INFORM February 2021 Volume 32 (2)

# INFORM

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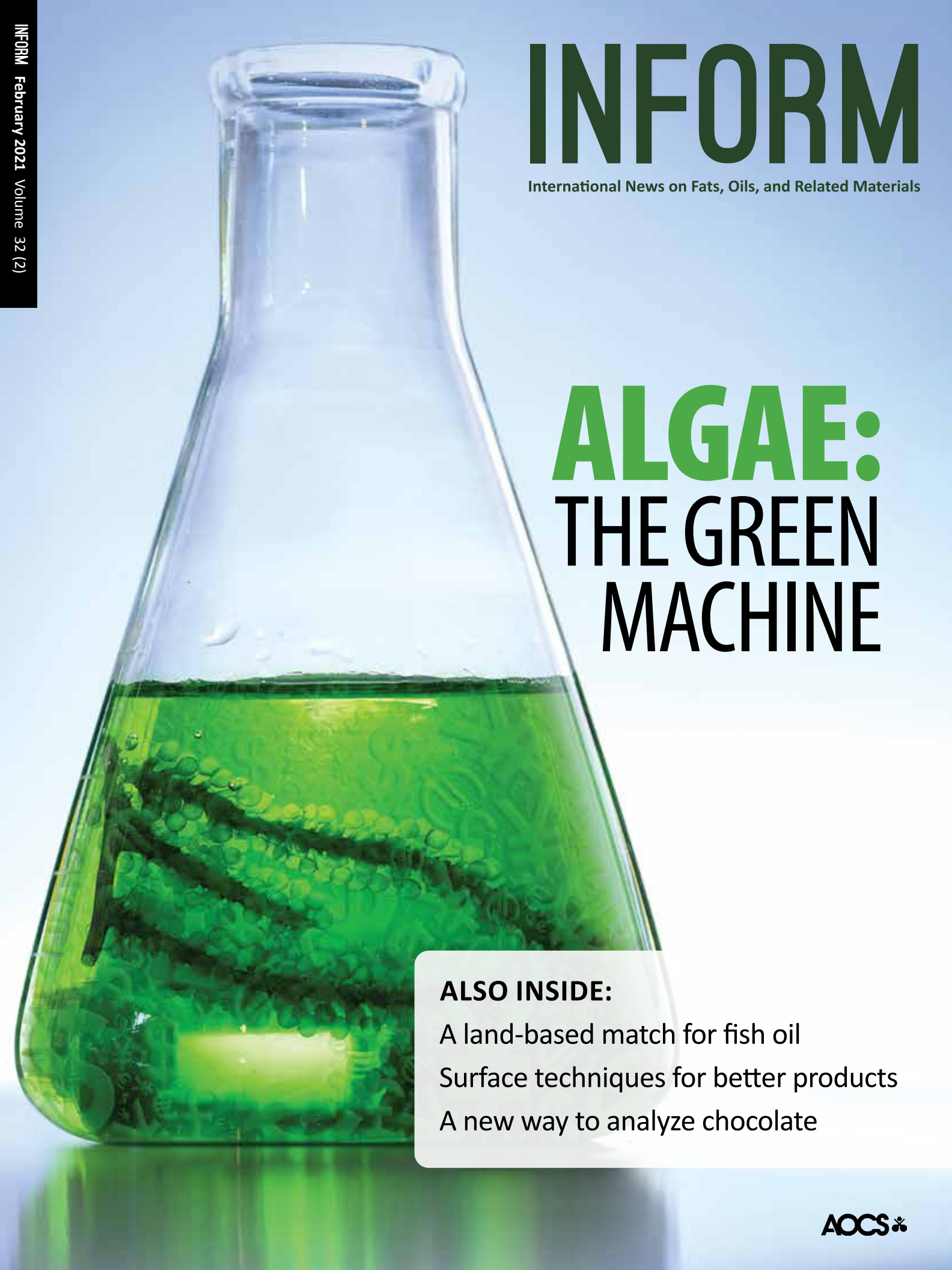

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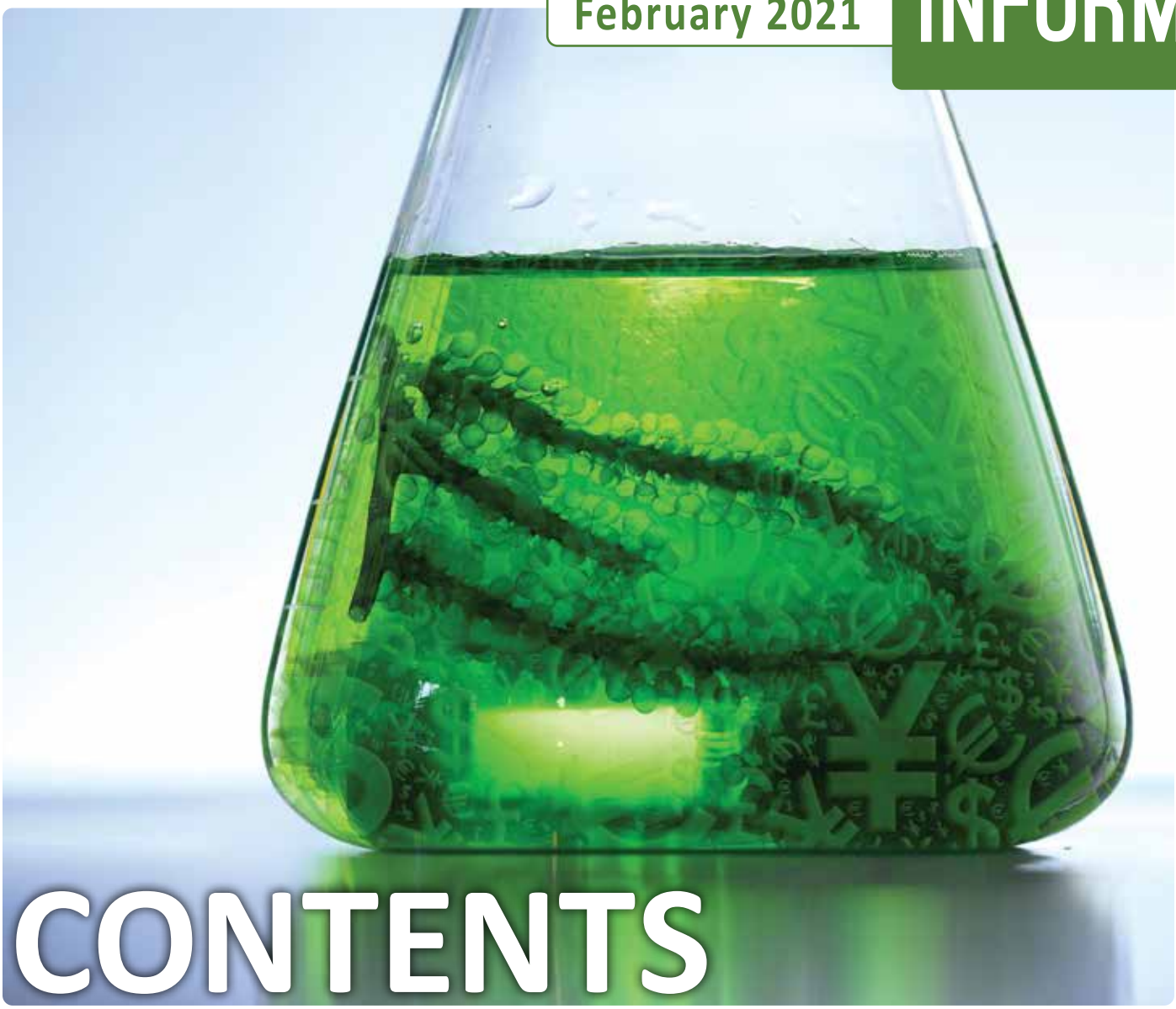

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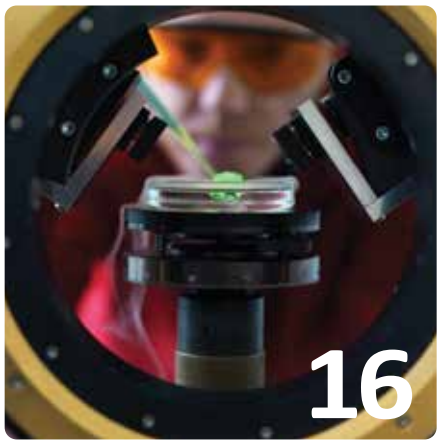


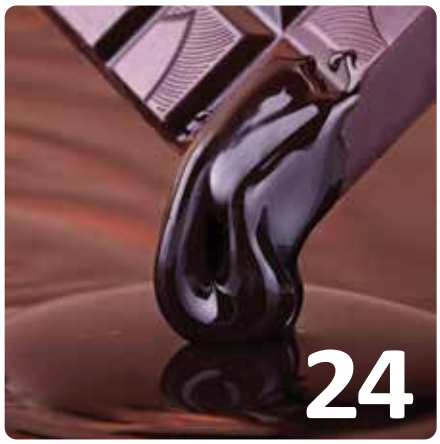





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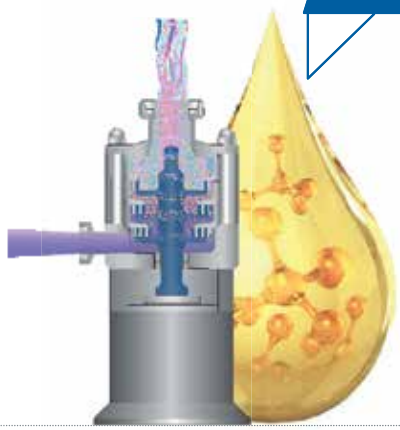

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# The green machine: commercializing microalgae products

Rebecca Guenard

Sales of plant-based foods are booming. The growing popularity of non-animal protein sources has led to their increased presence in grocery stores and fast-food restaurants. Even while venture capital investments declined due to the COVID-19 pandemic, start-ups focused on plant-based ingredients raised millions (https://tinyurl.com/plantbasedinvestments). In the Netherlands alone, there are more than 60 companies dedicated to the development of plant-based proteins (https://tinyurl.com/netherlandsplantbased). With so much lucrative potential in plant-based ingredients, some companies are trying to capitalize on algae as a unique source of protein and fatty acids.

- **Companies have tried to garner a valuable product from algae for decades; current start-up investments hint that its greatest potential is still in the food sector.**
- **Algae researchers are investigating its use as feed for agri- and aquaculture, while also continuing to optimize production of protein and omega-3s for humans.**
- **Cutting production costs will be the key to maintaining market success.**

Once primarily a niche ingredient, algae has gained recognition as a superfood. In addition to its nutritional value, algae is more sustainable than most types of agriculture. It can be grown without freshwater or pesticides and requires a tenth of the land for a comparable amount of biomass.

"Algae are the fastest growing plants," says Navid Moheimani, director of Murdoch University, Algae Research and Development Center in Perth, Australia. "You cannot find anything that grows faster than these organisms."

In the past two years, more effort has been focused on reducing the existing barriers that limit algae's use in food applications. In Europe, especially, algae is most often associated with biofuels. Western countries are less familiar with the range of algae species or their various tastes, texture, color, and aroma. Food companies are trying to bring this segment of the population onboard. Unfamiliar consumers are slowly gaining experience with algae as food thanks to the popularity of drink powders and protein bars that derive nutrition from species like chlorella and spirulina. Will algae farming finally gain a spot in mainstream agriculture? This article discusses the possibility.

## FROM FOOD TO FUEL TO FOOD

Before the routine use of ammonia fertilizers after the second World War, conventional crops produced a lower nutritional density than they do today. The excessive energy required for conventional agriculture and the spike in the postwar population led researchers to consider using algae as a food source (https://tinyurl.com/algaehistory). Algae researchers at the time were unsuccessful at finding a species that Westerners would willingly incorporate into their diets. Concurrently, research on conventional farming led to more efficient production of animals and grain. As a result, the industry shifted the purpose of algae farms to fuel production.

The advent of genomics, in the early 2000s, expanded algae's capabilities. Instead of being just a plant product, algae was viewed as a mini-factory. By modifying the DNA of single-celled species, referred to as microalgae, scientists coaxed the organisms to produce more triacylglycerols. Energy companies seized on microalgae as opportunity to offer an alternative to fossil fuels.

The reality of a depleting fossil fuel supply has since crystalized, yet biofuels have not proven cost-effective enough to gain long-term success. Nonetheless, a few energy industry hold-outs remain committed to producing fuels from algae lipids. In 2017, ExxonMobil announced that, with gene-editing, they could produce biofuels from algae at 10,000 barrels a day by 2025. However, they are the only major energy company still committed to microalgae biofuels. Other energy companies have given up on the pursuit.

Growing algae in large open-air ponds that make fuel production affordable introduces several complications (Fig.1, page 9). It is costly to stir the ponds mechanically so that all the organisms get exposed to the sun for photosynthesis. Scientists also worry that genetically altered strains may escape into the wild and cause havoc on natural ecosystems. Some means of physical containment must be assured. Ultimately, algae is just too expensive to produce at the scale needed to get enough oil for the transportation industry.

"It is very hard to compete with fossil fuels when it comes to price, because you do not pay to build them." says Moheimani. "You just extract them, crack them, and you have your products."

Most experts discourage investing resources in microalgae biofuels when they could be directed to more viable types of sustainable energy, like solar and wind (https://tinyurl.com/algaebiofuelsmyth). Research and investment dollars are now shifting back to algae production for human consumption, which has a greater potential for both public good and higher profit margins.

Start-ups with intentions to produce algae as a plant-based food ingredient are gaining millions from private equity firms. Government-funded research collaborations between public companies and academia also became popular in 2020. The Dutch Ministry of Education and Science funded a collaboration known as the "Microalgae for food" project to investigate

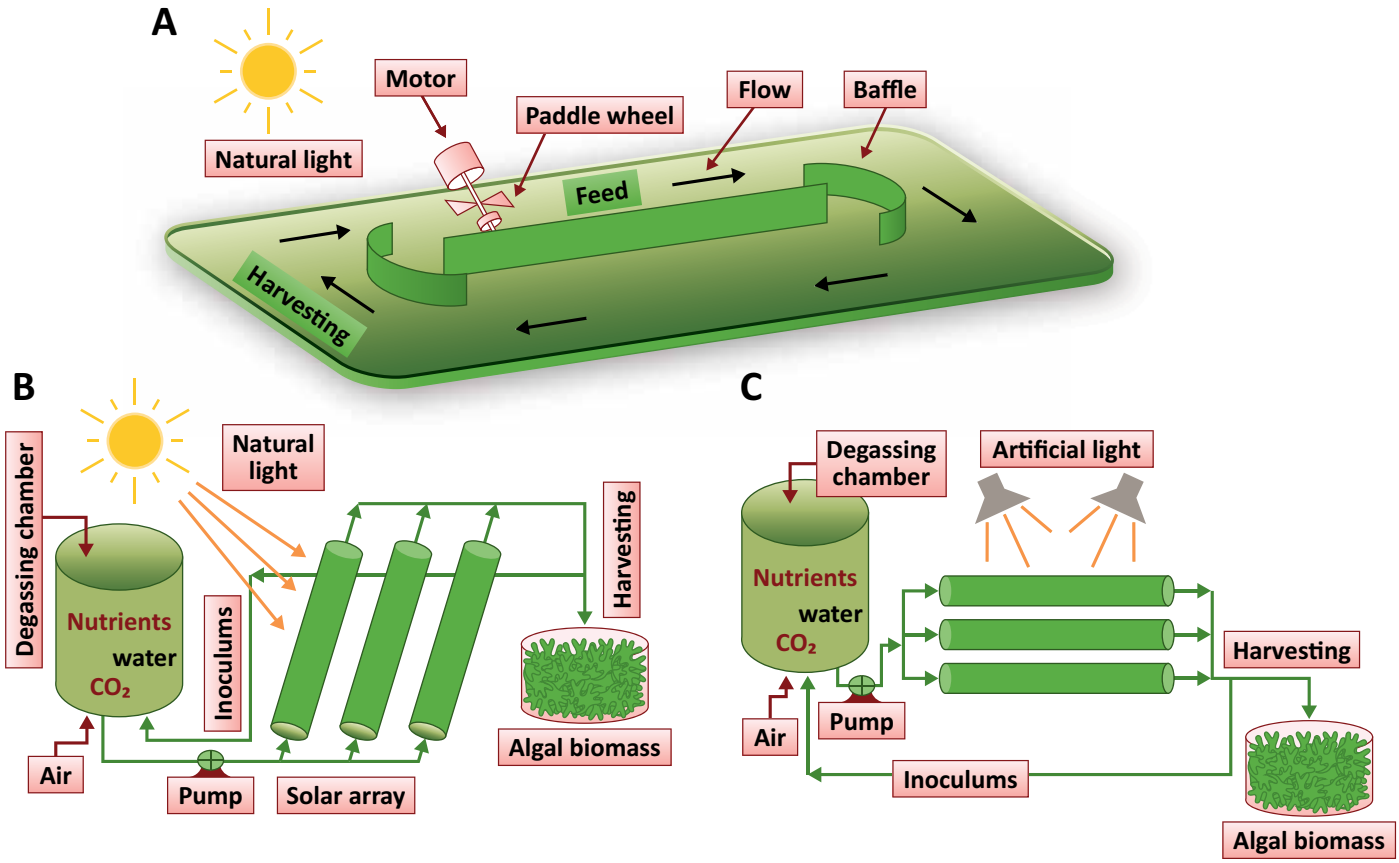


**FIG. 1. Different cultivation systems for large-scale production of algal biomass. (A) Open pond systems use natural solar light as a source of energy. Closed systems can use either (B) natural solar light or (C) artificial light as an energy source.** Source: Pathak, J., *Front. Environ. Sci. 6*:7,2018.

microalgae as a sustainable protein source (https://tinyurl.com/microalgaeforfood). Likewise, science institutes in New Zealand and Singapore are getting funding to evaluate protein production through a collaboration between their two countries. A growing number of organizations like these are investigating the many opportunities to increase algae's commercial value.

