and fats community.*

Rebecca Guenard

Locust and crickets have a fat profile suitable for cosmetics; black soldier flies do not. An article in the June 2018 issue of the *Journal of Cosmetic Science* describes how some insects have more potential as a skincare ingredient than others. On the food front, insects are gaining popularity as an alternative protein as food manufacturers look to replace meat-based ingredients. With regulatory status and consumer acceptance being potential hurdles, are insects likely to become a significant source of cosmetic and food ingredients?

Fats and oils are a major component of cosmetics. Linoleic acid heals dryness. Triglycerides soften skin. Skincare formulators rely heavily on mink as a source of these ingredients, but consumer discomfort over ethical treatment of mink for fur extends to this secondary product. Macadamia nut oil has a similar fatty acid profile, but competition with food use limits its application in cosmetics. Insects could be a viable alternative.

A team of scientists at Thomas More University College, Geel, Belgium, and the University of Antwerp, Antwerp, Belgium, raised black soldier flies in the lab and purchased frozen locust and crickets from local venders. They collected the fats from the insects by drying them in an oven, grinding them to a powder, and extracting the fat with petroleum ether (for larger volumes of dried material they used hexane). The insect fats contained shelf-life limiting phospholipids, which were removed with a degumming procedure. After further purification, the researchers ran a gamut of tests on the insect fats to determine spreadability, viscosity, and fatty acid profile. They also mixed the fats into a hand cream for comparison with creams made from mink- and plant-derived fats.

The researchers determined that, based on fatty acid content, cricket and locust fats are more suitable for use in cosmetics than black soldier flies. The flies have a high lauric acid content that may adversely affect skin lipid structure. On the other hand, the authors suggest that the flies could be used in soaps or shower gels, since their fatty acid profile mirrors those used in surfactants—although cleansers derived from flies may be a tough sell for consumers. In addition, when the researchers stirred cricket or locust fats into the formulation, the product darkened and emitted an odor.

These unfavorable outcomes revealed that more science is needed before insects become a valued cosmetics ingredient. The paper's authors noted that with further refining locust and cricket oils would lose their color and odor, concluding that insects should not be overlooked as a source of fats and oils for the cosmetics industry. By contrast, the food industry has rapidly adopted insects as a source for ingredients.

Eating insects is routine in many cultures, but it is just becoming trendy in atypical areas as consumers seek novel protein sources (https://doi.org/10.1002/aocs.12180). Insects also provide essential amino acids and other nutrition that can be ethically produced with a small environmental footprint. According to a report by the Food and Agriculture Organization of the United Nations the protein, vitamin, mineral, omega-3, and fatty acid content of mealworms is on par with fish,

and exceeds those of cattle and pigs (http://www.fao.org/docrep/018/i3253e/i3253e.pdf). The same report indicates that insect farms emit fewer greenhouse gasses and ammonia while requiring less land and water to produce an equivalent amount of protein as cattle or pig farms. In addition, when reared on organic waste, insects reduce environmental contamination.

Jarrod Goldin is president and co-founder of Entomo Farms, a cricket supplier in Ontario, Canada, that rears over 100 million insects a month. He says that in 2018, the company sold out of their supply of cricket powder and whole-roasted crickets. Consumers who strive for a healthful and sustainable lifestyle move past the ick-factor quickly, according to Goldin. "They define 'icky' or 'yucky' food as food that promotes diabetes, obesity, or heart disease," he says.

Bug-centric dishes are moving from the health and environmentally conscious to the mainstream, popping up on menus in restaurants, and even at ballparks. Toasted grasshoppers sell out at Seattle Mariners baseball games in Seattle, Washington (https://tinyurl.com/yd644fz3), and restaurant-goers in Belgium and the Netherlands can enjoy a burger made from buffaloworm and soy protein (https://tinyurl.com/y8r3xgay).

Bringing an insect dish to market is not without challenges. When the company that produces the insect burger, Bugfoundation, was founded in Osnabrück, Germany, in 2015, they made their burgers in Germany, but could not sell them there. Bugfoundation burgers were initially sold only in Belgium and the Netherlands, because regulations in those countries were more accepting of insects for human consumption.

References

Insects as an Alternative Source for the Production of Fats for Cosmetics, Verheyen, G. R., *et al*., *J. Cosmet. Sci.,* 69, 187–202, May/June 2018.

Applications of Insect-Derived Protein Ingredients in Food and Feed Industry, Lamsal, B., *et al*.,

J. Am. Oil Chem. Soc., Volume 95, Issue 8, pg. 875, 2018.

In 2015, the European Union updated its Novel Food Regulation to include "whole insects and their parts," eliminating the discrepancies in oversight among countries. Before the update, some countries within the EU were tolerant of insect ingredients, while others required premarket authorization. Now, throughout the European Union, all insect ingredients must now be authorized before going on the market. Bugfoundation's insect burger was temporarily grandfathered under a transition agreement that allows food sold legally prior to January 1, 2018, to remain legal until the manufacturer files for authorization. Under the new regulation, Bugfoundation is able to sell their insect burgers as frozen patties in Germany grocery stores.

Currently, five applications have been filed (two for cricket species, two for different mealworms, and one for mealworm larvae), but the EU has yet to add any insect to its list of authorized food.

In North America, edible insect regulation is less daunting. "Canada has specifically ruled that insects have a long enough history of safe use that they do not have to be declared a novel food. There is no need to prove its safety," says Goldin. In the United States, insects that are used for human food must be specifically grown for human consumption and cannot be wild-harvested. "Those are basically the two restrictions, because insects are already allowed in the food system in the US," Goldin says.

Entomo Farms increased its insect supply for 2019 to meet customer demands. Goldin says that 85 percent of the company's product is targeted for human consumption, but they are seeing significant growth in other markets, such as pet food and agriculture.

As consumers become accustomed to insects as ingredients and regulators update laws to meet this growing trend, food and cosmetics manufactures are more likely to introduce them into their products. It's like a California roll, Goldin explains. Before you know, it you are trying more and more sushi.

Olio is produced by Inform*'s associate editor, Rebecca Guenard. She can be contacted at rebecca.guenard@aocs.org.*

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Dealing with polymers under REACH

Regulatory Review is a regular column featuring updates on regulatory matters concerning oils- and fats-related industries.

Paul Ashford

In a world that is more sensitive than ever to the consequences of plastics accumulating in the environment, the regulatory focus has turned again toward polymers. Even the environmental NGOs recognize that these have important societal functions and that concerns should be focused on single-use plastics, where the throw-away culture is seen at its worst.

The simple point now being made is that there is no such place as "away." The basic inertness and longevity of most widely used thermoplastics make our decisions on material selection and disposal highly significant. There are increasing calls for a circular economy, but this is not yet happening to the extent it should, primarily because of the lack of regulation or financial incentive.

Taxation is being considered on single-use plastics to counter this, but the situation is made even more difficult by the complexity of the polymer arena. The number in commerce is estimated at between 400,000 and over one million.

The environmental fate of polymers is a major focus of attention in this situation, with particular concern emerging about microplastics and microfibers. While these are almost intractable issues when all sources are considered, regulators have realized that they can at least target the intentional addition of plastic microbeads to consumer-facing products in the cosmetics and personal care sector, where they are used as viscosity modifiers and application enhancers.

More surprising to the European polymer industry and its supply chain has been seeing REACH used as a vehicle for the introduction of a proposed Restriction in this area. Echa will be making a recommendation on this as early as January 2019. This follows a call for evidence and additional exchanges with industry earlier this year.