## SUSTAINABLE ANIMAL FEED

One common area of interest for microalgae experts is animal feed. Microalgal biomass contains an abundance of nutrients compared to other common feeds like corn and soybean (Table 1). Depending on the species, microalgae produce high concentrations of polyunsaturated fatty acids (PUFAs), $B_{12}$ vitamins, dietary fiber, sterols, antioxidants, and proteins (including all essential amino acids). While these are obviously crucial nutrients for humans, some researchers believe the best way to gain that benefit is indirectly—by feeding algae to animals.

"The biomass we produce contains 50% protein," says Moheimani. "Pig feed has only 5–10% protein." His group is assessing whether the microalgae could be added to the pig feed as a powder to boost its nutritional impact.

Belgian researchers are investigating whether chicken feed can be improved in a similar manner. The ValgOrize project (https://www.valgorize.eu/) was established by the European Regional Development Fund to enhance innovation in the algal sector. The primary focus of the project is on developing algae for human use, but researchers were interested in whether residues from such processing could be used as a supplement for chicken feed.

They conducted feed trials on both laying hens and broilers that included three different algae species. In a press release, the group reports that they are still analyzing the results in terms of digestibility of the feed and how it affects the taste of the resulting meat. They are also in the process of evaluating whether a 10% ratio of algae to feed is optimal as indicated by previous studies. However, they have determined that residual algae products need to be processed to reduce salt and mineral ash levels before they can be added to chicken feed (https://tinyurl.com/feedtrials).

"Without this treatment, the chicken's ability to digest the algae could be negatively affected, eventually resulting in a decreased efficient use of protein and energy," says Marta Lourenço, poultry researcher on the project.

Moheimani's team has not yet conducted feed trials for pigs. Since algae are not normally a food pigs consume, they first conducted *in vitro* studies. The study results gave the team confidence that pigs can digest algal biomass, and in the coming year Moheimani says his group will perform pig feed trials—although he feels there is more potential for microalgae use as aquaculture feed.

"Aquaculture prawns do not taste the same as prawns that come from the sea," Moheimani says. "That is because they do not eat their natural food when grown in prawn farms." He says his group is working with industrial partners to supply the

**TABLE 1. Comparison of the nutrition content in different types of commonly used animal feed.**
Source: Lum, K.K, *et al., J. Animal Sci. Biotechnol. 4*: 53, 2013.

| | Crude protein | Carbohydrates | Fat |
|---|---|---|---|
| **Soybeans** | 37% | 30% | 20% |
| **Corn** | 10% | 85% | 4% |
| **Wheat** | 14% | 84% | 2% |
| ***Anabaena cylindrica*** | 43–56% | 25–30% | 4–7% |
| ***Arthrospira maxima* (spirulina)** | 60–71% | 13–16% | 6–7% |
| ***Chlorella vulgaris*** | 51–58% | 12–16% | 14–22% |
| ***Spirogyra* sp.** | 6–20% | 33–64% | 11–21% |
| ***Synechococcus* sp.** | 73% | 15% | 11% |

algae prawns naturally prefer to eat, and to determine if algae feed improves the flavor of farmed seafood.

In three decades, the world population is projected to reach 9 billion. Feeding livestock as efficiently as possible will be essential to ensure that humans have the protein and other nutrients they need. However, in terms of omega-3s, sustainability would improve if humans went directly to the source.

## REMEDY FOR OVERFISHING

Earth will be out of seafood in 27 years, according to TheWorldCounts, a website that tabulates data associated with current global challenges (https://tinyurl.com/overfishing-stats). This statistic may represent an extreme case, but there is general agreement among scientists that the current global demand on fisheries is unsustainable (https://islandpress.org/books/oceans-2020). To reduce pressure on the fish market and capitalize on high-value omega-3s, researchers and corporations are racing to optimize microalgae's production of long-chain fatty acids.

Over the past decade, start-ups have established which microalgae species are most effective at producing omega-3s. Many of these small companies have since been acquired by the Dutch health and nutrition company, DSM (https://www.dsm.com). Production of principle fatty acids, such as gamma-linolenic acid, docosahexaenoic acid (DHA), arachidonic acid, and eicosapentaenoic acid (EPA), from microalgae are now a routine part of the billion dollar nutraceutical industry. These omega-6 and omega-3 fatty acids are primarily incorporated into infant formulas, but they have also been added to dairy products, table spreads, and mayonnaise.

Although certain species of microalgae produce 60% oil by weight (of which 40% is DHA) when fermented, solvent extraction and subsequent oil processing increases the cost of producing these omega-3s. Researchers are now focused on ways to bring microalgae production costs down.

## FERTILIZATION FROM WASTE

Algae may grow easily, but it does not grow everywhere. In the northern hemisphere, where sunlight is limited, microalgae production requires the controlled environment of closed photobioreactors. Moheimani says, in a climate like Western Australia algae can be grown more cost effectively in open ponds.

His group has optimized the value of their microalgae even further by growing the organisms on effluents from treated wastewater. "We developed a two-stage process," says Moheimani. "First, a bacterial process anaerobically digests wastewater solids, and then the contaminant-free effluent is transferred to the algae pond where it acts as fertilizer."

He says the process is not only a beneficial way to grow algae, but it could help small developing countries in Africa and southeast Asia, where water treatment to produce clean water is unaffordable. In addition, using wastewater to grow algae for profit prevents it from ending up in rivers and ground water, where it can produce a toxic algal bloom. "The future of the algae business is wastewater treatment," Moheimani says.

## *Information*

Distinct microalgae species for food—part 1: a methodological (top-down) approach for the life cycle assessment of microalgae cultivation in tubular photobioreactors, Schade, S. and T. Meier, *J. Appl. Phycol. 32*: 2977–2995, 2020.

Distinct microalgae species for food—part 2: Comparative life cycle assessment of microalgae and fish for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and protein. Schade, S., *et al.*, *J. Appl. Phycol. 32*: 2997–3013, 2020.

*Chlorella vulgaris* in a heterotrophic bioprocess: study of the lipid bioaccessibility and oxidative stability, Canelli, G., *et al.*, *Algal Res. 45*: 101754, 2020.

Chapter 19–"Microbial production of polyunsaturated fatty acids as nutraceuticals," by Ratledge, C. In *Woodhead Publishing Series in Food Science, Technology and Nutrition, Microbial Production of Food Ingredients, Enzymes and Nutraceuticals*, Editor(s): McNeil, B., *et al.*, Woodhead Publishing, Pages 531–558, 2013.

## COMMERCIALIZATION EFFORTS

Whether the algae business truly has a future ultimately depends on consumers. Many algae products have so far experienced a commercial success rollercoaster. The edible oil produced by algae fermentation that sold under the brand name *Thrive* is an example. In 2019, the Netherlands-based food ingredient company, Corbion, was initially successful in selling the cooking oil in Walmart stores throughout the United States. Then, in August 2020, the company's website read, "We are no longer selling *Thrive Culinary Algae Oil*. We were not able to achieve the commercial success needed to sustain the brand." Corbion still makes an algae oil product, called *AlgaWise*, for food manufacturers and it is considering bringing an algae butter to market in the future. For now, they have no direct-to-consumer products, but that may soon change.

Corbion formed a partnership, in 2019, with the Swiss company, Nestlé. A spokesperson for Nestlé said the companies are in a development phase and considering potential applications. "We are actively exploring the use of microalgae as an alternative protein and micronutrient source for exciting plant-based products," the spokesperson said in an email. Nestlé has already launched a few microalgae-centered products, including dog treats and human supplements.

After vexing the food industry for many years, is algae finally poised to become a mainstream source of food ingredients? The plant-based market boom and the COVID-19 pandemic might be the combination that pushes these organisms over the tipping point. Consumers aware that COVID-19 is a zoonotic disease (passing from animal to human) may be more inclined to adopt an animal-free diet, while supply-chain issues created by the virus have made shoppers even more conscientious about the traceability of their food ingredients. Finally, staying healthy and maintaining a rugged immune system are key consumer issues right now. Higher income consumers, who are less likely to have lost jobs due to shutdowns, may accept paying a little more for foods they believe offer the best health for their family. Rapid growth of the algae market is very likely in the next couple of years.

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

## AOCS MEETING WATCH

**May 3–14, 2021.** AOCS Annual Meeting & Expo, annualmeeting.aocs.org.

**October 5–7, 2021.** Plant Protein Science and Technology Forum, Millennium Knickerbocker, Chicago, Illinois, USA.

**May 1–4, 2022.** AOCS Annual Meeting & Expo, Hyatt Regency Atlanta, Atlanta, Georgia, USA.

**April 30–May 3, 2023.** AOCS Annual Meeting & Expo, Colorado Convention Center, Denver, Colorado, 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).

# Transgenic *Camelina sativa* oil: an effective source of both EPA *and* DHA in the human diet

**Annette L. West, Elizabeth A. Miles, Karen A. Lillycrop, Johnathan A. Napier, Philip C. Calder, and Graham C. Burdge**

- **Humans require both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to maintain cell function. Social and economic barriers prevent populations from achieving recommended intakes from fish, and the harvest of the primary source of dietary EPA and DHA is ecologically unsustainable.**
- **Transgenic plants which produce seed oils that contain EPA and DHA are a potential sustainable and scalable solution which can overcome the social and economic barriers to achieving recommended intakes. Most transgenic strains reported to date produce oils that contain either EPA or DHA (but not both) in appreciable amounts and so are nutritionally inadequate.**
- **A transgenic strain of *Camelina sativa* has been developed which produces balanced amounts of EPA and DHA, and is as effective as fish oil as a source of these fatty acids in the human diet. This novel crop has unique potential for meeting human nutritional needs as well as providing opportunities for agriculture and aquaculture, and for industries that require both EPA and DHA, such as the pharmaceutical industry.**

The omega-3 polyunsaturated fatty acids found in marine fish oils, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important components of the membranes of human cells and are required for normal cell function. The central nervous system, retina, and sperm are particularly enriched in DHA, while leukocytes and other tissues contain both EPA and DHA, albeit in smaller amounts. EPA and DHA influence cell function by conferring fluidity on the membrane phospholipid bilayer and by acting as substrates for the synthesis of lipid second messengers. Of particular importance, both EPA and DHA are substrates for the synthesis of specialized pro-resolving mediators, namely resolvins, protectins, and maresins, that are central to self-resolution of inflammation. Consequently, humans require an adequate supply of both EPA and DHA in their diet.

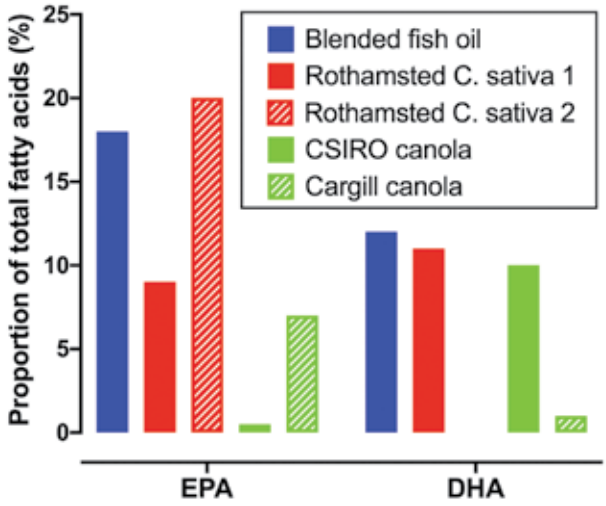


**FIG. 1. The proportions of EPA and DHA in the seed oils of transgenic plants.** Data from Tocher, *et al.*, 2019.

Despite the nutritional importance of EPA and DHA, consumption is often markedly below recommended intakes. For example, the UK Government recommends consuming 450 mg/day of EPA plus DHA to maintain health. However, typically the adult UK population consumes approximately 200 mg EPA plus DHA/day. Since EPA and DHA are only found in oily fish, and at substantially lower levels in meat and dairy foods, their consumption by individuals who follow a plant-based diet is close to zero. Further barriers to achieving recommended intakes include the cost of oily fish and fish oil and concerns about contamination with environmental pollutants. In addition, provision of EPA and DHA to meet demands places an unsustainable burden on marine ecosystems. (Salem and Eggersdorfer 2015) estimated that to provide the global population with 500 mg EPA plus DHA per day, there would be a shortfall of 1.1 million metric tons of EPA plus DHA from the marine harvest. Therefore, there is an urgent need for a sustainable and scalable source of EPA and DHA that overcomes current barriers to achieving recommended daily intakes.

The solution does not lie in increased consumption of vegetable oils which contain α-linolenic acid, because conversion to EPA is low in humans and DHA synthesis is negligible in men, although higher in women of reproductive age. It is unclear whether such capacity is sufficient to maintain adequate levels of EPA and DHA in tissues. Algal oils that contain EPA and DHA have been developed and used in infant milk formula, but they tend to have a high DHA content with small amounts of EPA. Moreover, the financial burden of upscaling the fermentation process is prohibitive such that algal oils are limited to niche applications.

Seed oils from transgenic plants that synthesize EPA and DHA are a potential solution to overcoming socioeconomic barriers to meeting recommended intakes which are sustainable and scalable to meet global demands. Four strains of plants have been developed, two based on canola and two derived from *Camelina sativa* (Fig. 1) (Tocher, *et al.*, 2019). The two transgenic canola oils developed by BASF/Cargill and the Commonwealth Scientific and Industrial Research Organisation

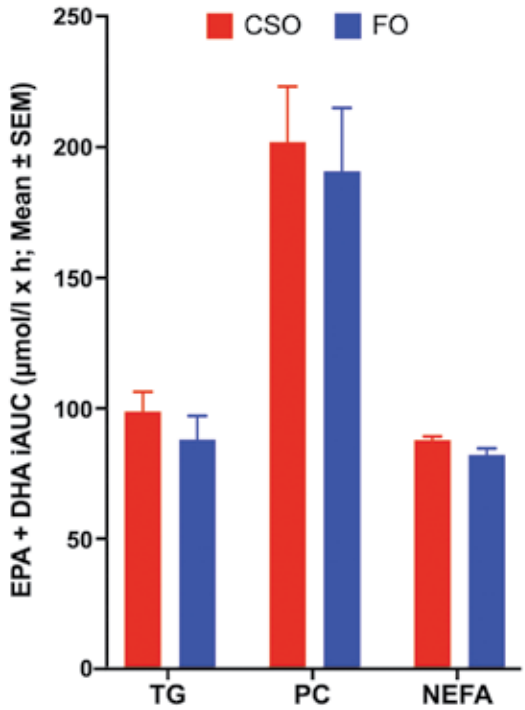


**FIG. 2. The incremental postprandial area-under-the-time x concentration curve of the incorporation of EPA + DHA into blood lipids. FO, blended fish oil; CSO, transgenic *Camelia sativa* oil; TG, triacylglycerol; PC, phosphatidylcholine, NEFA, non-esterified fatty acids.** Data from West, *et al.*, 2019.