Part of the surprise has arisen from a chemical regulation in effect being leveraged to address a physical environmental challenge. We have seen similar trends in the use of REACH to address the physical characteristics of materials in human health, with the classification of hazards relating to particulates with poor solubility. However, this raises the question about whether a substance-based regulation like REACH can be used to address classes of materials like plastics and particulates.

POLYMER IDENTITY: THE CHALLENGE FOR REACH

As regulators consider how to accommodate restrictions on microplastics within REACH, the discussion on what one is has moved center-stage. Debate on the definition is still ongoing, but most of it has focused on physical form. Little attention has been paid to the chemical composition outside of biodegradability and source (natural vs. synthetic), both of which are polymeric properties rather than identifiers.

Indeed, allocating polymer identifiers has been a challenge for the world's regulators for several decades now. The introduction of a global definition for a polymer—by the OECD in 1993 – has only added to the complexity of the challenge. One of the unintended consequences was the creation of a class of substances previously considered to be polymers, now being classed as "no longer polymers" (NLPs).

Most polymers in commerce are sold as mixtures: thermoplastics have additives like antioxidants, UV-stabilizers, plasticizers, and flame retardants, while thermosetting (reactive) polymers are likely to have solvents, catalysts, and other reactive species to facilitate further polymerization at later stages of the value chain. The OECD definition only addresses the polymeric component of such mixtures and speaks in terms of "monomer units" (the reacted form of the monomer) rather than the monomers themselves. This is consistent with other legal rulings under REACH, which have considered them as "substances in their own right" until they are polymerized.

RECONCILING POSITIONS

Some other world regulatory regimes have historically glossed over the polymer identity question by avoiding the need to distinguish between polymers as defined by the OECD and polymeric mixtures as sold on the market. REACH, however, forced a more rigorous investigation because of the need to decide what a "polymer substance" is in order to align with the Regulation's approach at substance level.

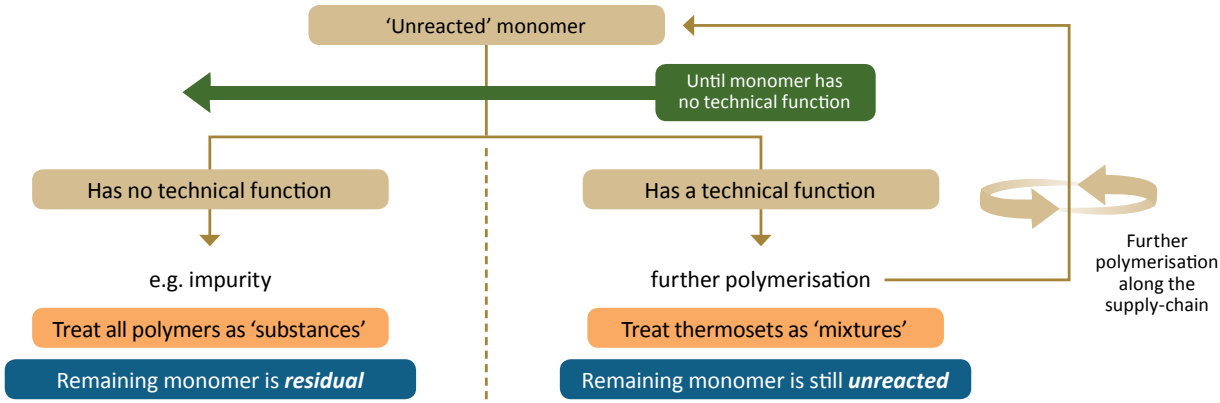

FIG. 1. Distinction between a "residual monomer" and an "unreacted monomer"

Echa and industry consulted on this when the agency sought to draft its original polymer guidance back in 2007. The dominant voice at that time was that of the thermoplastics industry.

It was understandably less concerned about residual monomers in its polymers, since these were typically present at very low levels in otherwise high molecular weight (MW) polymers. As such, they were considered to be impurities and, in line with the REACH substance definition, part of the polymer substance. The guidance therefore followed this template.

The same argument could be applied to fully cured thermoset matrices where the residual monomers would be at very low levels too. However, typically, thermosetting resins are sold by the manufacturer to the producer of the final article. Any monomer present is there to facilitate further polymerization in the next step of the process; it is not a residual impurity but there to perform a technical function. In line with legal interpretations of the monomer as a "substance in its own right" until polymerized, it would clearly be considered as part of a mixture.

After 2007, a number of thermoset industry trade associations pointed this out to Echa and it, plus some legal precedents, caused a reissuing of the Echa polymer guidance in 2012. However, the document did not manage to reconcile the two interpretations and an uninformed reader could consider it self-contradictory.

Against this backdrop, Cefic decided it needed to facilitate some further discussions between thermoset and thermoplastic polymer companies, in order to agree a consistent narrative that reflected both scenarios and avoided any contradictions of the type outlined. Dialogue between 2012 and 2014 led to an informal agreement on the distinction between a "residual monomer" (with no technical function) and an "unreacted monomer" (with one). In the former case, this would be an impurity in the polymer substance; in the latter, it would be a component in a polymer mixture (Fig. 1).

THERMOSETS AS A REACTIVE CONTINUUM

As discussed, thermosetting resins are processed along the value chain, with the polymer mixtures typically becoming less and less hazardous as MW increases and other mixture components, such as unreacted monomers, are consumed.

Since virtually all of these resins are classified according to "mixture rules," taking into account the hazard of the unreacted monomers, there is no lack of protection along the value chain. The classification, labelling, and packaging (CLP) Regulation and related communication through the provision of a safety data sheet (SDS) ensures this. The green section of Figure 1 illustrates MW build-up until any remaining monomer has no further technical function and becomes a "residual monomer."

On polymer identity, the regulatory challenge arises because there is no current way of delineating low MW polymers from high MW polymers of the same chemistry. Einecs does not extend to the naming and numbering of polymers. This means that the only available option is the Cas number system. Since this issue emerged, discussions have taken place with Cas about the application of their system to polymer identity.

However, their response is always that the system was introduced solely to distinguish between chemistries, not to differentiate polymers by MW. Thus those who use Cas to distinguish polymers on the basis of their hazard profile do so at their own risk. Echa has already noted this situation in the context of non-polymeric chemistry, but it takes on an even greater importance where there are no alternative nomenclature options.

ONGOING CHALLENGE FOR REACH

Article 138(2) of REACH provides for an ongoing review of the "risks posed" by polymers, with a view to identifying those that display equivalent concern to other substances. This clause has acted as a trigger for a number of studies requested by the European Commission. The first was conducted in 2012 by RPA, which sought to gain an overview of the polymer sector and the hazards and risks associated with it.

In 2014, Bio-Intelligence was commissioned to assess the potential for grouping polymers (to reduce the sheer number to be considered) and criteria to define "polymers of low concern." This principle had been adopted in other parts of the world, although not with the same sensitivities about specific polymer identity issues. Bio-Intelligence was therefore able to assemble a significant body of information on approaches adopted elsewhere, although this only tangentially addressed the central issue facing REACH.

In the Commission's latest initiative, a further study has been launched to determine the criteria for "polymers of concern." This clearly plays more closely to the agenda identified under Article 138(2) but stresses even more the need for clarity on how polymer substances are identified. It will not be possible to gloss over the identity issue as may have been possible for "polymers of low concern." Much more will be at stake.

Indeed, with substance identity profiles (SIPs) being such an integral part of the REACH registration process for non-polymers, there may be much debate among polymer manufacturers and importers about sameness criteria in any resulting pre-Siefs or equivalent. To some extent, the Commission foresees this in the scope of the study, which it breaks down into three areas:

- to find criteria based on hazard properties and exposure for deciding whether a polymer is of concern or not;
- to look at ways of grouping polymers (or substance ID for polymers); and
- to find the appropriate information requirements for polymers of concern (also in the function of tonnage bands).