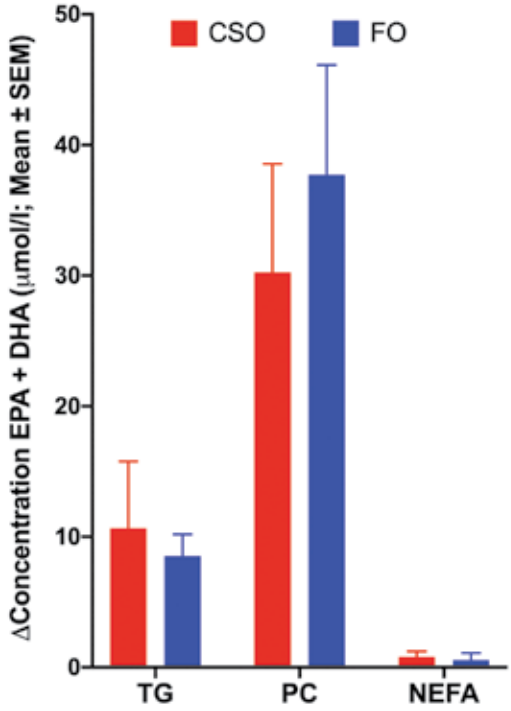


**FIG. 3. The change in EPA + DHA concentration in blood lipids after 8 weeks dietary supplementation with either FO (blended fish oil) or CSO (transgenic *Camelia sativa* oil). TG, triacylglycerol; PC, phosphatidylcholine, NEFA, non-esterified fatty acids.**
Data from West, *et al.*, 2020b.

**FIG. 4. A field trial of the transgenic strain of *C. sativa* used to produce the seed oil for West, *et al.* 1999, 2020a,b**

(CSIRO)/Nuseed have EPA : DHA ratios of 7 :1 and 0.5 : 10, respectively. The seed oil from one transgenic strain of *C. sativa* developed by Rothamsted Research has an EPA : DHA ratio of 20 : 0 (Fig. 1). Since humans need to consume both EPA and DHA, seed oils that contain predominately either EPA or DHA with small amounts of the other fatty acid have limited potential for meeting human dietary requirements, either via direct consumption or indirectly via an animal feed ingredient. However, the other transgenic strain of *C. sativa* developed by Rothamsted Research produces an oil with similar proportions of EPA and DHA (9 : 11) which approximates the relative amounts of EPA and DHA in commercial fish oils such as those harvested from the Northern Hemisphere's oceans (Fig. 1) and so is a potential alternative to oily fish as a source of both EPA and DHA in the human diet.

Building on these achievements, a recent study funded by the Biotechnology and Biological Sciences Research Council, UK, investigated whether the seed oil from the transgenic strain of *C. sativa* with an EPA : DHA ratio of 9 :11 was as good a dietary source of these fatty acids in humans as a commercially prepared blended fish oil. Analysis of the triacylglycerol (TG) composition of the test oils showed complete divergence in the contribution of individual molecular species to the total lipid content of the oils (West, *et al.*, 2020a). Thus, there was a possibility that the bioavailability of EPA and DHA could differ between these oils because of the selectivity of pancreatic lipase for *sn*-1,3 positions. Therefore, the incorporation of EPA and DHA into blood lipids was measured over an eight-hour period after consuming test oils providing 450 mg EPA plus DHA, the daily amount recommended by the UK government. Participants were healthy men and women aged 18–30 years and 50–65 years who took part in a double-blind crossover trial (West, *et al.*, 2019). The results showed that the incorporation of EPA and DHA from transgenic *C. sativa* oil into blood TG, phosphatidylcholine (PC), and non-esterified fatty acids was equivalent to that from fish oil (Fig. 2). There were no differences between sexes, but postprandial incorporation of EPA and DHA into TG and PC was greater in older compared to younger participants.

A follow-on trial tested whether dietary supplementation with *C. sativa* oil was as good at increasing blood EPA and DHA concentration as fish oil. A mixed group of healthy men and women (aged 20 to 74 years) consumed 450 mg EPA plus DHA/day for 8 weeks provided by either transgenic *C. sativa* oil or blended fish oil in a single-blind crossover study (West, *et al.*, 2020b). Both EPA and DHA concentrations increased significantly in plasma lipids, but there were no significant differences between test oils in the magnitude of change in any lipid class that was measured (Fig. 3). Moreover, both test oils induced significant improvement in the omega-3 index, a biomarker of cardiovascular disease risk.

The transgenic *C. sativa* oil was well-tolerated in both phases of the trial. One participant withdrew because the individual was unable to tolerate the taste of the fish oil. These findings demonstrate that the seed oil from a transgenic *C. sativa* plant that contains an almost 1: 1 ratio of EPA : DHA,

and hence is superior to other transgenic oils that contain only EPA or DHA (or a disproportionately high EPA : DHA ratio), is an effective replacement for fish oil in the human diet. Importantly, field trials in the United Kingdom, United States, and Canada (Fig. 4, previous page) demonstrated the robustness and stability of the transgenic trait without unintended accumulation of EPA and DHA in non-seed tissues (Han, *et al.*, 2020). Together, the evidence demonstrates that transgenic *C. sativa* seed oil which contains EPA and DHA is an effective, scalable, and sustainable replacement for oily fish and fish oil in the human diet, and is without adverse ecological impact. Potential applications, in addition to human nutrition, include food supplements (especially those marketed to consumers who chose not to consume animal products), medicines (the preparation of which will require less clean up than for fish oil), and aquafeed (we previously demonstrated that this transgenic *C. sativa* oil is an effective direct substitute for fish oils in aquafeed diets, supporting the normal growth and development of a range of commercially farmed fish species including salmon, sea bass and sea bream, and agriculture, which stands to benefit from the potential this novel robust crop offers.

Collectively, these data demonstrate the very significant potential of transgenic *C. sativa* oils containing biologically relevant amounts of both EPA and DHA.

## Further reading


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# Surface techniques for a successful transformation to the natural space

Tobias Halthur and Anna Stenstam

One of the most powerful forces driving development today is the desire to produce more environmentally friendly, biodegradable, and sustainable products. Consequently, manufacturers are making a huge effort to replace petroleum-based ingredients, like surfactants, polymers, and packing materials, with "green" alternatives from sustainable sources. At the same time, the demand for products that are "free from" specific ingredients is increasing due to legislation (such as the upcoming limits of 1 ppm dioxane in personal care and household products, effectively limiting the use of ethoxylated surfactants). Shifting public opinion and growing consumer preferences for "sulphate-free" or "free from parabens" products also need to be considered.

- **Is it time to transform your formulation using sustainable materials?**
- **Surface techniques can provide the crucial information you need to make the leap based on mechanistic understanding of your present and future products.**
- **The key to a successful tranformation to the natural space is to know and understand the fundamentals of how your formulation works today.**

Regardless of the reasons, these transformations are difficult for the industry to make. How do you transform your high-performance formulation with new sustainable ingredients and still maintain function and performance? That is almost impossible to do by simply replacing a surfactant and/or polymer with a greener alternative. Maintaining a high performance and long shelf life as more and more effective ingredients are banned or disliked requires a more holistic and systematic approach.

## UNDERSTAND HOW YOUR FORMULATION WORKS TODAY

The key to a successful transformation is to know and understand the fundamentals of how your formulation works today. What are the mechanisms at play for creating that perfect coating, deposition of active, or cleaning effect that your products have right now, and that you want to replicate for tomorrow? To understand these mechanisms, you must investigate and understand the molecular interactions in your formulation (in the bulk) as well as what happens when the formulation comes into contact with the surfaces where it performs (skin, hair, tile, fabrics, or fibers, just to mention a few). This article focuses on what happens at those performance-critical surfaces and how surface techniques such as ellipsometry, Quartz Crystal Microbalance (QCM-D), and Atomic Force Microscopy (AFM) can give you the edge needed for a deeper understanding and a smoother transfer to new sustainable products.

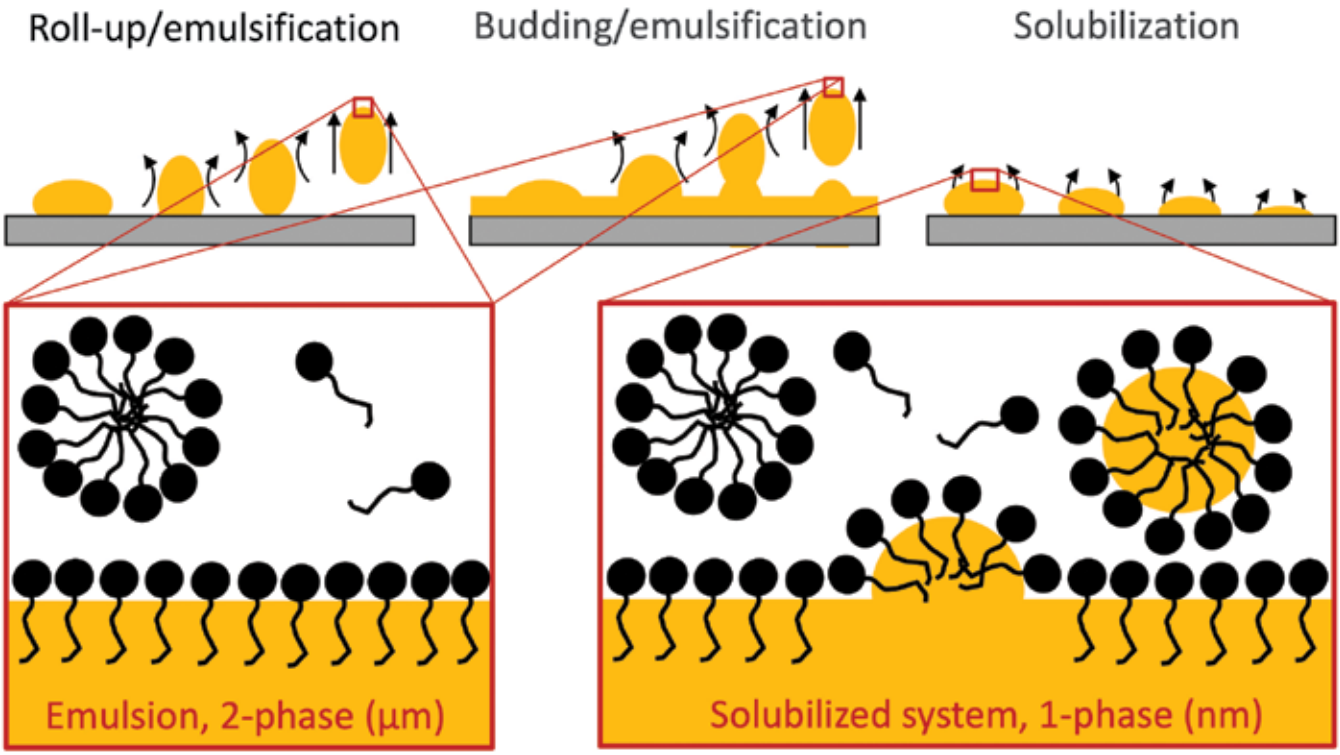



**FIG. 1. Simplified schematic of cleaning mechanisms. Both roll-up and budding leads to emulsification of the dirt and a 2-phase bulk system, in contrast to solubilization, where the dirt is solubilized inside micelles yielding an isotropic 1-phase system.**

Development of new products, such as cleaning products, almost always involves measuring a product's effects, like taking before and after pictures or simple measurements of tiles that are fouled with model grease such as oil, wine, proteins, or other residues. While these meaurements are a great marketing tool for convincing customers that your product works, they do not tell you *why* a new formulation works or *why* it is not as good as the old formulation.

The advantage of using surface-sensitive techniques and instruments, on the other hand, is that they can give you crucial information about what actually happens on the surface and, if interpreted correctly, give you valuable clues about how to alter your formulation or which type of ingredients you should look for.

## THE POWER OF SURFACE TECHNIQUES

**QCM-D** (see "QCM-D basics", page 18) is a mechanically based technique that provides information about adsorption/desorption as well as viscoelastic properties *in-situ* for molecularly thin films on surfaces. When QCM-D is used to investigate the removal of grease from hard surfaces (hard surface cleaning), the data will not only provide a value of how effectively the grease is removed, but also about the cleaning mechanism. This means that you will be able to see if the surfactant system is swelling the grease prior to removal, and if there is a "roll-up" or "budding" effect with emulsification, or mainly solubilization (Fig. 1). Once you understand your data, you can investigate and compare the mechanism for different types of surfactants and, ultimately, find the perfect candidate, or even mixtures, to achieve the optimal cleaning effect.

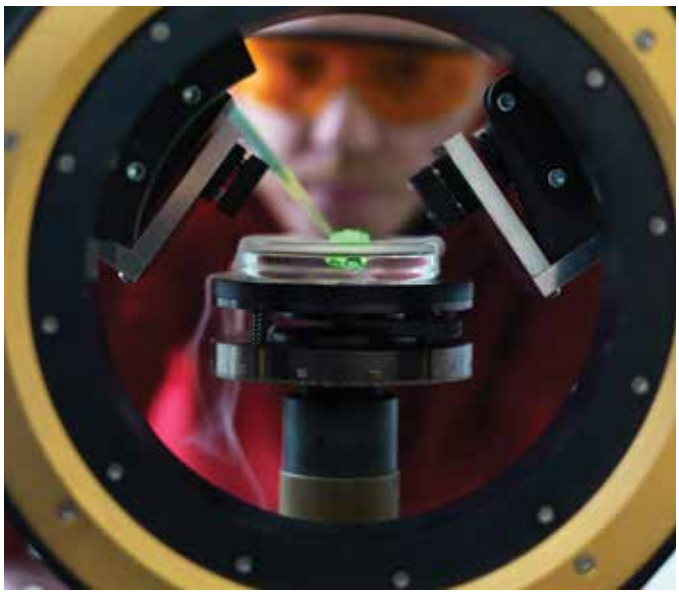


**FIG. 2. Ellipsometry instrument**

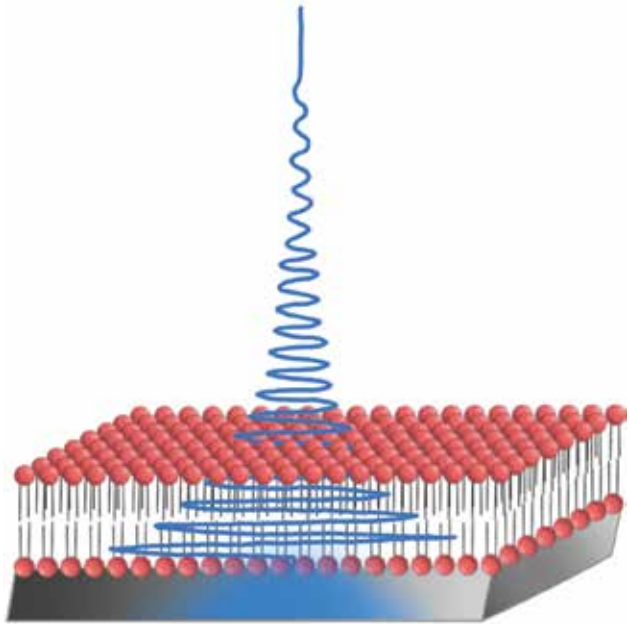
**Ellipsometry** (see "Ellipsometry basics", page 19) is an optical technique (Fig. 2) that, similarly to QCM-D, will provide information regarding adsorbed/desorbed mass for thin films with very high accuracy *in-situ*. Ellipsometry will not provide mechanical film properties like QCM-D does, but you can

## Quartz Crystal Microbalance (QCM) basics

QCM is a mechanical instrument with roots in oxide and metal deposition research, where it was originally only used in vacuum to yield mass deposition values with high sensitivity and accuracy. The heart of the instrument is a piezoelectric quartz crystal substrate that oscillates in shear mode when an AC-current is applied across it. The resonance frequency (and overtones) of the oscillation is directly propotional to the mass of the crystal. Thus, small changes in the resonance frequency can be transferred into a mass change (adsorption or desorption) occurring at the substrate interface according to the Sauerbry relation.