The consultant on this project, Wood, will need clear guidance on the substance identity question, before even starting to determine the hazard and exposure properties of the polymers being considered. There will also be a need to cross-read between information obtained on polymers "as sold" (typically mixtures, even for thermoplastics where additives are used) and the hazard profiles of the substances themselves.

CONCLUDING THOUGHTS

REACH imposes a level of substance specificity on chemicals not seen in other comparable regulations. When applied to polymers, any assessment under Article 138(2) will require very clear guidance on how a substance is identified. While thermoplastics are the dominant form in terms of volume, thermosetting and other reactive polymers are dominant in number.

Accordingly, dealing with the naming of reactive systems will be a key issue, which is likely to require going beyond the existing Cas framework.

The latest polymer study will need to address this early in its development. Although it is anticipated to run until the end of 2019, industry experience suggests that discussions on polymer identity at substance level may take much longer. The challenges are very different to those posed by the wider plastics discussion, which is focused solely on physical characteristics.

Therefore, it will be important not to confuse or conflate the two issues.

Paul Ashford is managing director of Anthesis-Caleb, a global sustainability services and solutions consultancy.

AOCS MEETING WATCH

May 5–8, 2019. AOCS Annual Meeting & Expo, America's Center Convention Complex, St. Louis, Missouri, USA.

October 8–11, 2019. 18th AOCS Latin American Congress and Exhibition on Fats, Oils and Lipids, Bourbon Cataratas Convention & Spa Resort, Foz do Iguaçu, Brazil.

November 5–7, 2019. AOCS Annual Pulse Science and Technology Forum. Courtyard by Marriott, Toronto, Canada.

November 8–10, 2019. 2nd AOCS China Section Conference: Health, Advanced Processing, and Value-Added Utilization, Zhujiang (Pearl River) Hotel, Guangzhou (Canton), China.

April 26–29, 2020. AOCS Annual Meeting & Expo, Palais des congrès de Montréal, Montréal, Québec, Canada.

May 2–5, 2021. AOCS Annual Meeting & Expo, Oregon Convention Center, Portland, Oregon, USA.

May 1–4, 2022. AOCS Annual Meeting & Expo, Hyatt Regency Atlanta, Atlanta, Georgia, 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: +1 217-693-4831).

LATAM: Emulsifiers in all food categories

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

Leslie Kleiner

Many food products require the use of emulsifiers in their formulation. I was curious about the use of emulsifiers in LATAM, and consulted the Innova database to learn about the top 15 emulsifiers across all food categories in that region. Below are the findings for all new and reformulated products within all food market segments launched between December 2013 and December 2018.

Q: What are the top 15 emulsifiers used in food products in LATAM? In how many new product launches or reformulations were they used?

The most commonly used emulsifiers—used alone or in conjunction with other emulsifier(s)—are listed below.

Name	Product number count
Soy lecithin	22,453
Distilled monoglyceride	3,879
Mono- and diglycerides	3,652
Mono- and di-glycerides of fatty acids	3,590
Glycerin	2,728
Lecithin (origin not described)	2,312
Gum arabic	1,466
Sunflower lecithin	1,400
Disodium diphosphate	1,185
Gelatin	910
Sodium citrate	760
Sodium caseinate	511
Disodium orthophosphate	380
Sorbitan tristearate	333
Sodium triphosphate	281

Q: What are the top 15 market segments and top 15 subcategories with respect to use of the emulsifiers listed (category/ product number count)?

The top market segments in descending order are: bakery (8,938), confectionery (7,774), cereals (2,205), dairy (1,381), baby and toddlers (934), sports nutrition (768), desserts and ice cream (723), hot drinks (592), snacks (542), soft drinks (423), ready meals and side dishes (319), supplements (298), spreads (288), clinical nutrition (234), and sauces and seasonings (202).

For the subcategories, the distribution is: sweet biscuits/cookies (5,682), chocolate blocks (2,468), cereal and energy bars (1,605), chocolate pieces-unwrapped (1,439), cakes/pastries and sweet goods (1,377), individually wrapped chocolate pieces (1,284), savory biscuits/crackers (812), baby formula/milk (751), other chocolate confectionery (709), cold cereal (577), dairy-based ice cream and frozen yogurt (549), baking ingredients and mixes (538), bread and bread products (529), chocolate bars (500), sports powders (481).

Q: Which are the top 15 brands with respect to emulsifier use? (includes product count)

The top 15 brands are Bauducco (331), Quaker (230), Lindt (207), Hersheys (205), Bimbo (197), Marinela (193), Great Value (184), Trident (184), Costa (172), Arcor (165), Kisses (153), Nestle (151), Pozuelo (144), Cacau Show (142), Bon o Bon (141).

Q: Where are the items from the top 15 market subcategories produced?

Most of the items are produced within LATAM; however, some are produced in North America and Europe. The breakdown (by country/number of items produced) is: Mexico (3,138), Brazil (2,935), Unites States (1,325), Argentina (1,083), Colombia (1,080), Chile (530), Costa Rica (418), Ecuador (329), Peru (329), Germany (297), Spain (281), Italy (226).

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.

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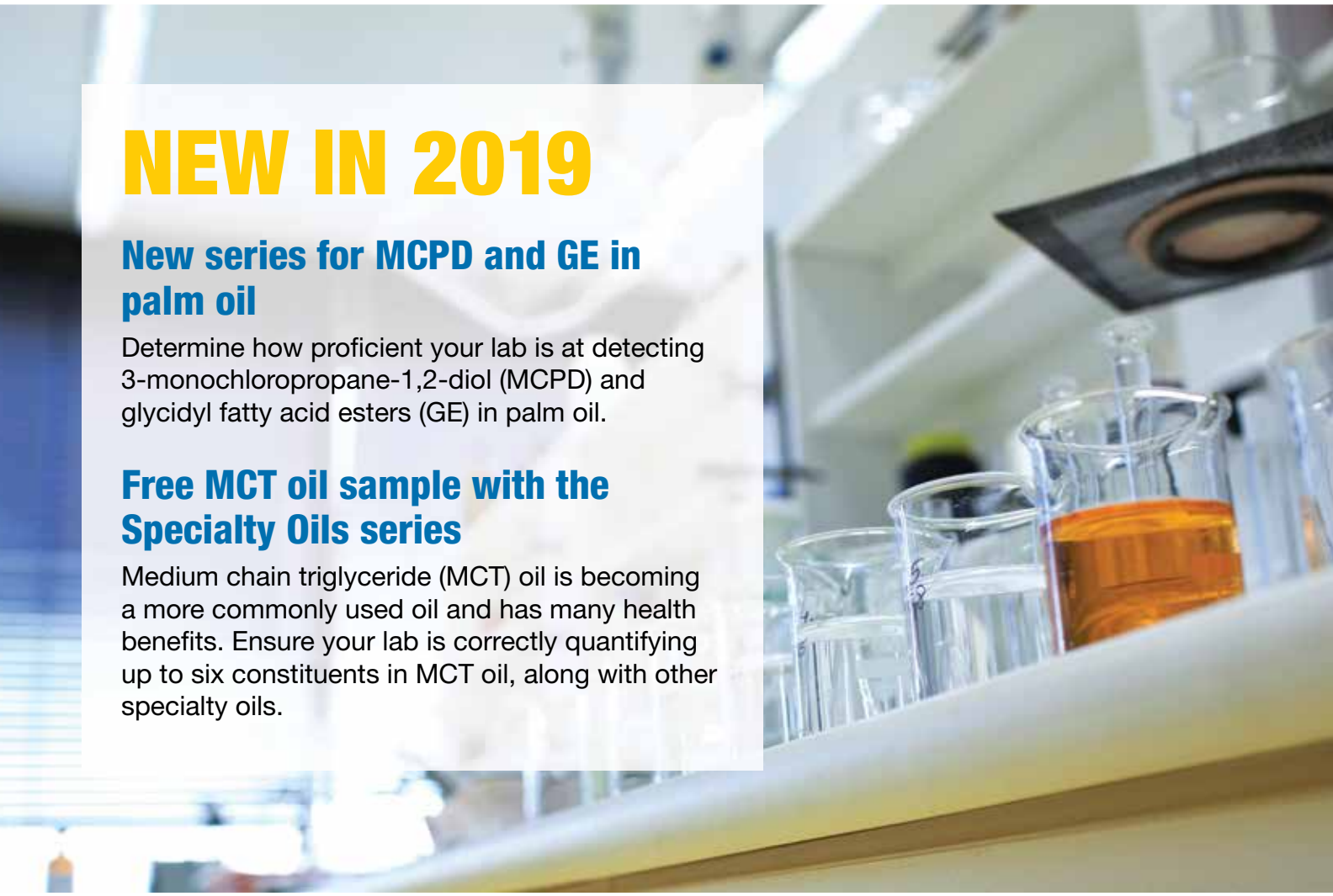

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Volunteerism and professional development

Member Spotlight is a regular column that features members who play critical roles in AOCS.

If there is anyone who perfectly illustrates how volunteering for AOCS meshes perfectly with professional development, that person may just be Susan Seegers.

On the job at Bunge North America while wearing her manager of technical operations hat, she oversees two main laboratories that support product developers and researchers dealing with oils, grains, and milled products such as flours. For AOCS, she serves in two primary capacities: as chair of the Laboratory Proficiency Program (LPP) and as chair of the Flavor and Oxidation Subcommittee of the Uniform Methods Committee (UMC).

Seegers estimates that she spends no more than an hour a week (if that) conducting her volunteer work. In addition, as both LPP chair and subcommittee chair, she attends a yearly meeting held in conjunction with the AOCS Annual Meeting & Expo (AME).

"My main responsibility for LPP is to look at all the different proficiency programs AOCS administers to see how many participants are utilizing them and to analyze which are the most effective. In a nutshell, we make sure AOCS is providing services that are needed in industry, government, and academia." Beyond that, Seegers and AOCS staff members work to predict what upcoming needs LPP participants will have.

Seegers also reviews any appeals generated by participants after their LPP results are posted. "Most often, participants appeal typos, which we don't correct. They can also appeal if they had an equipment issue or they think there was something wrong with their sample," explains Dawn Shepherd, AOCS laboratory program manager.

Seegers' work with the Uniform Methods Committee keeps her up to date on the latest work being done by analysts and researchers on flavor and oxidation. "I keep my ear to the ground for people wanting to bring forward new methods, either by presenting at the AME or to the UMC."

Seegers became involved simply by going to meetings and asking, "Can I do something?" It helped that her manager encouraged her participation. "You just have to put yourself out there and go for it," Seegers says, adding—with a laugh—that "AOCS has never said no!

"Everything that I'm doing for AOCS directly relates to my work," she notes. "Plus, chairing technical sessions at the AME provides another avenue to see what's coming and what people are talking about. It's a great way to keep current. Beyond that, volunteering is another form of networking with everyone from instrument makers to analysts with other companies to contract labs."

Fast facts

Name	Susan Seegers
Joined AOCS	2006
Education	M.S. in analytical chemistry (2010, Governors State University) and M.S. in quality assurance (2011, Southern Polytechnic State University)
Job title	Manager, Technical Operations
Employer	Bunge North America (Chesterfield, Missouri, USA)
Role in AOCS	Chair, Laboratory Proficiency Program; chair, Flavor and Oxidation Subcommittee of the Uniform Methods Committee
High-fat Indulgence	Gooey caramel brownie with ice cream
Most memorable AOCS experience	Having the chance to interact with people of differing backgrounds by being at a dinner with colleagues from 10 different countries.
Other involvement	Annual Meeting & Expo session chair, Technical Service Value Center

PATENTS

Mixture of fatty acids and palmitoylethanolamide for use in the treatment of inflammatory and allergic pathologies

Migliaccio, R., *et al*., US10149827, December 11, 2018

The present invention relates to a mixture containing up to two fatty acids selected from palmitic acid, oleic acid, stearic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, azelaic acid, and myristic acid and palmitoylethanolamide. In one embodiment of the present invention said mixture is characterized in that at least one of said up to two fatty acids is saturated. The present invention also relates to the use of the aforesaid mixture in the treatment of inflammatory and allergic pathologies.

Pouring batching device for fat-containing substances, in particular for chocolate

Veglio, D., Live-Tech SRL, US10154677, December 18, 2018

A pouring batching device (1), in particular for chocolate, in dies is described, comprising a case (2) inside which at least one cylindrical recess is obtained, in which a longitudinal shaft (7) is housed, to which at least one rotary piston (3) is fastened, means adapted to control the flow of said chocolate, wherein the case (2) is placed below a tank containing chocolate to be poured and is open towards its top, locating a hopper (8) adapted to put in contact an internal volume of the tank with the recess of the case (2), so that a mass of chocolate can easily descend by gravity inside the case (2) even without a sucking effect by the rotary piston (3).

Edible products having a high cocoa polyphenol content and improved flavor, and the milled cocoa extracts used therein

Anderson, B.A., *et al*., Mars, Inc., US10155017, December 18, 2018

Milling dry extracts containing cocoa polyphenols (CPs) to reduce the particle size improves the flavor of edible products (e.g., foods, medical foods, nutritional supplements, and pharmaceuticals) or additives containing the milled cocoa extracts. The products, e.g., chocolates, are less astringent and less bitter. The mean particle size after milling is less than about 15 microns, preferably less than about 10 microns, and most preferably less than about 5 microns. The total CP content of the milled extracts is at least about 300 milligrams and preferably about 300 to about 700 milligrams per gram of milled extract. The additives consist essentially of (i) the milled high CP cocoa extract and (ii) a fat (e.g., cocoa butter), an oil (e.g., vegetable oil), or a syrup (e.g., corn syrup).

Disorders implicating PUFA oxidation

Shchepinov, M.S., Retrotope, Inc., US10154978, December 18, 2018

Some aspects of the invention provide for a method of treating hepatic disorders, lipidemias, and cardiac-related risk factors using polyunsaturated fatty acids which are modified in certain positions to attenuate oxidative damage by Reactive Oxygen Species (ROS) and/or suppress the rate of formation of reactive products and toxic compounds.

Lipid compositions containing bioactive fatty acids

Remmereit, J., *et al*., Sciadonics, Inc., US10154979, December 18, 2018

Provided herein is technology relating to lipid compositions containing bioactive fatty acids and particularly, but not exclusively, to compositions and methods related to the production and use of structured lipid compositions containing sciadonic and/or pinoleic acid alone or in combination with other bioactive fatty acids including, but not limited to, eicosapentaenoic acid, docosahexaenoic acid, conjugated linoleic acid, and non-.beta.-oxidizable fatty acid analoges such as tetradecylthioacetic acid.