Two decades ago, this technique was refined and adjusted for use in liquid (solid/liquid interface) by researchers at Chalmers Institute of Technology in Sweden, and was later commercialized under the Swedish brand Q-Sense. The main problem with using QCM in liquid (or any non-vacuum situation) is that the ambient will affect the oscillation and also introduce a damping factor referred to as the dissipation. In practice, this means that the mass measured in liquid is the total mass oscillating with the crystal (adsorbed layer plus trapped liquid), also referred to as the hydrated mass, in contrast to the "dry" mass measured in ellipsometry. In fact, the hydration of an adsorbed layer can be obtained by combining ellipsometry and QCM data collected at the solid/liquid interface.

The dampening problem has become an advantageous feature, since the dissipation can be seen as a measure of how much the adsorbed layer interacts with the bulk, and thus these instruments are referred to as QCM-D (QCM with Dissipation measurements). For instance, a flat and rigid layer will give low dissipation whereas a viscous, thick and "fluffy" layer will give high dissipation and is therefore related to the viscoelastic properties of the film. It should further be stated that the Sauerbrey relation is strictly only valid for thin rigid films,



**Schematic illustration of the QCM ossilation signal propagating and dissipating through a lipid bilayer adsorbed to a sensor surface and out to the bulk. The lipid bilayer being only an illustration of one possible substrate to analyze. Model dirt and cellulose nanofibers are other examples of thin films that can be studied.**

and more complex modeling is needed for fluffy viscous layers. Newer instruments often have data modeling software for calculating mass and thickness, as well as viscosity and shear modulus. In our opinion, this data is difficult to verify, but most of the time it is enough to draw conclusions based on the raw data and how signals change in various environments (eg., pH, ionic strength, etc.), which can be used to compare different substrates and ingredients and/or formulations.

instead gain information regarding the thickness and refractive index of the film, which also relates to its packing density. For example, ellipsometry is a very effective tool for investigating coacervate deposition from shampoo formulations onto model surfaces. Coacervates are polymer-surfactant complexes formed during rinsing (dilution) that should deposit and stay attached to the hair. This process can be effectively mimicked in an ellipsometer cuvette by continuously diluting the formulation and seeing at what dilution and to what extent deposition occurs. These types of measurements not only allow you to test various surfactant polymer combinations and concentrations, but also the effect of water quality parameters, such as water hardness, temperature, and pH—all of which play crucial roles during this very dynamic and often kinetically trapped process.

**AFM** (AFM basics", page 20), which is mainly used as a high-resolution and surface-sensitive microscope, can be used to visualize your surface and actually see what your coacervate coating looks like once it has deposited on a surface, or how a fouled surface looks after it has been cleaned (Fig. 3). It will tell you if there are any grease residuals remaining in patches, or if you have achieved perfect cleaning. One should also not forget that AFM is not only a microscope giving high-resolution topographical images, but it can also provide surface force data (both normal and frictional forces). This means that you can, for instance, measure changes in surface friction as well as adhesion forces and mechanical properties (such as stiffness and elasticity). All of this data can be combined with information from QCM-D and ellipsometry to provide a more complete picture of what is going on at the surface.

## LIMITATIONS AND HOW TO CIRCUMVENT THEM (AT LEAST SOME)

*Studying your substrate: What surfaces can you use?*

Both ellipsometry and QCM-D are limited to measurements of flat model surfaces. Ellipsometry requires a highly reflective flat surface, such as glass, silica, or metal, but measurements can also be made at the air/liquid, or even liquid/liquid interface. With QCM-D, the sensor *is* the surface that you monitor (see "QCM basics"). On the other hand, QCM-D sensors and the substrates used for ellipsometry can be modified to better mimic your true substrate. By coating them with polymeric films, you can create a range of plastic surfaces with varying hydrophobicities. These, in turn, can be further modified by wet chemistry or plasma treatments to introduce specific functional groups. Through vapour deposition, coatings can also be made to introduce thin metal and oxide layers—even on QCM-D sensors, which means that both ellipsometry and QCM-D can be used to mimic hard surface cleaning of glass (silica), metal surfaces (eg., stainless steel, carbon steel, titanium), as well as plastic surfaces. For this application, it is also possible to use model-fouling coatings in which the model substrate is spin-coated with grease (eg., fat, wax, or cooked polymer-

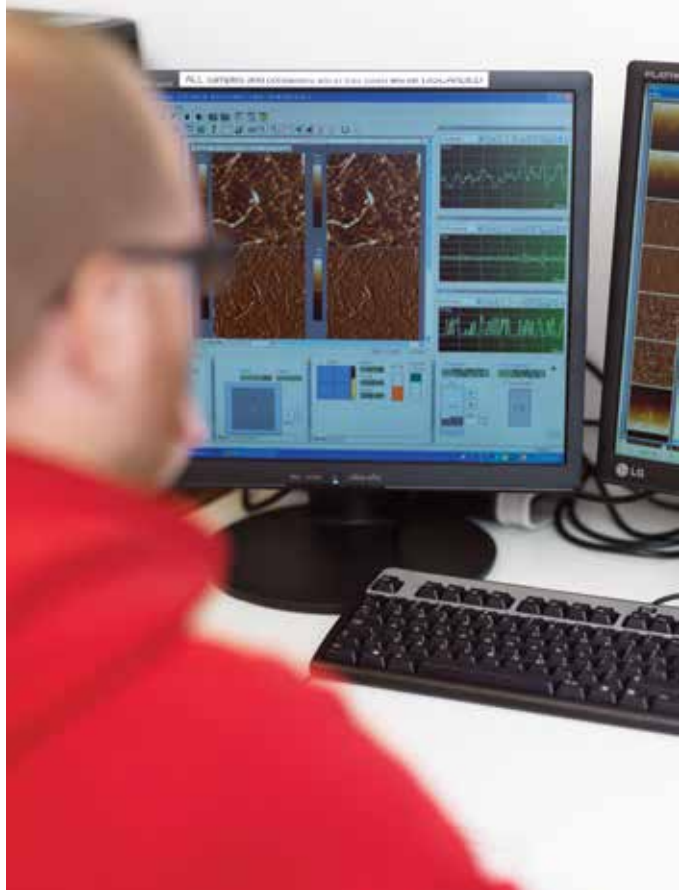


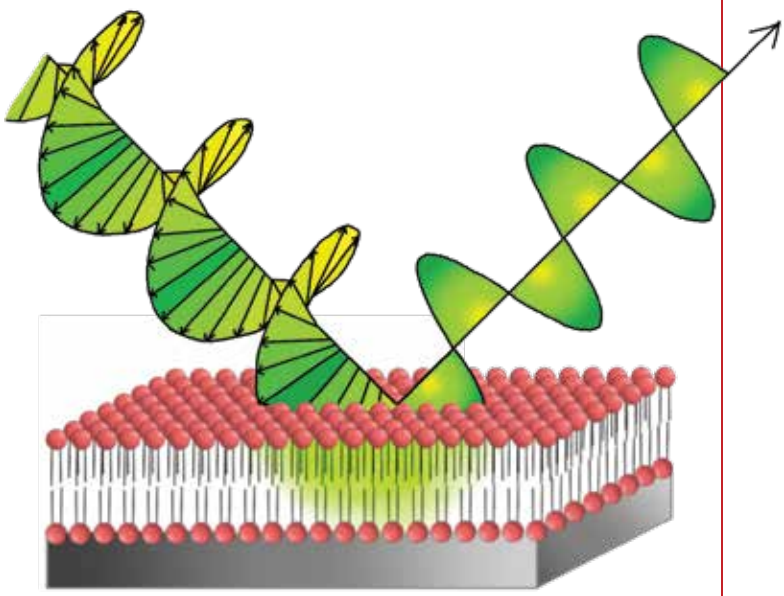
**FIG. 3. Author Tobias Halthur uses Atomic Force Microscopy.**

## Ellipsometry basics

Ellipsometry is an optical technique that is traditionally used by physicists to accurately determine thickness and optical properties of thin metal or oxide layers (solid/air interface)—during vapor deposition, for example. The technique makes use of the change in polarization of light as it is reflected on a surface/substrate. Physical properties (eg., thickness, refractive index, and dielectric constants) for thin films can be determined with high resolution (at the Angstrom level for thickness).

This classical materials instrument can be converted to measure *in-situ* at the solid/liquid interface, which can be used to investigate adsorption and desorption processes for molecularly thin layers such as surfactant surface self-assembly and polymer and protein adsorption, among other applications. An optical contrast (difference in refractive index) is needed for accurate measurements. This means it is difficult to get accurate thickness and refractive index data for diffuse low-density layers, although the product of the thickness and refractive index (optical density) can easily be converted to adsorbed "dry" mass with relative high accuracy even for "fussy" layers.

Such *in-situ* measurements in liquid have been performed for roughly three decades at four Swedish universities, and some of the data has been validated with other techniques, such as neutron reflection. However, the technique has not been picked up to any great extent outside Sweden, and there is no commercial instrument available for *in-situ* measurements in liquid.



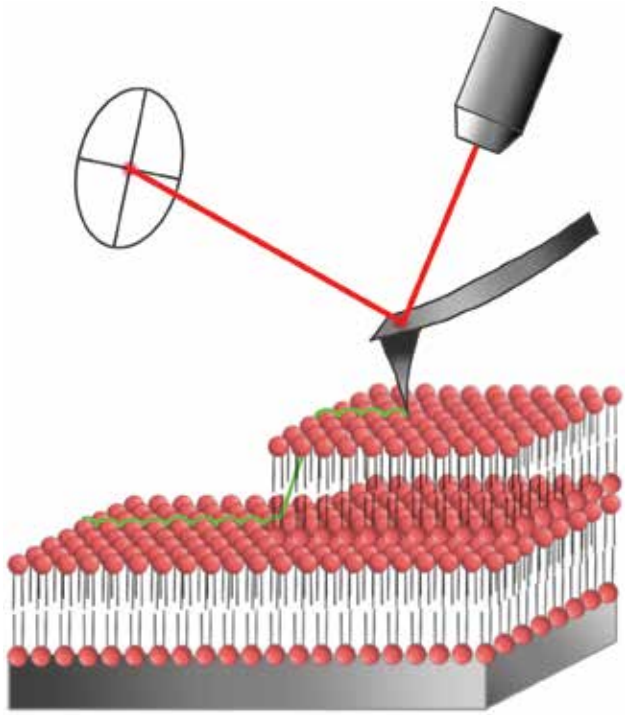
**Schematic illustration of how the incident elliptically polarized light is reflected and transformed to plane polarized light when interacting with a lipid bilayer adsorbed to a substrate. The lipid bilayer being only an illustration of one possible substrate to analyze. Coacervate deposition and model dirt removal are other examples of thin films and phenomena that can be investigated.**

## Atomic Force Microscopy (AFM) basics

AFM is a Scanning Probe Microscopy (SPM) technique. The common feature of all SPM techniqes is that a probe (often a sharp pointy tip) is scanned in contact or close proximity to the substrate investigated. For AFM, the tip is located at the very edge of a thin flexible cantileaver and can be compared to the needle and tip on the pickup of an old vinyl record gramophone player, although at much smaller scale. A laser is aimed at the topside of the cantilever, and the reflection is calibrated to hit the center of a detector. When the tip approaches a surface, small atomic or molecular forces (attractive or repulsive) will cause the cantileaver to bend, which offsets the reflected laser beam from the center of the detector. This can be used to calculate the force pulling or pushing on the tip (provided that the spring constant for the cantilieaver is known).

When scanning the tip across the surface (x-y plane), either in contact or ossilating mode (bouncing on the surface or on repulsive forces from the surface), a feedback loop maintains a constant force (contact mode) or amplitude (ossilation mode) by rapidly adjusting the height (z-direction) of the cantileaver. This provides topographical data of the sample, which can be used to create a 3D image of the surface with nm resolution (especially in z-direction). The deflection (in contact mode) or amplitude (ossilation mode) data fed to the feedback loop will provide a high-contrast image in which even small features and details are seen. In addition, the damping of the ossilation when the tip is bounced on a surface will be picked up as a phase change, which can provide some mechanical contrast in the image if there is a difference in elasticity for different locations or structures on the surface.

AFM can also be used for normal force measurements, which was its original purpose. In this case, the tip is not scanned across the surface. Instead, the bending of the cantileaver can be monitored as it approaches and is retracted from the surface. This will provide information regarding the presence and magnitude of attractive or repulsive electrostatic forces (long-range) and Van der Waahls forces (which are always attractive and short range) as well as adhesion forces and pull of force once the tip is retracted from the surface.



**Schematic illustration of how the AFM cantileaver and tip probes the surface of lipid bilayers on a solid support, tracing out the step hight in the transition from one to two bilayers. The lipid bilayer being only an illustration of one possible substrate to analyze. As mentioned in the text, almost any surface can be investigated with this technique, including real hair and textile fibers as well as packaging materials and specifically manufactured model surfaces, just to mention a few.**

There are also frictional force applications in which the tip is slided across the substrate in a sideways conformation, and the torsional twisting of the cantileaver is analyzed at various applied normal forces. If applied on molecularly smooth surfaces, these measurements can provide a molecular frictional coefficient.

ized oils), egg proteins, carbohydrates, or mixtures therof to simulate fouled dirty surfaces. At CR, we have also developed a protocol to coat surfaces with cellulose nanofibers suitable for QCM-D and ellipsometry to mimic cotton fabric, hydration, swelling and oxidation of it, as well as cleansing.

It is more difficult to mimic biological substrates, such as hair or skin, but model substrates with similar physiochemical attributes can be used. A hydrophobic substrate could mimic virgin hair, for example, while a hydrophilic and negatively charged substrate, such as silica, is commonly used to imitate dyed or damaged hair. More complex "biological" substrates, such as supported lipid bilayers, which mimic cell membranes, are also frequently used.

AFM, on the other hand, can operate directly on most true surfaces—although most instruments may have size or weight limitations. Fine structures can sometimes be difficult to see if there is an overlaying larger roughness on the surface or limited access for the probe to get into groves or "valleys," whereas ridges are more easily accessible. It is also crucial that the surface investigated is firmly fixated, wich makes measurements on fibers (hair and textiles) more challenging, but not imposible.

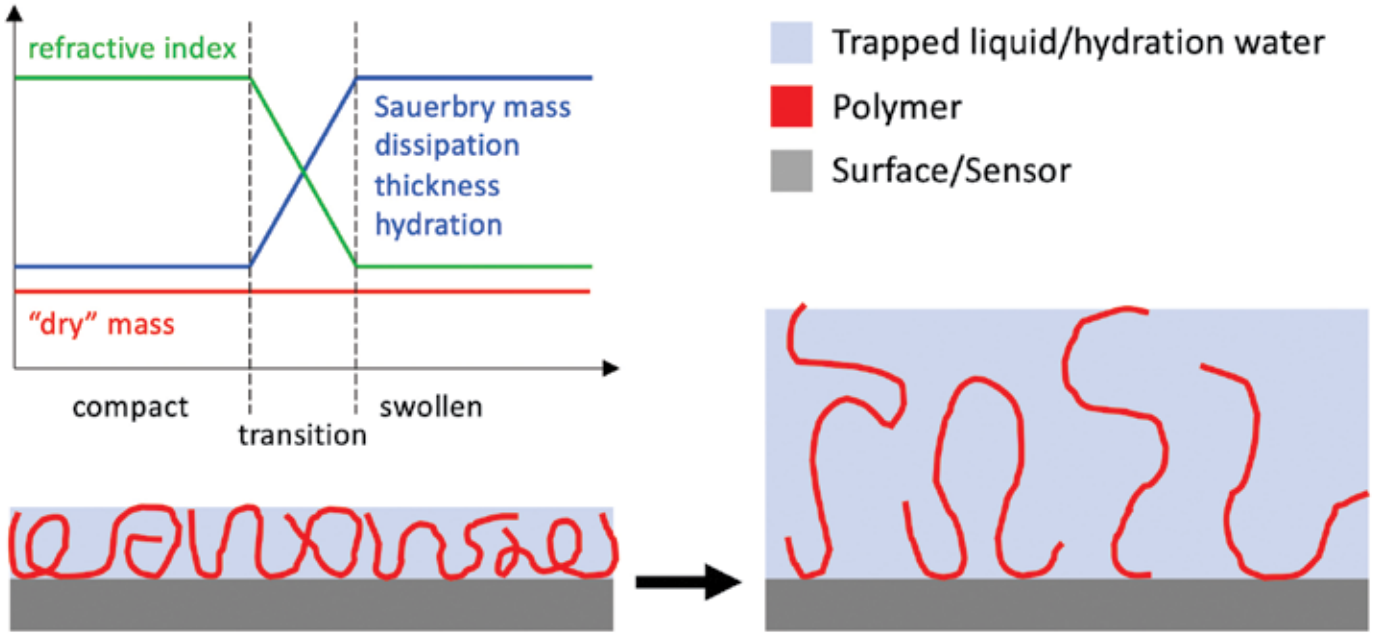



**FIG. 4. The key difference between ellipsometry and QCM can be seen in this illustration of a polymer layer swelling from thin and rigid (left) to a thick and more viscous and "floppy" structure (right), such as swelling of dirt before budding starts or swelling and uptake of water in a nanocellulose film due to a change in temperature, pH, or other external stress. In ellipsometry, this transition is registered as an increase in thickness and decrease in refractive index with maintained constant "dry" mass, whereas QCM-D senses an increased hydrated mass and dissipation from the increased water interaction, ~~top left~~.**

### *Studying your formulation: What medium can you use?*

For ellipsometry to work, you need a reasonably clear and transparent fluid so that the optical signal is not scatted, whereas a turbid fluid is perfectly fine for QCM-D measurements. Ellipsometry data will always provide the true mass of your film ("dry mass"), whereas QCM-D will provide the total mass associated with the sensor (adsorbed layer + trapped liquid), often referred to as the hydrated mass (Fig. 4). This means that you can determine the hydration of your film by combining ellipsometry and QCM-D measurements—knowledge that can be very valuable when devloping diverse applications, such as care products, and for understanding film integrity when evaluating packaging material.

**The most important limitation for QCM-D is that it is very sensitive—not only to changes on the surface, but also to liquid bulk properties.** Thus, small changes in bulk viscosity will affect the signal and can be easily misinterpreted as a mass change. To avoid drawing the wrong conclusions, it is important to learn how to interpret data from this seemingly simple instrument.

### Ex-situ *measurements*

We have chosen to focus on *in-situ* measurements for both QCM-D and ellipsometry because they provide the best and most accurate data when using these two techniques. Also, *in situ* measurements are often preferred, because they provide insight into the processes that occur during manufacturing or use, and can provide kinetic information. However, it should be noted that both techniques can also be used for *ex-situ* measurements. *Ex-situ* measurements are sometimes needed to circumvent limitations of the techniques, such as when the bulk viscosity is too high for the liquid to give reliable data or be pumped into the QCM-D cell, or if turbid samples are needed for ellipsometry.

The opposite is the case for AFM. *Ex-situ* before and after measurements in the same area or on separate but identical samples are easier to perform, and usually provide much higher resolution. That said, measurements can be performed *in-situ* in liquid, if needed, although this is more difficult and will never provide the same kinetic data as QCM-D and ellipsometry.

## PUTTING IT ALL TOGETHER WITH A PRACTICAL EXAMPLE

So, let's say we are developing a new hard surface cleaning product and want to evaluate the cleaning effect and mechanism of various natural, biodegradable surfactants to obtain the optimal mixture for a new sustainable cleaning formulation. The first step is to develop one or several model ceramic or metal surfaces coated with the type of material that you want your product to clean (cooked grease, proteins, carbohydrates, or mixtures therof). Spin-coating is often used for this purpose (Fig. 5, page 22).

Next, you investigate the appearance and quality of the coating using AFM, while measuring its mass using *ex-situ*

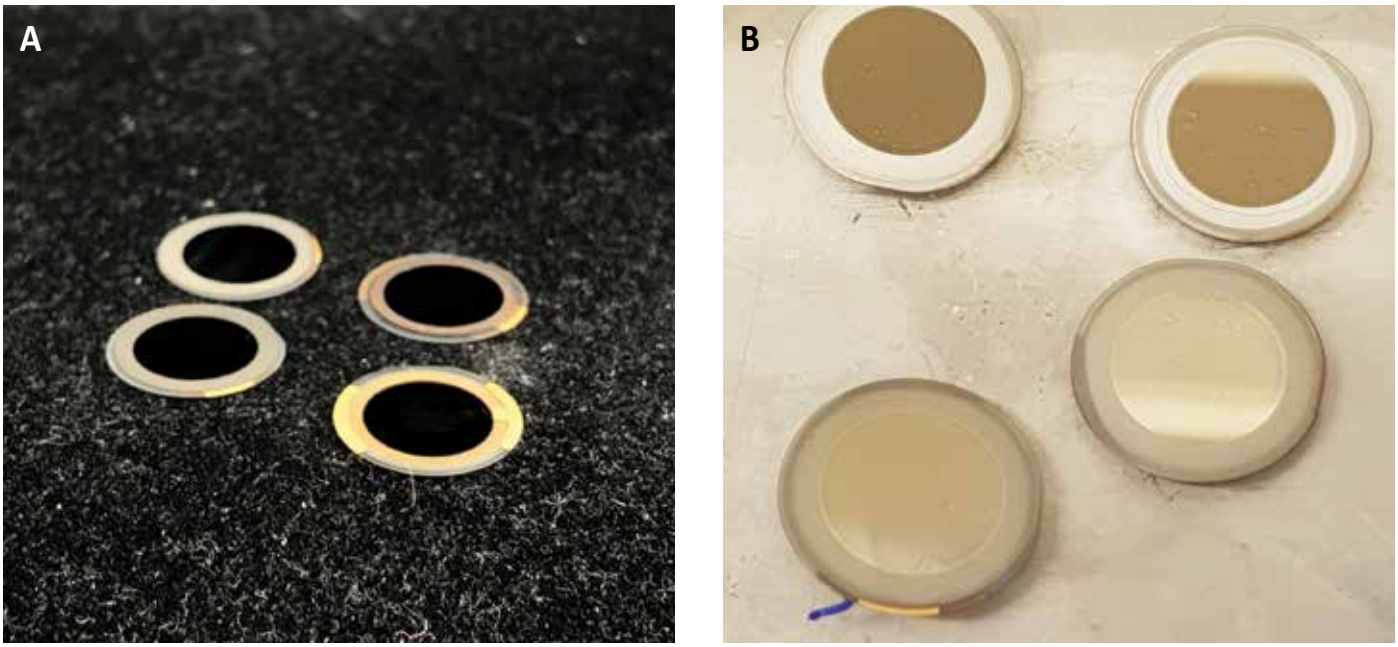



**FIG 5. a. Uncoated QCM sensors; b. QCM sensors spin-coated with model grease**

## Surface techniques produce tangible results.

Here are some results the team at CR Competence has been able to guide companies toward, with the use of surface techniques:

- Improved coacervate deposition—by optimizing deposition and co-deposition of oils and actives, using ellipsometry;
- Better cleaning and degreasing—through mechanistic understanding of how various surfactants and mixtures remove grease, using QCM-D;
- Bench marking of mild and efficient bleaching of textile—by studying cellulose fibers, their swelling, and oxidation, by QCM-D and AFM;
- Corrosion protection—by studying film formation on stainless steel with different film forming additives, using QCM-D;
- Reduced process losses—by developing protocols to block adsorption of high- affinity, low-concentration active ingredients to process tubing;
- Improved adhesion—by using AFM to study surface modification of aluminum for better lamination.
- Better understanding of protein, carbohydrate, and lipid fouling on food packaging materials—by adsorption measurements of actual food products on polyethylene coated sensors using QCM-D.
- Effect of milk fouling on processing equipment—by QCM-D adsorption measurements on stainless-steel sensors in milk with various heat pre-treatments.

QCM-D in air and liquid—both before and after the coating is applied. You can then use a combination of QCM-D and ellipsometry *in-situ* to investigate the effect that various surfactants at different pH and ionic strenghs have on your fouled surface. This can thus provide you with information regarding the kinetics and efficiency of cleaning, as well as the underlying mechanisms: surfactant adsorption, followed by swelling, budding, and/or solubilization of the model "dirt" (Fig. 1).

Finally, you can investigate the appearance of the fully or partly cleaned substrate using AFM, which can also determine the mechanical properties of the remaining dirt, such as elasticity, adhesion forces, and friction coefficient. In addition, this might even give you information about abrasion on the surface material from solvents or extreme pH values. The in-depth data you collect with these surface techniques will give you a fundamental understanding of the mechanisms of action needed to optimize your formulation and make rational selections of new ingredients and additives for your next product.

*Tobias Halthur is Chief Scientific Officer at CR Competence and adjunct university researcher at Malmö University in Sweden. He is an expert in surface characterization and modification techniques, which he has worked with for 20 years, since earning a PhD at KTH, Royal Institute of Tehcnology and the Institute of Surface Chemistry in Stockholm. At CR, he has utilized the techniques for the benefit of a vast variety of companies.*

*Anna Stenstam is CEO and co-founder of CR Competence and Honorary Doctor at the faculty of Engineering at Lund University. She has a PhD in physical chemistry (protein-surfactant interactions). With CR, she aims to make advanced methods and deep understanding available for all companies.*

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# Exothermal analysis of cocoa butter, cocoa liquor, and chocolate mass with MultiTherm TC™

Yuantong Zeng, Peter Braun, Jürg Peter Keller, Konstantinos Paggios, Julia Jacob, and Stefanie Pirhalla

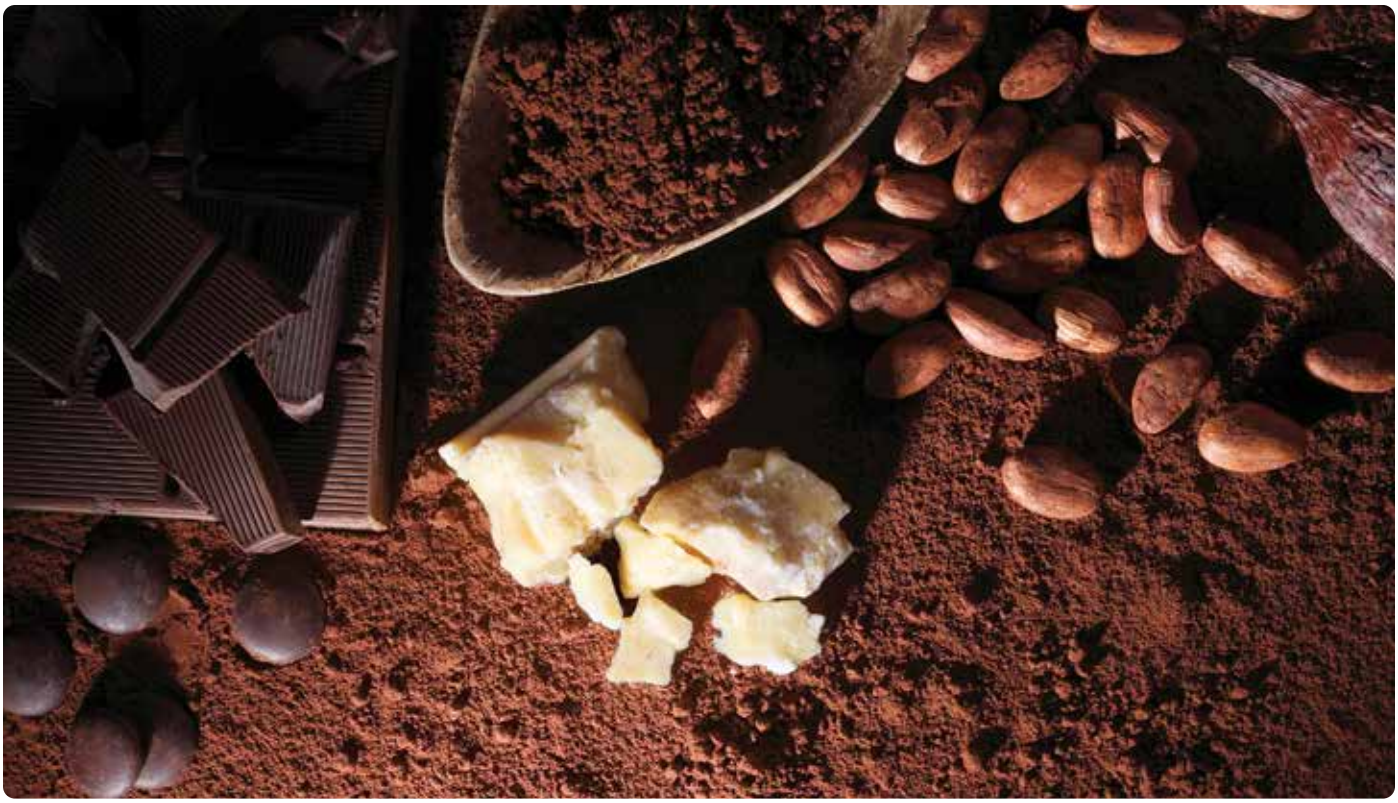
Cocoa butter is the crystallizing part of the cacao bean, but because chocolate often contains cocoa liquor (a mixture of cocoa butter and cocoa solids) the hardness and the melting characteristics of chocolate depend on the crystallization ability of both the cocoa butter and cocoa liquor that are used. Many factors influence the crystallization ability of cocoa butter, including the origin and genotype of the cacao bean, ripeness of the cacao fruit, harvesting and fermentation conditions, processing methods (roasting, alkalizing, deodorizing), and storage conditions (see "Quick review of chocolate production and quality parameters," page 28).

- **The melting characteristics of chocolate are usually evaluated by determining the crystallization behavior of cocoa butter, but variations in room temperature and mechanical stirring can influence the results.**
- **Because chocolate often contains cocoa liquor, its melting characteristics depend on the crystallization of the liquor as well as the butter.**
- **This article describes a new approach for determining cocoa butter, cocoa liquor, and chocolate cooling curves that also minimizes the influence of ambient temperature.**

## METHODS USED TO EVALUATE CRYSTALLIZATION

In 1899, Shukoff introduced a vacuum-jacketed flask to determine the solidification temperature of fats. The cooling curve (temperature versus time) analysis with a Shukoff flask (OICCC analytical method 110–1988) is still the most-used method for determining cocoa butter crystallization behavior. The Shukoff flask with vacuum jacket was a great invention. However, the main weakness of most Shukoff measuring instruments is the sensitivity of the temperature probe in ambient conditions. Variations in room temperature influence recorded sample temperatures and, thereby, the resulting measurements. Also, the method itself calls for the temperature of the cooling medium to be set to 0°C with iced water. This subjective temperature is too low for the desired expansion of the exothermal reaction of cocoa butter. Consequently, in practice, the cooling temperature is often changed to 10°C or 15°C to obtain a more distinct cooling curve.

It is difficult to compare the results of Shukoff-analysis at different cooling temperatures. Plus, the Shukoff cooling curve method uses only information about crystal growth to evaluate a sample's crystallization properties, without taking information about nucleation into account. Purified cocoa butters often exhibit a delayed but stronger nucleation, and, therefore, weaker crystal growth.



However, the crystallization of purified cocoa butters in the presence of chocolate mass (which provides a huge amount of solid particle surface) is excellent. Such samples can potentially be evaluated as poor quality using the Shukoff method.

In contrast, the Jensen-method (British Standard BS684) determines the cooling curve by stirring. The fat sample is stirred with an up-down stroke every 15 seconds during cooling at 17°C (Timms 2003). The main drawback of this method is that the reproducibility of the Jensen cooling curve is influenced by the mechanical stirring movement.