Compositions and methods for treating chronic inflammation and inflammatory diseases

Bannister; Robin M., *et al*., Infirst Healthcare Ltd., US10155042, December 18, 2018

The present specification discloses pharmaceutical compositions, methods of preparing such pharmaceutical compositions, and methods and uses of treating a chronic inflammation and/or an inflammatory disease in an individual using such pharmaceutical compositions.

Solid, heterogeneous catalysts and methods of use

Singh, I.P., *et al*., SBI BioEnergy, US10155717, December 18, 2018

Solid mixed catalysts and methods for use in conversion of triglycerides and free fatty acids to biodiesel are described. A batch or continuous process may be used with the catalysts for transesterification of triglycerides with an alkyl alcohol to produce corresponding mono carboxylic acid esters and glycerol in high yields and purity. Similarly, alkyl and aryl carboxylic acids and free fatty acids are also converted to corresponding alkyl esters. The described catalysts are thermostable, long lasting, and highly active.

Polypeptides with lipase activity and polynucleotides encoding same

Li, M., *et al*., Novozymes A/S, US10155935, December 18, 2018

Provided are isolated polypeptides with lipase activity and polynucleotides encoding the polypeptides. Also provided are nucleic acid constructs, vectors, and host cells comprising the polynucleotides, and methods of using the polypeptides.

Freeze-dried, aerated dairy or dairy-substitute compositions, and methods of making thereof

Peterson, S., *et al*., Nestec S.A., US10159261, December 25, 2018

Freeze-dried, aerated yogurt products that include dairy or dairy-substitute compositions and an emulsifier are disclosed, along with methods of producing and using same.

Biodiesel-resistant PVC/NBR rubber composition

Herbert, C.G., *et al*., Ford Motor Co. BRASIL LTDA, US10160313, December 25, 2018

A PVC/NBR rubber composition includes (A) 100 PHR polyblend (33–45 ACN) PVC/NBR, (B) 20–50 PHR conductive carbon black, (C) 1–100 PHR carbon black, (B) being more electrically conductive than (C), and (D) 5–30 PHR synthetic amorphous silica, wherein the rubber composition has electrical resistivity of less than 1.times.10.sup.6.OMEGA. measured according to SAE J2260.

Method for producing phospholipid-containing composition, and phospholipid-containing composition

Shigeeda, M., *et al*., Kaneka Corp., US10160984, December 25, 2018

A method for producing a phospholipid-containing composition which includes 10% by weight or more of phosphatidylserine based on the whole phospholipid-containing composition, a content of a polyunsaturated fatty acid being from 10 to 40% by weight based on the total amount of constituent fatty acids, the method including the following steps (1) and (2) in this order, and the following steps (3) and (4) in this order inexpensively and stably supplies a phospholipid-containing composition which includes phosphatidylserine to which a large amount of the polyunsaturated fatty acid is bonded at the 2-position thereof. Step (1): performing an esterification reaction of a polyunsaturated fatty acid with lysophospholipid using phospholipase A2 (PLA2) to obtain phospholipid. Step (2): adjusting an activity of PLA2 in the phospholipid to 10 U/g (phospholipid) or less after the step (1). Step (3): performing a base exchange reaction of a mixture including the phospholipid and serine in the presence of phospholipase D (PLD) to form a phospholipid-containing composition which includes phosphatidylserine. Step (4): separating the composition.

Method of removing impurities from natural ester, oil-based dielectric fluids

Han, S.J., *et al*., Union Carbide Chemicals & Plastics Technology LLC, US10163542, December 25, 2018

The method of manufacturing a natural ester, oil-based electrical insulation fluid by contacting refined, bleached, optionally winterized, and deodorized natural ester oil, e.g., soy oil, with an absorbent is improved by using as the absorbent a synthetic silicate absorbent comprising an alkali and/or alkaline earth metal, e.g., magnesium.

Methods of reducing or preventing oxidation of small dense LDL or membrane polyunsaturated fatty acids

Mason, R.P., Amarin Pharmaceuticals Ireland Ltd., US10172818, January 8, 2019

In various embodiments, the present invention provides methods of treating and/or preventing cardiovascular-related disease and, in particular, a method of reducing or preventing small dense LDL ("sdLDL") oxidation in a subject, the method comprising administering to the subject a pharmaceutical composition comprising eicosapentaenoic acid or a derivative thereof.

Fused gene, vector, transgenic plant, method for manufacturing vegetable fat or oil, method for constructing transgenic plant, and kit for constructing transgenic plant

Ohta, H., *et al*., Tokyo Institute of Technology, US10174333, January 8, 2019

1) A fused gene including a nucleic acid sequence which affects the biosynthesis or accumulation of neutral lipid and a phosphorus deficiency-responsive expression control sequence which is operably linked to the nucleic acid sequence and controls the expression of the nucleic acid sequence, 2) a transgenic plant which contains the fused gene, 3) a method for manufacturing vegetable fat or oil, including a cultivation step of cultivating the transgenic plant, and 4) a method for manufacturing vegetable fat or oil in which the cultivation step is a step of cultivating the transgenic plant in a phosphorus-deficient state are provided.

Patent information was compiled by Scott Bloomer, a registered US patent agent and Director, Technical Services at AOCS. Contact him at scott.bloomer@aocs.org.

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Subcritical extraction of oil from black and white chia seeds with n-propane and comparison with conventional techniques

Hrnčič, M.K., *et al.*, *J. Supercrit. Fluid. 140*: 182–187, 2018, https://doi.org/10.1016/j.supflu.2018.06.017.

Subcritical fluid extraction from Chia seeds (*Salvia hispanica* L.) using n-propane as a solvent and classical extractions using Soxhlet and ultrasonic extraction in n-hexane were performed to obtain oil-rich extract. Influence of elevated operating pressure (up to 300 bar) and temperature (40°C and 60°C) on the extraction yield and extract composition is presented. Higher solvent density contributed to higher extraction yield, which increased from 14.38% to 20.8%. Extraction kinetic curves were modelled using Brunner's equation, and the model has been proved to fit well to the results. Compositions of extracts obtained by different methods were analyzed and compared by gas chromatography (GC). Presence of palmitic, stearic, oleic, linoleic, and linolenic free fatty acid has been confirmed. The highest proportion of linolenic (almost 60%) and linoleic acid is attained in oils obtained by subcritical propane extraction.

Effect of different microwave power setting on quality of chia seed oil obtained in a cold press

Özcan, M.M., *et al.*, *Food Chem. 278*: 190–196, 2019, https://doi.org/10.1016/j.foodchem.2018.11.048.

This study was conducted to investigate the impacts of microwave heating treatments at different powers (0, 180, 360, 540, 720, and 900Watts) on the quality attributes of chia seed oil. Linoleic acid contents of the chia seed oil heated in microwave oven changed between 19.21% (900 W) and 21.17% (control), respectively ($p < 0.05$). Linolenic acid contents of heated chia seed oils varied between 66.84% (900 W) and 68.71% (control). alpha-tocopherol and beta-tocopherol contents of the chia oil samples varied between 47.71 mg/100 g (900 W) and 51.17 mg/100 g (control) to 62.58 mg/100 g (900 W) and 67.81 mg/100 g (control), respectively. While caffeic acid contents of the oils change between 0.27 mg/g (900 W) and 3.84 mg/g (control), rosmarinic acid contents of chia seed oils were found between 1.32 mg/g (900 W) and 3.17 mg/g (control). Results reflect a change in the chemical structures of the chia oil. Overall, much care should be taken when roasting chia seeds in microwave to avoid losses in the bioactive components of chia oil.

Effect of roasting treatment on the chemical composition of sesame oil

Ji, J., *et al.*, *LWT– Food Sci. Technol. 101*: 191–200, 2019, https://doi.org/10.1016/j.lwt.2018.11.008.

Sesame oil is rich in highly concentrated bioactive components including tocopherols, phytosterols, and lignans (e.g., sesamolin, sesamin, and sesamol). The chemical composition and quantification of oil extracted from roasted sesame seeds was investigated and compared with unroasted sesame oil in relation to health-promoting and potentially harmful substances (particularly polycyclic aromatic hydrocarbons, abbreviated as PAHs). With roasting, the peroxide value and color development of oils was elevated obviously while the total tocopherols and sesamolin decreased steadily. The acid values in the current experiment were expected to grow as the roasting time increased at the same temperature, and the acid value decreased in the first 30 min of roasting at 160°C. Increased roasting temperature or time facilitates sesamol formation in sesame oil. The fatty acid profiles are almost independent of roasting conditions. There was a significant increase in PAHs with elevated temperatures and extended times. In general, favorable sensory qualities accompanied by a beneficial healthy composition of sesame oil may be attributed to the treatment by roasting at a temperature from 160°C to 180°C and a roasting time of less than 20 min.

Black sesame pigment extract from sesame dregs by subcritical CO_2: extraction optimization, composition analysis, binding copper, and antioxidant protection

Bai, L., *et al.*, *LWT– Food Sci. Technol. 100*: 28–34, 2019, https://doi.org/10.1016/j.lwt.2018.10.040.

Black sesame pigment (BSP) is a natural product with good bioactivity. The massive accumulation of sesame dregs (SDs) from sesame seed processing provides a rich source of BSP. This study investigates the activity of BSP derived from SDs. A BSP yield of 3.58 ± 0.08% was achieved via extraction with subcritical CO_2 under optimal conditions. BSP effectively bound Cu *in vitro* at a pH of 7.0 (R > 90%). Furthermore, binding activity was observed in *Saccharomyces cerevisiae* (43.78% reduction in intracellular Cu, BSP at 200 microgram/mL). In addition, BSP conferred antioxidant protection in mutant yeast strains lacking superoxide dismutase and glutathione synthase by decreasing lipid peroxidation and reactive oxygen species (ROS). Superoxide dismutase and glutathione synthase protect against ROS by decreasing lipid peroxidation con-

ferred by BSP. For decreasing intracellular ROS, only glutathione synthase seemed to be involved.

Optimization of the production of structured lipid by enzymatic interesterification from coconut (*Cocos nucifera*) oil and sesame (*Sesamum indicum*) oil using Response Surface Methodology

Sivakanthan, S., *et al.*, *LWT– Food Sci. Technol. 101*: 723–730, 2019, https://doi.org/10.1016/j.lwt.2018.11.085.

Blends of coconut (*Cocos nucifera*) oil and sesame (*Sesamum indicum*) oil were enzymatically interesterified using aqueous lipase derived from *Rhizomucor miehei* and the reaction conditions, namely, temperature (45–65°C), time (16–48 h), and mass ratio of oils (CO:SO; 70:30–50:50) were optimized using Response Surface Methodology (three-factor, three-level central composite design). Degree of interesterification (DI), and the ratio of monounsaturated and polyunsaturated fatty acids (MUFA:PUFA) of triacylglycerols were used as response variables. The linear effects of all factors were significant for the DI while for MUFA:PUFA, the linear effect of oil ratio and interaction effect of time and oil ratio showed significant effects. The conditions, temperature; 57°C, time; 16 h and weight ratio of oil (coconut oil:sesame oil); 50:50 were found to be the optimum. The R2 value for DI and MUFA:PUFA ratio were 0.80 and 0.82, respectively. Models fitted for both DI and MUFA:PUFA ratio were significant with non-significant lack of fit. Produced structured lipid exhibited superior nutritional, physical and chemical properties than its raw counterparts. Therefore, the constructed models and data provide useful information to produce structured lipid from interesterification of coconut oil and sesame oil in up-scaled level. The produced novel lipid containing beneficial fatty acids from both oils could be used to produce healthy fat-based products.

Nanoencapsulation of hydrophobic and low-soluble food bioactive compounds within different nanocarriers

Rezaei, A., *et al.*, *Food Hydrocoll. 88*: 146–162, 2019, https://doi.org/10.1016/j.foodhyd.2018.10.003.

There are many hydrophobic or poorly soluble nutrients and bioactive compounds which are essential for human health, such as phenolic compounds, carotenoids, essential oils, essential fatty acids, and insoluble vitamins. The low bioavailability and sustainability of these compounds are the main challenges for their use in the pharmaceutical and food industries. Nanoencapsulation can be a favorable approach for protecting hydrophobic food bioactive compounds against unsuitable circumstances and enhance their bioavailability. In this review, several nanoencapsulation delivery systems for hydrophobic compounds, such as inclusion complexes through cyclodextrins, amylose, and yeast cells, as well as nanogels, nanoemulsions, nanofibers, nanosponges, nanoliposomes, and nanoparticles made with lipids and biopolymers are discussed. Also, the toxicity and safety aspects of the nanocarriers loaded with hydrophobic food bioactive compounds has been covered. Different studies on encapsulation of hydrophobic food bioactives have shown that by incorporating them into sophisticated nanocarriers, promising and favorable results can be achieved such as improvement in water solubility, antioxidant, and other health-promoting properties; *in vitro* gastrointestinal release profile; and better protection against process and environment harsh conditions such as light, oxygen, high temperatures, and humidity.

Why are the majority of active compounds in the CNS domain natural products? A critical analysis

Sonali, S., *et al.*, *J. Med. Chem. 61*: 10345–10374, 2018, https://doi.org/10.1021/acs.jmedchem.7b01922.

Natural products are a rich source of chemical diversity that represent years of structural optimization through evolutionary processes. If 20 natural products can generate more than 400 clinically approved CNS drugs, then think of what can happen if we could harvest and understand the natural products that have yet to be explored.

Small-molecule natural products (NPs) have a long and successful track record of providing first-in-class drugs and pharmacophore

(scaffolds) in all therapeutic areas, serving as a bridge between modern and traditional medicine. This trajectory has been remarkably successful in three key areas of modern therapeutics: cancers, infections, and CNS diseases. Beginning with the discovery of morphine 200 years ago, natural products have remained the primary source of new drugs/scaffolds for CNS diseases. In this perspective, we address the question: Why are the majority of active compounds in the CNS domain natural products? Our analysis indicates that ~84% approved drugs for CNS diseases are NPs or NP-inspired, and interestingly, 20 natural products provided more than 400 clinically approved CNS drugs. We have discussed unique physicochemical properties of NPs and NP-inspired vis-à-vis synthetic drugs, isoform selectivity, and evolutionary relationship, providing a rationale for increasing focus on natural product driven discovery for next-generation drugs for neurodegenerative diseases.

Recent advances in the medicinal chemistry of liver X receptors

El-Dien, B., *et al.*, J. *Med. Chem. 61*: 10935–10956, 2018, https://doi.org/10.1021/acs.jmedchem.8b00045.

Liver X receptors, more commonly represented as LXR, are important targets for which there are few known natural ligands. Finding a natural ligand that binds tightly to this receptor is an enviable goal, as most natural ligands do not bind well enough to be considered as promising leads.