Ziegleder (1985) described an isothermal DSC-Method for analyzing the crystallization behavior of cocoa butter. With this method, the DSC crystallization curve (heat flow versus time) is recorded at constant cooling temperature (20°C or 21°C), and various times along the DSC crystallization curve are used to evaluate quality. DSC is an excellent instrument for analyzing the crystallization and melting behavior of cocoa butter and chocolate, but it is mainly used for research purposes rather than routine measurement because it is laborious, expensive, and requires expertise and technical skills.

The crystallization behavior of cocoa butter can also be characterized by determining the solid fat content (SFC) with NMR measurement (Standard methods: IUPAC 2.150, AOCS Cd 16b–93). To do so, the samples must be tempered to stabilize their crystal structure. They are then measured at a wide range of temperatures (typically at 20°C, 25°C, 30°C, and 35°C). The SFC-analysis is extremely time-consuming and also not suitable for fast, routine measurement.

The MultiTherm TC is a newly developed approach for the determination of cocoa butter, cocoa liquor, and chocolate cooling curves. This instrument is specially designed to minimize the influence of ambient temperature. The cooling temperature is also selected to maximize the expansion of the exothermal reaction of cocoa butter (17.6°C), cocoa liquor (19.0°C), and chocolate mass (20°C). The so-called Buhler Crystallization Index (BCI) is calculated based on the information of the entire cooling curve, including the nucleation and crystal growth portions of the curve.

## PRINCIPLE BEHIND MULTITHERM TC™

The measuring principle is based on a calorimetric, exothermal analysis of cocoa butter during the cooling process. The measuring instrument is equipped with a thermoelectric element. The cooling temperature is set to 17.6°C for cocoa butter, 19°C for cocoa liquor, and 20°C for chocolate mass. The released crystallization energy causes the temperature of the sample to rise. The temperature profile of the sample is then acquired over time and recorded.

A disposable aluminum cup (volumetric capacity 15 ml) is used as a sample holder, and an air gap between the aluminum cup and the cooling cell serves as an insulation layer for the heat transfer. This allows the sample to be cooled down gently from the beginning. In this way, the inflection point temperature for the crystal nucleation (TN) can be uniquely determined as an inflection point on the recorded cooling curve (Fig. 1, page 26). During the main crystallization (crystal growth) phase, the temperature increase of the sample is more pronounced due to the insulation layer. The reproducibility of the measurements is also significantly improved.

To reduce the influence of ambient temperature on the measurement result, the measuring cell and the temperature sensor are completely shielded with insulating material (plastic). The holding bracket of the temperature sensor is especially

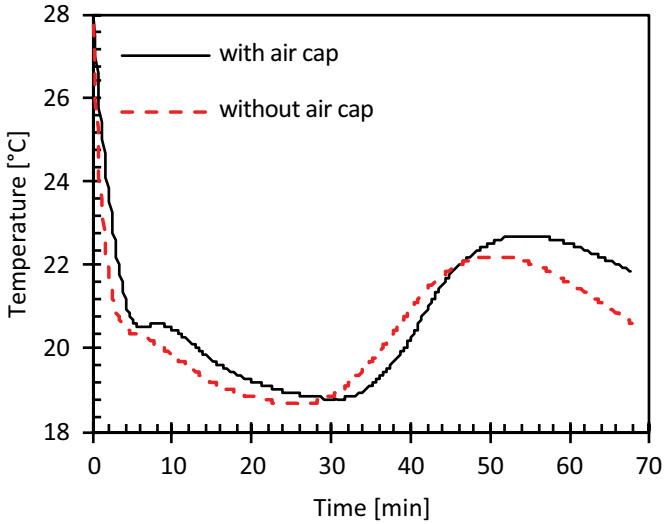



FIG. 1. Cooling curve of a cocoa butter sample with and without air gap at a cooling temperature of 17.6°C

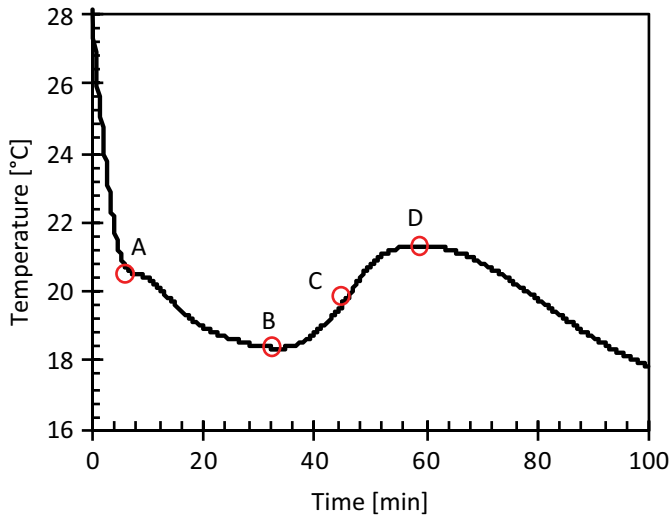



FIG. 3. Cooling curve of a cocoa butter sample

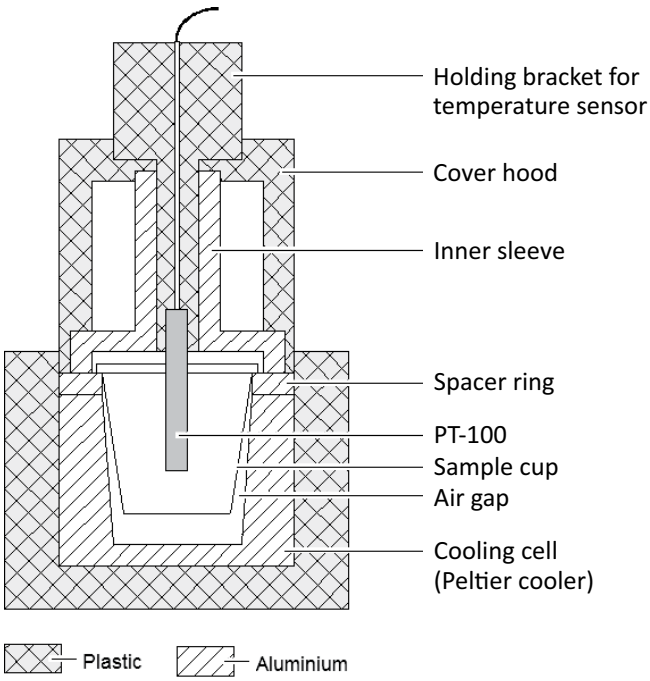



FIG. 2. Schematic diagram of the measuring equipment

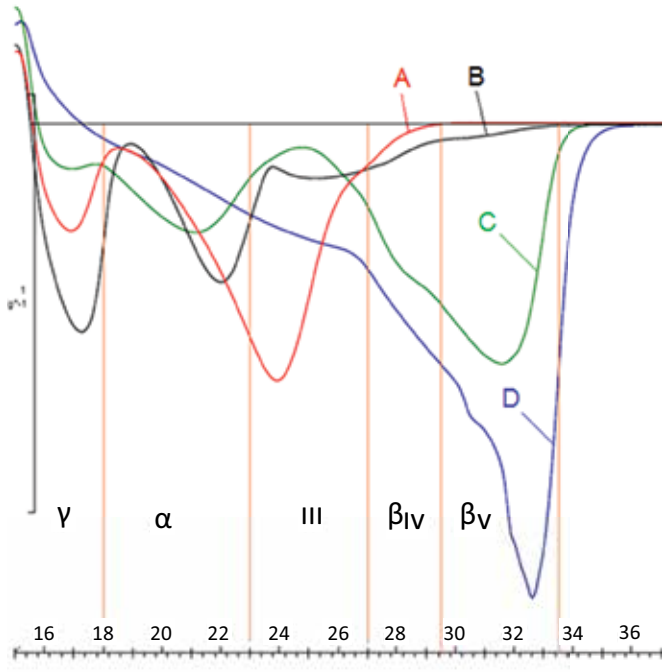



FIG. 4. DSCmelting curve of the cocoa butter sample after different cooling times (A, B, C, and D)

tempered by means of an aluminium inner sleeve, in which the temperature approaches the cooling cell temperature (Fig. 2).

An installed internal computer controls the cooling temperature of the cooling cell, acquires the temperature profile of the sample over the measuring time, and evaluates the quality parameters afterward.

## COOLING CURVE OF COCOA BUTTER: NUCLEATION AND CRYSTAL GROWTH

The cooling curve of cocoa butter can be seen in Figure 3. A cocoa butter sample was cooled down with MultiTherm TC™ at a cooling temperature of 17.6°C. The cooling curve shows several characteristic points. These include: A. first inflection point; B. minimum temperature; C. second inflection point; D. maximum temperature.

To analyze the development of the crystal modification of the crystallized cocoa butter, a small amount of the cooled sample (~20mg) was taken after defined time points (A, B, C, and D), and its melting behaviour was analyzed by means of DSC measurement (Differential Scanning Calorimetric, heating rate: 4°C/min). The melting temperature of the sample relates directly to the modification of cocoa butter crystal. The endothermic DSC melting curve provides information on the development of crystal modification of the cocoa butter sample during the cooling process (Fig. 4).

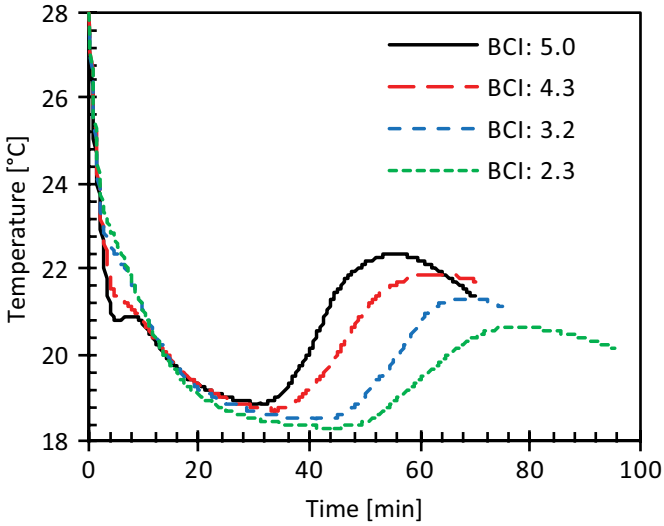



**FIG. 5. Different nucleation phases of the cooling curves of cocoa butter**

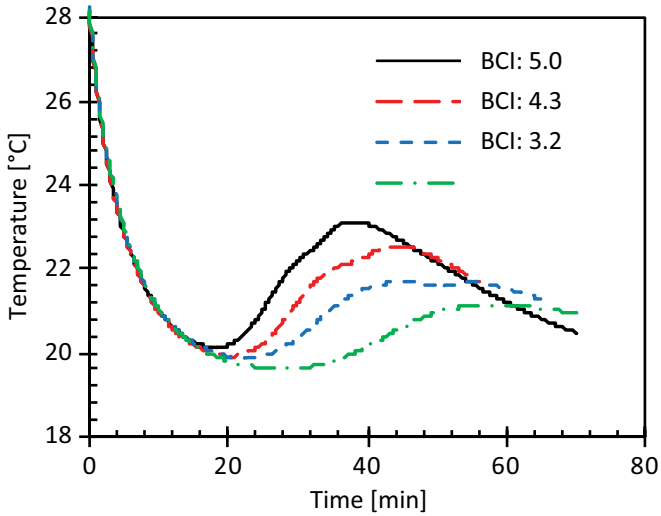



**FIG. 6. Cooling curve of recombined cocoa mass**

The nucleation of cocoa butter starts at a sample temperature of 21.7°C. A first inflection point (Point A) can be seen at 20.5°C. This inflection point represents the moderate release of crystallization energy of unstable alpha crystals, which mainly form at the sides of the aluminum cup. A gradual crystal transformation from unstable alpha crystals to stable beta V crystals takes place until a minimum temperature is reached (Point B). At this point, the crystal growth of stable beta V crystals starts, which is initiated by the accumulation of a certain amount of already transformed beta V crystals. The released crystallization energy causes a strong temperature rise of the sample even though it is cooled from outside. This temperature increase subsequently activates the transformation of unstable alpha crystals to stable beta V crystals. The second inflection point (Point C) is reached when the released heat per time unit is at its maximum. Afterward, the temperature rise continues to dominate until a maximum temperature is reached (Point D). The sample temperature drops again from this point on.

## EVALUATION AND PRESENTATION OF THE RESULTS

### *Direct evaluation of the cooling curve*

The following measuring points are evaluated directly based on the recorded cooling curve:

- First inflection point during the nucleation phase
  Temperature in [°C]: TN
  Time in [min]: tN
- Second inflection point during the crystal growth phase

  Gradient of the maximum temperature rise $Q = \frac{dT}{dt}$

  The Q value describes the maximum intensity of the crystal growth during the solidification.
- Minimum and maximum temperature (TMIN, tMIN and TMAX, tMAX)

### *Indirect evaluation of the Buhler Crystallization Index™ (BCI™)*

A new crystallization index, referred to as BCI, can be calculated based on the complete cooling curve information. The sequence of crystal growth, as well as the time, temperature, and energy characteristics of the nucleation, are taken into account for this calculation. A strong nucleation leads to a weak crystallization heat development during later crystal growth, because the heat has been partly released during the formation of unstable alpha crystals.

When comparing the cooling curves of different cocoa butter samples, the starting time, temperature, and intensity of nucleation can be very different (Fig 5). This is due to differences in the purity level of cocoa butter grades. Homogeneous nucleation occurs with much more difficulty in very pure cocoa butter. In the case of heterogeneous nucleation, the crystallization is catalyzed easily by foreign surfaces, e.g., fine cocoa particles in cocoa butter. In cocoa mass and in chocolate mass, the heterogeneous nucleation dominates due to the abundance of fine solid particles.

To calculate the crystallization index (BCI), a database of cooling curves of more than 70 different cocoa butter examples were collected. These cocoa butters were mixed systematically with low-fat cocoa powder (11% fat) in the ratio of 1:1. These recombined cocoa masses had a fat content of ~55%. The nucleation pattern in the cooling curves (measuring temperature: 19°C) of different recombined cocoa masses were very similar. A homogeneous nucleation was not noticeable, and the heterogeneous nucleation dominated for recombined cocoa masses (Fig. 6).

Based on a simple, dynamic cooling model, the change of crystallization enthalpy is continuously estimated. This allows the thermal features of the cooling curve to be precisely determined, as demonstrated by points A, B, C, and D, and the derived features in Figure 3. A regression analysis of the features of cooling curves of cocoa butter and their recombined cocoa mass make it possible to select and weight the import-

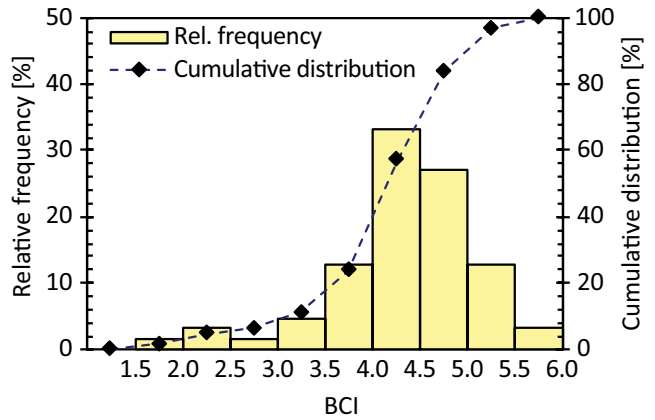



**FIG. 7. Relative frequency and cumulative distribution of the BCI™ values of cocoa butter samples**

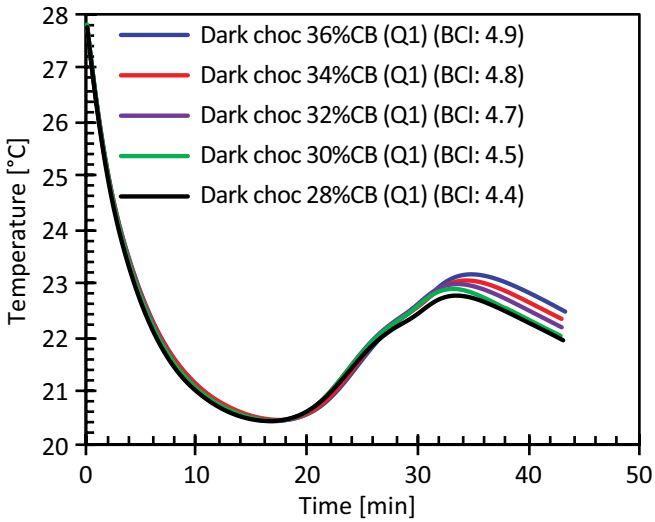



**FIG. 8. Cooling curve of dark chocolate mass with different cocoa butter content**

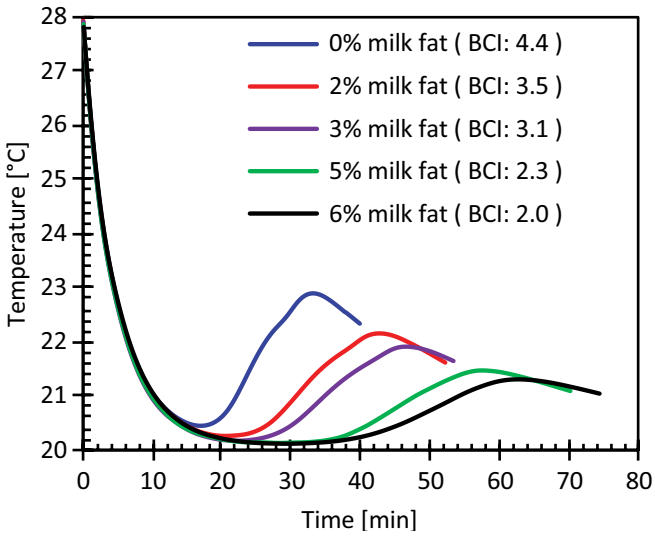



**FIG. 9. Cooling curve of dark chocolate mass with different milk fat content**

**TABLE 1. Interpretation of the BCI™ values**

| BCI™ value | Interpretation | Relative frequency |
|---|---|---|
| > 5.0 | Very fast crystallization, very hard | 16% |
| 4.0 … 5.0 | Good crystallization ability | 60% |
| 3.0 … 3.9 | Moderate crystallization ability | 18% |
| < 3.0 | Very slow crystallization, very soft | 6% |

ant features for calculation of the BCI. The BCI of cocoa butter and of its recombined cocoa mass must be the same.

## BCI™ OF COMMERCIALLY AVAILABLE COCOA BUTTER SAMPLES

More than 100 cocoa butter samples were collected in the years 2008–2012. The quality of all samples have since been analyzed with MultiTherm TC™. The BCI™ values of all the samples were between 1.6 and 6.

Figure 7 shows the relative frequency and cumulative distribution of the BCI™ values. More than 33% showed a BCI™ value between 4.0 and 4.5. Since this was the value that occured most frequently, a BCI™ value in the range 4.0 to 4.5 was interpreted as average quality (Table 1).

## BCI-MEASUREMENT FOR COCOA LIQUOR AND CHOCOLATE MASS

Since cocoa liquor is an important raw material for producing chocolate, the crystallization quality of chocolate mass also depends on the characteristics of the cocoa liquor that is used.

### More about chocolate

How is chocolate made, and why is milk chocolate softer than dark chocolate? The May 2012 issue of *Inform* magazine will help you get up to speed on chocolate. Look for the issue with the box of bon bons on the cover at https://www.aocs.org/stay-informed/inform-magazine/inform-archives.

The BCI-value of cocoa liquor and chocolate mass can be also analyzed with MultiTherm. The cooling temperature should be set to 19°C for cocoa liquor and 20°C for dark- and milk-chocolate mass.

Braun and Pirhalla (2012) showed that the crystallization ability (BCI) of chocolate mass depends mainly on the crystallization ability of the cocoa liquor and cocoa butter that are used. The total cocoa butter content of the chocolate mass also had an impact on the BCI-value (Fig. 8). The BCI of chocolate mass decreases remarkably with increasing milk fat content (Fig. 9). On the other hand, the particle size showed no impact on the crystallization of chocolate mass.

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*Jürg Peter Keller is a professor and program manager of MAS Automation Management at FHNW—University of Applied Sciences and Arts Northwestern Switzerland, School of Engineering.*

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# Physicochemical properties of Andiroba (*Carapa guianensis*) and Pracaxi (*Pentaclethra macroloba*) oils

**Fernanda Luisa Lüdtke, Larissa Magalhães Grimaldi, Thais Jordânia Silva, Kamila Ramponi Rodrigues de Godoi, Mayanny Gomes Silva, Renato Grimaldi, and Ana Paula Badan Ribeiro**

The Amazon forest presents a diversity of plant species that are sources of oils and fats with unique physicochemical characteristics. Oils extracted from plant species such as Andiroba (*Carapa guianensis*) and Pracaxi (*Pentaclethra macroloba*) may be suitable for developing new fat products for use in food and cosmetics (Bezerra, *et al.*, 2017; Pereira, *et al.*, 2019).

- **This article looks at two oils from the Amazon—andiroba and pacaxi.**
- **Both oils are currently used as ingredients in natural skin and hair products and have pharmacological effects, but could they also be used to develop new fats for food?**
- **Here we explore the unique pharmacological potential, composition, characteristics and properties, and behavior of these two unusual oils.**

## ANDIROBA OIL

Andiroba (*Carapa guianensis*) is among the most important and well-known Amazonian trees. Obtaining oil from the seeds (Fig. 1) is a practice that has increased, due to the oil's unique characteristics and properties. The pharmacological potential of andiroba oil has been confirmed by many studies, arousing considerable interest in the pharmaceutical and cosmetic industries (Ferrari, *et al.*, 2007; Mendonça, *et al.*, 2007).

**FIG. 1. Andiroba seeds** (Costa, P.P.P.R., 2012)

**TABLE 1. Quality and identity parameters of andiroba oil**

| Properties | |
|---|---|
| Acid value (mg KOH/g) | 3.63 |
| Peroxide value (meq.$O_2$/kg) | 4.72 |
| Iodine value (g $I_2$/100 g) | 62.18 |
| Saponification value (mg KOH/g) | 194.28 |
| Free fatty acids (%) | 1.82 |

**TABLE. 2. Fatty acid composition of andiroba oil**

| Fatty acid | g.100g$^{-1}$ |
|---|---|
| Lauric acid (C12:0) | 0.11 |
| Myristic acid (C14:0) | 0.18 |
| Pentadecylic acid (C15:0) | 0.15 |
| Palmitic acid (C16:0) | 27.34 |
| Palmitoleic acid (C16:1) | 0.95 |
| Margaric acid (C17:0) | 0.13 |
| Margaric-oleic acid (C17:1) | 0.26 |
| Stearic acid (C18:0) | 8.79 |
| Oleic acid (C18:1) | 48.44 |
| Vaccenic acid (C18:1) | 0.92 |
| Linoleic acid (C18:2) | 10.29 |
| Linolenic acid (C18:3) | 0.25 |
| Arachidic acid (C20:0) | 1.48 |
| Gadoleic acid (C20:1) | 0.14 |
| Behenic acid (C22:0) | 0.40 |
| Lignoceric acid (C24:0) | 0.27 |
| **∑ saturated** | **38.85** |
| **∑ unsaturated** | **61.15** |

Table 1 presents the quality and identity parameters of andiroba oil. Andiroba oil was in agreement with specifications of the *Codex Alimentarius* (1999) to acid (4.0 mg KOH/g) and peroxide values (15 meq.$O_2$/kg). Iodine and saponification values are correlated with the intrinsic properties of a sample, such as carbonic chain length and degree of unsaturation (*Codex Alimentarius*, 1999). Andiroba oil presented an iodine value of 62.18 (g $I_2$/100g), which is related to unsaturation of fatty acids (FA); see Table 2 (Shahidi, 2005). The shorter the fatty acid chain, the higher the saponification value, so andiroba oil presents a value of 194.28 (mg KOH/g) for this parameter (Rohman, *et al.*, 2017).

The main fatty acids in andiroba oil are palmitic and oleic acids, which correspond to 75% of the total FA composition; a relevant content of linoleic and stearic acids was also found, as shown in Table 2.

Approximately 67% of andiroba oil is composed by triacylglycerols (TAG), primarily monosaturated TAG (43.46%). Palmitic oleic oleic (POO) is the main TAG (21.10%) and includes two major fatty acids (oleic and palmitic). The lipid classes of andiroba oil are shown in Table 3.

Table 4 presents the parameters of andiroba oil's thermal behavior. A graph of the melting behavior shows one endothermic peak with a final temperature of 23.62°C, confirming that the oil is liquid at 25°C. Alternatively, the crystallization curve presents two exothermic peaks. Since andiroba oil is composed of 38.85% of saturated fatty acids (SFA) (palmitic and stearic acids, mainly), peak 1 is related to this fraction. Peak 2 is related to the unsaturated oil fraction, which includes oleic and linoleic acids.

**TABLE 3. Lipid classes of andiroba oil**

| Lipid Classes | % |
|---|---|
| TAG | 67.29 |
| DAG | 11.27 |
| MAG+FA | 21.44 |

*TAG:triacylglycerol; DAG: diacylglycerol; MAG: monoacylglycerol; FA: fatty acid

**TABLE 4. Thermal parameters of andiroba oil**

| Parameter | Toc | Tpc | ΔHc | Tfc |
|---|---|---|---|---|
| Melting-peak 1 | -22.31 | -6.21 | 56.24 | 23.62 |
| Crystalization-peak 1 | 12.75 | 0.13 | 32.91 | -30.74 |
| Crystalization-peak 2 | -44.74 | -47.56 | 5.69 | -51.40 |

*Onset temperature (To (°C)); peak temperature (Tp (°C)); enthalpy (ΔH (J/g)) and final temperature (Tf(°C)).

## PRACAXI OIL

Pracaxi (*Pentaclethra macroloba*) is a typical Amazon plant with little-explored lipid compounds. The plant is a natural source of behenic acid, which is obtained from the seeds (Fig. 2). Pracaxi oil is used as an ingredient in oil blends, soaps, moisturizers, exfoliators, skin cleaners, conditioners, shampoos, and other cosmetics (Teixeira, *et al.*, 2020). However, its physiochemical properties give it the potential to have broad applications (as the sole lipid material or in blends) in shortenings (margarine), confectionery (cakes and icing fats), cookies, other baked goods, ice cream, and other foods (Bezerra, *et al.*, 2017).

**FIG. 2. Pracaxi seeds** (Silva, J.L., 2018)

**TABLE 5. Quality and identity of pracaxi oil**

| Properties | |
|---|---|
| Acid value (mg KOH/g) | 6.08 |
| Peroxide value (meq.$O_2$/kg) | 2.09 |
| Iodine value (g $I_2$/100 g) | 67.86 |
| Saponification value (mg KOH/g) | 154.20 |
| Free fatty acids (%) | 3.05 |

Table 5 presents the quality and identity of pracaxi oil. Pracaxi oil was in agreement with specifications of the *Codex Alimentarius* (1999) to peroxide value (15 meq.$O_2$/kg); the acid value exceeded the maximum limit established, which can be attributed to the pracaxi cultivation and harvest conditions. Iodine and saponification values were 67.86 (g $I_2$/100 g) and 154.20 (mg KOH/g), respectively. These are in agreement with previous studies (Pereira, *et al.*, 2019; Serra, *et al.*, 2019).

Pracaxi oil presented 65.84% of unsaturated fatty acids (UFA), mainly oleic acid (50.08%); and 34.3% of saturated fatty acids (SFA); see Table 6. A relevant content of long-chain SFAs was found, represented by behenic (16.77%) and lignoceric (11.44%) acids. Behenic acid is a long-chain SFA with a low caloric value and has consequently been used to reduce calories in oils and fats. This FA reduces digestion of triacylglycerols by inhibiting pancreatic lipase, which increases excretion and low absorption of TAG in the intestine (Kojima, *et al.*, 2013). Thus, FA composition of pracaxi oil suggests that it can be useful in the production of low-calorie products (Pereira, *et al.*, 2019). Pracaxi oil showed 93.46% of TAG in the lipid classes that were found (Table 7).

As oleic acid is the main FA present, pracaxi oil showed high proportion of TAG with long-chain fatty acids . TAG containing behenic and lignoceric acids were also founded. Similar results was observed by Pereira *et al.* (2019), suggesting that pracaxi oil can be used to develop new products with specific characteristics.

Thermal analysis showed that pracaxi oil behaves in a way that is typical of vegetable oils (Table 8). Both crystallization and melting curves exhibited two distinct peaks, probably due to the variety of TAGs in the composition of this oil and differences in the carbon chain length and degree pf saturation (Table 6). The crystallization and melting peaks confirmed that this oil is liquid at ambient temperature.

Since saturated triacylglycerols crystallize at higher temperatures than unsaturated ones, the peak 1 of crystallization started at 21.79°C (onset temperature) and may be associated with monounsaturated and diunsaturated TAG. Peak 2 started at -43.78°C, the crystallization temperature of triunsaturared TAG.

Peak 1 of melting is probably related to the triunsaturated and diunsaturated TAG, which have lower melting temperatures (-15 to 22.8°C). The final temperature of peak 2 of melting (26.41°C) indicates that this oil was fully melted close to 25°C, which is characteristic of saturated TAG (O'Brien, 2009).

**TABLE 6. Fatty acid composition of pracaxi oil**

| Fatty acid | g.100g$^{-1}$ |
|---|---|
| Lauric acid (C12:0) | 0.08 |
| Myristic acid (C14:0) | 0.07 |
| Palmitic acid (C16:0) | 1.60 |
| Palmitoleic acid (C16:1) | 0.12 |
| Margaric acid (C17:0) | 0.07 |
| Margaric-oleic acid (C17:1) | 0.03 |
| Stearic acid (C18:0) | 3.13 |
| Oleic acid (C18:1) | 50.08 |
| Linoleic acid (C18:2) | 13.02 |
| Linolenic acid (C18:3) | 0.14 |
| Arachidic acid (C20:0) | 1.14 |
| Gadoleic acid (C20:1) | 1.49 |
| Behenic acid (C22:0) | 16.77 |
| Erucic acid (C22:1) | 0.82 |
| Lignoceric acid (C24:0) | 11.44 |
| Nervonic acid (C24:1) | 0.14 |
| **∑ saturated** | **34.3** |
| **∑ unsaturated** | **65.84** |

**TABLE 7. Lipid classes of pracaxi oil**

| Lipid Classes | % |
|---|---|
| TAG | 93.41 |
| DAG | 4.24 |
| MAG+FA | 2.35 |

*TAG:triacylglycerol; DAG: diacylglycerol; MAG: monoacylglycerol; FA: fatty acid.

*The authors of this article are with Fats and Oils Laboratory—UNICAMP (University of Campinas), Brazil. For more information, contact Kamila Ramponi Rodrigues de Godoi at kamila.ramponi@hotmail.com.*

**TABLE 8. Thermal parameters of pracaxi oil**

| Parameter | To | Tp | ΔH | Tf |
|---|---|---|---|---|
| Melting-peak 1 | -27.59±2.51 | -16.85±0.13 | 8.41±1.53 | -4.50±1.41 |
| Melting-peak 2 | -3.93±0.60 | 14.93±0.01 | 42.68±8.72 | 26.41±1.10 |
| Crystallization-peak 1 | 21.79±0.00 | 6.18±0.18 | 50.04±9.31 | -27.16±0.30 |
| Crystallization-peak 2 | -43.78±0.30 | -50.37±0.11 | 3.45±0.80 | -57.49±0.80 |

*Onset temperature (To (°C)); peak temperature (Tp (°C)); enthalpy (ΔH (J/g)) and final temperature (Tf(°C)).

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# A dietary fatty acid that may tweak energy metabolism

Deena B. Snoke and Martha A. Belury

Metabolic syndrome (MetS) is a threatening health issue in contemporary societies throughout the world. The diagnosis of MetS is based on a set of clinical measurements, including obesity, hyperglycemia, hypertension, and dyslipidemia. MetS diagnosis predicts for increased risk of several metabolic diseases which are leading causes of mortality worldwide: cardiovascular disease, type 2 diabetes mellitus, stroke, nonalcoholic fatty liver disease, dementia, and a variety of cancers (Fig. 1). Over the past several decades, the prevalence of MetS has increased to 1 in 3 adults in the United States and 1 in 4 adults globally. Depending on genetics, age, ethnicity, race, socioeconomic status, and other factors, this prevalence has been cited to range from as low as 10% to as high as 84% [1].