Nuclear hormone receptors represent a large family of ligand-activated transcription factors that include steroid receptors, thyroid/retinoid receptors, and orphan receptors. Among nuclear hormone receptors, the liver X receptors have emerged as very important drug targets. These receptors regulate some of the most important metabolic functions, and they were also identified as anti-inflammatory transcription factors and regulators of the immune system. The development of drugs targeting liver X receptors continues to be a challenge, but advances in our knowledge of receptor structure and function move us forward, toward achieving this goal. This review highlights the latest advances in the development of synthetic LXR modulators in the primary literature from 2013 to 2017. In this review, we place great emphasis on the structure and function of LXRs because of their essential role in the drug design process. The structure–activity relationships of the most active and promising synthetic modulators are discussed.

Experimental evidence of the presence of bimolecular caffeine/catechin complexes in green tea extracts

Mattoli, L., *et al.*, *J. Nat. Prod. 81*: 2338–2347, 2018, https://doi.org/10.1021/acs.jnatprod.8b00168.

This study sheds light on bioavailability of caffeine from different sources and in the presence of different chemistries. The findings suggest that caffeine alone and caffeine from tea are two distinct chemical entities or complexes. This could mean that caffeine and caffeine-catechine complexes play different physiological functions—perhaps a topic of further research.

TECHNICAL UPDATE

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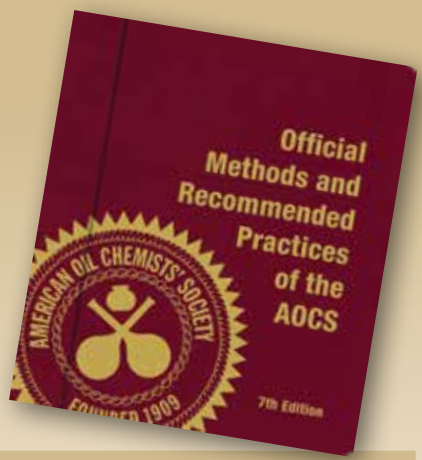

The following 5 AOCS Official Methods have been declared surplus. They will be published with an asterisk in the next edition of the AOCS Official Methods book and removed a year later. Surplus methods are still available from AOCS at https://www.aocs.org/attain-lab-services/methods/methods/search.

Method number	Methods to be declared surplus	Year of initial publication	Reason for declaring surplus
Aj 1-86	Aflatoxins in Corn, Minicolumn Method	1986	Antiquated manual method, replaced by disposable immunoaffinity tests
Ca 4-25	Soluble Mineral Matter and Fatty Acids, Combined as Mineral Soap	1925	Not likely to be used, elemental analysis more likely
Cd 5-40	Reichert-Meissl, Polenske, and Kirschner Values, Modified AOAC Methods	1940	Not likely to be used, replaced by GC methods
Tz 1a-78	Activity of Hydrogenation Catalysts	1978	Specific to Partially Hydrogenated Oil
Tz 1b-79	Selectivity of Hydrogenation Catalysts	1979	Specific to Partially Hydrogenated Oil

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A hypothesis on the peculiar pharmacological behavior of biologically active natural compounds is based on the occurrence of molecular interactions originating from the high complexity of the natural matrix, following the rules of supramolecular chemistry. In this context, some investigations were performed to establish unequivocally the presence of caffeine/catechin complexes in green tea extracts (GTEs). 1H NMR spectroscopy was utilized to compare profiles from GTEs with caffeine/catechin mixtures in different molar ratios, showing that peaks related to caffeine in GTEs are generally upfield shifted compared to those of free caffeine. On the other hand, ESIMS experiments performed on GTE, by means of precursor ion scan and neutral loss scan experiments, proved unequivocally the presence of caffeine/catechin complexes. Further investigations were performed by an LC-MS method operating at high-resolution conditions. The reconstructed ion chromatograms of the exact mass ions corresponding to caffeine/catechin species have been obtained, showing the presence of complexes of caffeine with gallate-type catechins. Furthermore, this last approach evidenced the presence of the same complex with different structures, consequently exhibiting different retention times. Both MSE and product ion MS/MS methods confirm the nature of caffeine/catechin complexes of the detected ions, showing the formation of protonated caffeine.

Chemical profiling and multivariate data fusion methods for the identification of the botanical origin of honey

Ballabio, D., *et al*., *Food Chem*. *266*: 79–89, 2018, https://doi.org/10.1016/j.foodchem.2018.05.084

Validating the authenticity and origin of natural products is an important but complex task due to the numerous variables that govern the chemical composition of a natural product grown at any given place. Specific methodologies are needed to identify the geographic origin of each product correctly. Here authors have combined different tools to get a more precise answer for honey. Similar methodologies could potentially be developed for other natural products.

The characterization of 72 Italian honey samples from 8 botanical varieties was carried out by a comprehensive approach exploiting data fusion of IR, NIR, and Raman spectroscopies; Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-MS); and electronic nose. High-, mid-, and low-level data fusion approaches were tested to verify if the combination of several analytical sources can improve the classification ability of honeys from different botanical origins. Classification was performed on the fused data by Partial Least Squares- Discriminant Analysis; a strict validation protocol was used to estimate the predictive performances of the models. The best results were obtained with high-level data fusion combining Raman and NIR spectroscopy and PTR-MS, with classification performances better than those obtained on single analytical sources (accuracy of 99% and 100% on test and training samples, respectively). The combination of just three analytical sources assures a limited time of analysis.

Protective effect of beta-lactoglobulin against heat-induced loss of antioxidant activity of resveratrol

Guo, Y. and P. Jauregi, *Food Chem*. *266*: 101–109, 2018, https://doi.org/10.1016/j.foodchem.2018.05.108

Heat destroys or reduces the efficacy of many products. Resveratrol is an important multifunctional compound implicated in many physiological functions. A complex with beta-lactoglobulin that improves reservatrol's water solubility and protects it from heat is a welcome finding, as these enhancements make reservatrol more available for physiological functions in animals and humans. This study may also suggest that natural compounds work better when used together with other chemicals, which reinforces the case for natural botanical extracts in herbal medicine.

Resveratrol exhibits many health benefits; however, low water solubility and instability under processing conditions such as heating can be some of the main challenges for its processing and formulation. Here the complexation of beta-lactoglobulin (β-Lg) with resveratrol was investigated to improve its solubility and stability. The solubility of resveratrol in water was determined as 7 mg/100 ml. Resveratrol-β-Lg nanoparticles (181.8 nm) were produced at pH 6 and 75°C for 45 min. Heating resveratrol solutions at 75°C for 45 min resulted in isomerization of resveratrol and reduced antioxidant activity. However, resveratrol-β-Lg nanocomplexes which had undergone the same heat treatment exhibited improved antioxidant activity. Heating under pasteurization conditions led to similar results and both native β-Lg and nanoparticles exhibited a protective effect against heat-induced chemical changes in resveratrol, resulting in enhanced antioxidant activity. Fluorescence measurements revealed strong interactions of resveratrol with both native protein and nanoparticles.

Biorefining of industrial hemp (*Cannabis sativa* L.) threshing residues into cannabinoid and antioxidant fractions by supercritical carbon dioxide, pressurized liquid and enzyme-assisted extractions

Kitrytė, V., *et al*., *Food Chem*. *267*: 420–429, 2018, https://doi.org/10.1016/j.foodchem.2017.09.080.

The legalization of hemp production under the 2018 US Farm Bill has opened the door to using the therapeutic benefits of the hemp plant and its metabolites, which are free from psychoactive components. In the coming years, more research and resources are expected to go into understanding hemp and its metabolites.

C. sativa threshing residues were biorefined by consecutive supercritical carbon dioxide (SFE-CO_2) pressurized liquid (PLE) and enzyme-assisted extractions (EAE). SFE-CO_2 at optimized parameters yielded 8.3 g/100 g of lipophilic fraction containing 0.2 and 2.2 g of cannabidiol and cannabidiolic acid per 100 g of threshing residues, respectively. The recovery of cannabinoids from

plant material was >93%. PLE gave 4.3 and 18.9 g/100 g of flavonoid-containing polar extracts, while EAE added 20.2% (w/w) of water-soluble constituents and increased the release of mono- and disaccharides by up to 94%. Antioxidant capacity of non-polar and polar fractions was in the range of 1.3–23.5 mg gallic acid equivalents/g DW and 0.6–205.2 mg Trolox equivalents/g DW, with the highest activities of PLE-EtOH/H_2O extract. The combined SFE-CO_2, PLE, and EAE reduced antioxidant capacity of starting plant material by 90–99%, showing that suggested multistep fractionation procedure is efficient in the recovery of a major part of the antioxidatively active constituents from hemp threshing residues.

Natural biomaterial-based edible and pH-sensitive films combined with electrochemical writing for intelligent food packaging

Zhai, X., *et al.*, *J. Agric. Food Chem.* *66*: 12836–12846, 2018, https://doi.org/10.1021/acs.jafc.8b04932.

Preserving written text in the wake of spoilage is a big plus that eliminates guess work.

An edible and pH-sensitive film combined with electrochemical writing was developed by using gelatin, gellan gum, and red radish anthocyanins extract for intelligent food packaging. The composite film showed an orange red-to-yellow color change in the pH range of 2–12. The tensile strength, ductility, and barrier abilities to ultraviolet (UV) light and oxygen of the films were improved as the concentration of red radish anthocyanins increased. Multicolor patterns were successfully drawn on the films by using the electrochemical writing method. The composite films, which acted as gas sensors, presented visible color changes in the presence of milk and fish spoilage, while the written patterns were well-preserved. Accordingly, this composite film with written patterns could be an easy-to-use indicator with great potential for monitoring food spoilage as a part of an intelligent packaging system.

Resveratrol alleviates rheumatoid arthritis via reducing ROS and inflammation, inhibiting MAPK signaling pathways, and suppressing angiogenesis

Yang, G., *et al.*, *J. Agric. Food Chem.* *66*: 12953–12960, 2018, https://doi.org/10.1021/acs.jafc.8b05047.

Resveratrol is a multifunctional natural product with limited use due to low water solubility and a poor understanding of its mechanisms. Various products in the market claim to alleviate rheumatoid arthritis (RA) and muscular/joint pains via MAPK, AMPK, or simply through better known antioxidant and anti-inflammatory pathways. It is possible that all of these pathways contribute to resveratrol's efficacy, but more studies are needed to

increase the compound's water solubility and stability to heat and oxygen before its therapeutic potential can be fully assessed.

Rheumatoid arthritis (RA) is a systemic autoimmune disease primarily affecting joints and is featured by chronic synovial inflammation and angiogenesis. We employed a bovine type-II collagen (BIIC)-induced Sprague–Dawley rat arthritis model and an *in vitro* RA model based on interleukin (IL)-1beta-stimulated rat synovial cells (RSC-364) to explore the preventive effect of resveratrol on RA and the underlying mechanisms. We found that resveratrol ameliorated BIIC-elicited synovitis and RA-related pathological hallmarks, such as inflammatory cell infiltration and angiogenesis in the synovial tissue. Also, BIIC-stimulated rats displayed increased serum levels of proinflammatory cytokines and reactive oxygen species (ROS), as manifested by elevated serum malonaldehyde contents combined with reduced superoxide dismutase activity. It is noteworthy that resveratrol abolished BIIC-induced ROS and inflammation, confirming the antioxidative and anti-inflammatory actions of resveratrol in the context of RA. Furthermore, immunoblotting indicated that resveratrol downregulated the increase in the levels of hypoxia-inducible factor-1alpha (HIF-1α) and that of the activated phosphorylation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase in IL-1beta-stimulated RSC-364 cells. Moreover, we observed that resveratrol-treated RSC-364 cells displayed both G0/G1 cell-cycle arrest and enhanced levels of apoptosis. Altogether, the present evidence established the preventive role of resveratrol in RA progression. Mechanistically, resveratrol inhibits MAPK signaling pathways, likely by reducing ROS accumulation, to suppress the inflammatory response and cell proliferation and to provoke cell apoptosis in the synovial tissue, along with mitigation of HIF-1α-mediated angiogenesis. Thus resveratrol appears to hold great potential for clinical translation as a novel RA therapeutic.

Industrial Applications

History of human-powered oil expeller: a literature review

Sheikh, S.M. and K.S. Zakiuddin K.S., In: Zhang B., Ceccarelli M. (eds) *Explorations in the History and Heritage of Machines and Mechanisms. History of Mechanism and Machine Science*, vol 37. Springer, Cham, 2019, https://doi.org/10.1007/978-3-030-03538-9_7.

This paper is a vibrant introduction to both traditional and improved methods of extracting vegetable oil from oilseeds. The operating parameters and mechanisms of different designs and progressive developments are reviewed to determine the scope of improvements in human powered oil expelling that can be made in rural areas.

Desolventizing kinetics of oilseed meals with superheated hexane

Faner , S.A., *et al*, J. *Food Proc. Eng.*, 2019, https://doi.org/10.1111/jfpe.12987.

The desolventization kinetics of oilseed meals with superheated solvent was investigated experimentally and theoretically. A bench-scale equipment was designed and built to measure continuously the rate of mass loss and the solid temperature during desolventization of meal particles. Experiments were carried out with sunflower and soy meals using superheated hexane vapor as desolventizing fluid and temperatures ranging from 90°C to 135°C. Experimental data were used to determine the vapor-particle heat transfer coefficient under different conditions, which compare reasonably with predictions of dimensionless correlations for solid particles. A relatively simple mathematical model to represent the desolventization kinetics of individual meal particles, based on heat transfer in a receding front system, was developed. The model was used to analyze the effect of temperature, particle diameter, shrinkage, and mass transfer on desolventizing rate. Prediction of desolventization kinetics of sunflower and soy meals compared satisfactorily against experimental results at different temperatures.

Hydrothermal catalytic processing of waste cooking oil for hydrogen-rich syngas production

Nanda, S., *et al.*, *Chem. Eng. Sci. 195*: 935–945, 2019, https://doi.org/10.1016/j.ces.2018.10.039.

Substantial amounts of waste cooking oil are obtained worldwide from household and catering enterprises because of deep-frying and other cooking activities. Supercritical water gasification is considered as an aqueous phase reforming process to produce hydrogen enriched syngas from biomass and other organic wastes. In this study, waste cooking oil was gasified at variable temperatures (375–675°C), feed concentration (25–40 wt%) and reaction time (15–60 min) to investigate their effects on syngas yield and composition. Maximum yields of hydrogen (5.16 mol/kg) and total gases (10.5 mol/kg) were obtained at optimal temperature, feed concentration, and reaction time of 675°C, 25 wt% and 60 min, respectively. At 5 wt% loading, Ru/Al_2O_3 enhanced hydrogen yield (10.16 mol/kg) through water-gas shift reaction, whereas $Ni/Si\text{-}Al_2O_3$ improved methane yield (8.15 mol/kg) via methanation reaction. The trend of hydrogen production from catalytic supercritical water gasification of waste cooking oil at 675°C, 25 wt% and 60 min decreased as $Ru/Al_2O_3 > Ni/Si\text{-}Al_2O_3 > K_2CO_3 > Na_2CO_3$. The results indicate the recycling potential of waste cooking oil for hydrogen production through hydrothermal gasification.

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