- **Individuals with metabolic syndrome exhibit higher risk of mortality and chronic positive energy balance.**
- **Recent studies have shown that while replacing saturated fats with mono- and polyunsaturated fats is beneficial to metabolic health, polyunsaturated fats seem to exhibit greater benefit.**
- **The fatty acyl composition of the mitochondrial phospholipid cardiolipin and its effect on muscle energy metabolism may be an important link to the beneficial health effects of consuming polyunsaturated fats.**

Energy balance is defined as an equal amount of energy intake (by consumption of food) and expenditure (by biological function and daily activities). Many individuals with MetS have chronic positive energy balance in which they take in more energy than they expend. This surplus energy is stored as adipose tissue and results in increased body mass. While they take in more energy than they expend, individuals with greater body mass also require more energy to regulate and maintain nutrient and blood supply to tissues and carry out necessary biological functions. Factors that increase obesity, and therefore increase the risk of MetS, include over-consumption of food, lack of physical activity, unresolved psychological stress, and poor sleep quality. Efforts to identify modifiable dietary and other environmental factors that decrease risk of MetS and its related comorbidities in healthy individuals are important to reducing the incidence of MetS and its related comorbidities. Furthermore, as personalized nutrition becomes a more popular scientific approach to improve health outcomes, identifying biological factors or biomarkers that may guide an individual's nutrition recommendations to reduce disease risk, improve overall health, or optimize athletic performance are of growing interest in the scientific community.

## DIETARY OILS: MORE THAN JUST A SOURCE OF CALORIES

Dietary fats are the most energy-dense macronutrient, with a total of 9 kilocalories of energy per gram—more than twice as much energy per gram as proteins and carbohydrates, which contain 4 kilocalories of energy per gram. In addition to the role of fats as an energy source for daily activities, different types of dietary fats serve as signaling molecules that mediate whole-body energy metabolism. The signals that fats can affect will dictate how an individual's body uses and stores calories for energy. Furthermore, many types of dietary fats are the building blocks of cellular membranes, which provide a physical barrier that separates the body's cells from their extracellular environment.

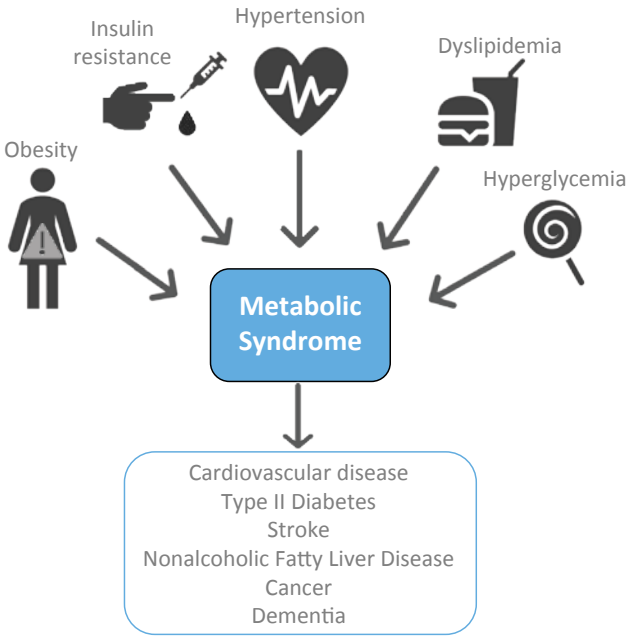


**FIG. 1. Clinical measurements of metabolic syndrome and increased risk of comorbidities. MetS consists of several clinical observations that include obesity, insulin resistance, hypertension, dyslipidemia, and hyperglycemia. Individuals with MetS have increased risk of developing a host of metabolic diseases, including cardiovascular disease, type 2 diabetes mellitus, nonalcoholic fatty liver disease, and cancer, as well as all-cause mortality.**

The types of fats consumed in the diet have an impact on an individual's health. According to US Centers for Disease Control and Prevention National Health and Nutrition Examination Survey (NHANES) data from 2017, the fat in the typical American diet currently consists of 33% saturated fats from food sources such as processed foods, baked goods, and meat products. As seen in Figure 2, roughly 34% is comprised of monounsaturated fats (primarily oleic acid) from such primary food sources as poultry, dairy, and nuts, while about 24% is comprised of polyunsaturated fats (primarily linoleic acid) coming from food sources such as seeds, nuts, and plant-based oils [2]. A recent meta-analysis of 15 randomized control trials and ~59,000 participants confirmed what has long been suggested about saturated fat consumption: that reducing saturated fats in the diet reduced the risk of combined cardiovascular events by 21% [3]. A different meta-analysis of 30 observational studies and clinical trials showed that replacing saturated fats with mono- or polyunsaturated fats was beneficial, but not to the same degree [4]. While replacement with monounsaturated fats improved high-density lipoprotein (HDL) cholesterol only, polyunsaturated fats improved HDL cholesterol but also decreased obesity, triglyceride levels, and risk of metabolic syndrome development. Yet, an important question remains: How exactly do polyunsaturated fats elicit all these positive benefits?

This is the exact question researchers in the lab where I worked as a graduate student under the supervision of Professor Martha Belury have been trying to answer for the past several years. In a study by Norris, *et al.*, published in 2009, we observed that linoleic acid-rich oil supplementation in postmenopausal obese women had positive effects on health outcomes. At the time, these findings were shocking: While these women had significant reductions in adipose tissue and fasting glucose levels, they also had a significant increase in lean body mass. This raises the question: Is it possible that the primary polyunsaturated fat consumed in the diet—linoleic acid—may serve an important role in muscle that leads to improvements in metabolic health?

## CARDIOLIPIN: POSSIBLE LINK TO IMPROVE METABOLISM

Another cellular membrane that dietary fats can influence is that of the mitochondria: an important eukaryotic organelle necessary to produce large amounts of cellular energy for movement that constitutes daily activity. Cardiolipin (1,3-bis(sn-3'-phosphatidyl)-sn-glycerol; CL) is a unique glycerophospholipid found only in mitochondrial and bacterial membranes [5]. CL serves as a lipophilic "anchor"' within the membrane, offering structural support for the protein machinery necessary to support energy needs of tissues with high energy demand, such as heart and skeletal muscle.

CL is most well-studied in the heart, where the majority of CL contains four chains of linoleic acid (tetralinoleoyl-CL; Fig. 3, page 36). Changes in CL acyl chain composition and how these species are remodeled have been implicated in several metabolic diseases, including heart failure, diabetic cardiomyopathy, and cancer. While these changes to CL and its remodeling are well-documented in the heart, little is known about the desired metabolic profile of CL in skeletal muscle—a tissue with high energy demand and a major regulator of glucose disposal—on measures of whole-body metabolic function.

The types of dietary fats consumed have also been reported to influence CL quantity and species, with a majority of studies investigating the influence of dietary fat on changes

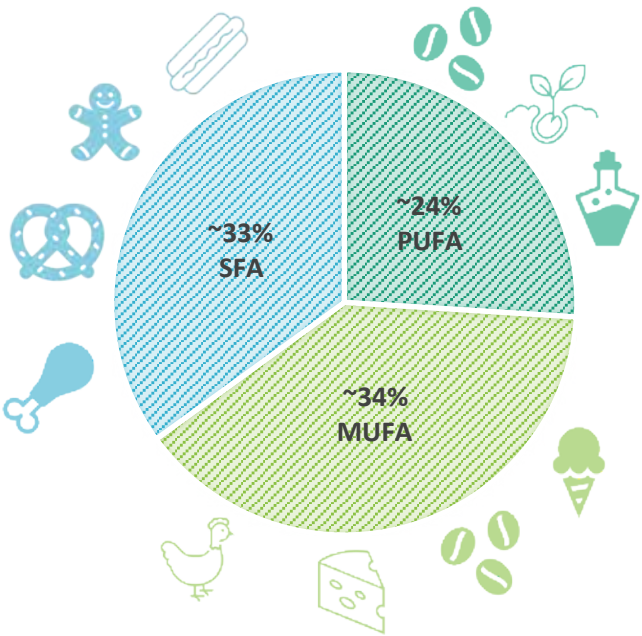


**FIG. 2. The typical distribution of the fats eaten in the United States. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.**

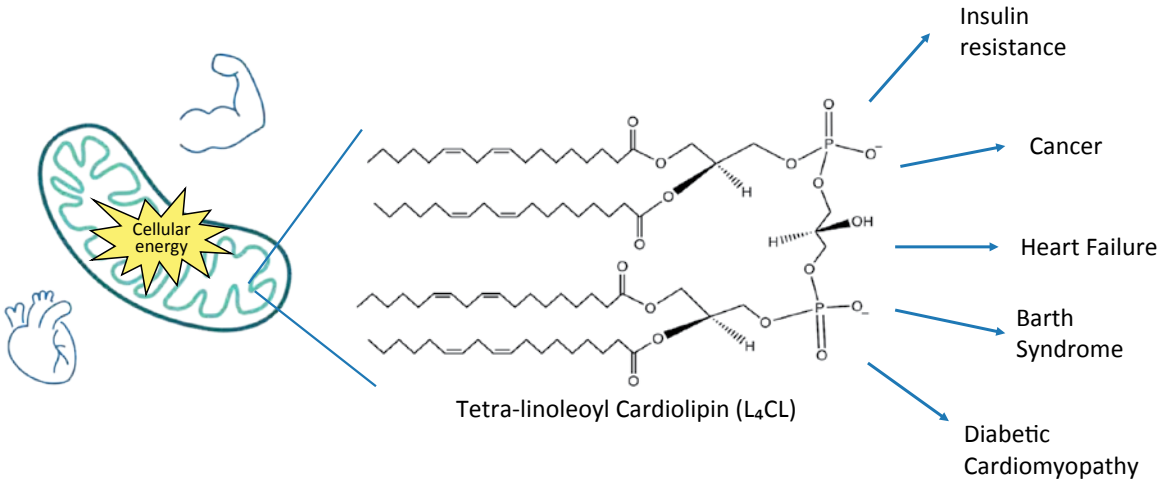


**FIG. 3. Cardiolipin (CL) is a phospholipid in the mitochondria that supports machinery used to make ATP energy. It is found in the inner mitochondrial membrane, where it structurally supports the machinery necessary for cellular energy production. This machinery is particularly important in tissues with high energy demands, such as heart and skeletal muscle. In the heart, 80–90% of CL contains four chains of linoleic acid. Changes to CL speciation have been implicated in a variety of metabolic conditions including insulin resistance, cancer, heart failure, Barth syndrome (a genetic disorder that results in non-functional CL remodeling), and diabetic cardiomyopathy.**

to CL speciation in liver and heart tissues [6]. Although mono- and polyunsaturated fats have been documented to show beneficial metabolic effects when replacing saturated fat in the diet, a comparison of how these fats may impact skeletal muscle CL has not been reported. Because of the role of skeletal muscle in regulating whole-body energy metabolism, it is important to understand how dietary fat composition influences CL in skeletal muscle and characterize how these changes impact whole-body energy metabolism. Perhaps CL is a key biological factor that may be responsible for the positive effects of polyunsaturated fats observed in studies that have been widely published and are rapidly accruing in recent years. In determining how CL responds to dietary fat composition, this could be used as a tool to determine an individual's risk of developing MetS-related health complications, or as a biomarker to develop a personalized approach to building and maintaining muscle mass and a healthy metabolism.

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*Deena Snoke completed her B.S. in Biology at Keene State College, New Hampshire, USA. There, she was first exposed to scientific research, spending three years in the undergraduate research lab of Professor Susan Whittemore. Snoke has spent the past six years at Ohio State under the supervision of Professor Martha Belury, where she recently defended her Ph.D. dissertation titled, "Investigations of lipophilic bioactive dietary components to improve aspects of metabolic dysregulation in mice." Snoke's graduate program experience was enriched by her leadership in her graduate organization as well as three predoctoral fellowships, including her recognition as the 2019–2020 AOCS Smouse Memorial Fellow. She is now a postdoctoral fellow in the lab of Professor Michael Toth at The University of Vermont, where she will study factors that mediate skeletal muscle atrophy during cancer treatment.*

*Martha Ann Belury earned her B.S. in nutrition /dietetics and her Ph.D. in biological sciences from the University of Texas at Austin. As the Carol S. Kennedy endowed professor in nutritional sciences at Ohio State University, she conducts translational research to identify mechanistic targets of dietary fatty acids that affect energy metabolism and inflammation. Along with many wonderful collaborators and student mentees, Belury has co-authored over 120 peer-reviewed publications in dietary modulation of energy metabolism and inflammation related to cancer cachexia, obesity, type 2 diabetes, and sarcopenia. She is Vice President-Elect of the American Society for Nutrition and an Elected Fellow in the American Association for the Advancement of Science (AAAS).*


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# Using fats and oils to replace worn body parts

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

**Rebecca Guenard**

Human joints routinely deteriorate from injury, aging and disease. Biomaterials designed to fill-in damaged areas can help; however, as effective as they are, it is difficult to improve upon the original cartilage or bone. Still, bone transplants are the second most common (blood is first) type of tissue replacement. Over 2 million grafting procedures are performed worldwide annually. In the coming years, this number is expected to increase with the longer life expectancy of a growing population.

Two recent reports on artificial joint repair show promise for improving on current technology. In the first, hydrogels were induced to form more natural cartilage when exposed to a magnetic field. A second research group grew a jaw bone from adipose tissue. An imminent clinical trial to test the later technology in humans indicates that scientists may finally have figured out how to stimulate the body to regrow more authentic tissue.

Building a joint is complicated since artificial tissue must function under load-bearing conditions. Ideally, scientists would like to retrieve cells from the patient and grow them on a biocompatible structure to be implanted into the body, where they independently develop into all the critical parts of a joint. The joint requires strong connective tissue that can bind to bone and withstand a range of forces, as well as stretch to various lengths. Additionally, cartilage covers the bone at the joint interface, distributing all the pressure across the joint. The cell density of this cartilage is concentrated closer to the bone and remains an unsolved clinical challenge.

A group of researchers at the University of Pennsylvania in Philadelphia, USA, have developed a means of joint tissue repair that addresses the thin layer of cartilage that often suffers damage (https://doi.org/10.1002/adfm.201807909). Inspiration came from self-healing polymers with embedded microcapsules that rupture when the material is sliced, releasing a polymerizing catalyst that repairs the area. The diverse team of engineers and physicians constructed poly(D,L-lactide-co-glycolide) acid hydrogels containing microcapsules filled with a growth factor necessary for the *in situ* formation of cartilage.

The microcapsules were formulated to have a tunable response to the dynamic mechanical stress that musculoskeletal tissues commonly experience by adjusting the ratio of lactic and glycolic acids. Equal proportions of the acids made a gel whose contents are released quickly, and higher lactic acid concentrations resulted in a gel that maintains the integrity of microcapsules longer.

Having proven that hydrogel microcapsules could be used long-term to deliver treatment to damaged cartilage, the team experimented with enhancing the biomimicry of their gels. Specifically, one of the limitations of engineered tissue is that cells are evenly distributed in the material; however, in native tissue there is a gradient, with cells concentrated toward the surface in contact with bone (https://doi.org/10.1002/adma.202005030). Previous researchers added metallic particles to the cells and exposed them to a magnetic field to separate them. Since such methods risk damaging the cells, the group instead added a magnetic liquid to their hydrogel solution. Once the cells and microcapsules were arranged in their desired placement, the magnetic liquid diffused out when the gel was cross-linked, leaving an engineered tissue similar to the real thing.

"These magneto-patterned engineered tissues better resemble the native tissue, in terms of their cell disposition and mechanical properties, compared to standard uniform synthetic materials or biologics that have been produced," said Robert Mauck, professor of orthopedic surgery and bioengineering at Penn Medicine, in a press release (https://tinyurl.com/pennpressrelease). "By locking cells and other drug-delivering agents in place via magneto-patterning, we are able to start tissues on the appropriate trajectory to produce better implants for cartilage repair."

The Penn team believes their technology may someday be used to fix cartilage damage or generate new cartilage on joint surfaces. A group at Columbia University, in New York, USA, is also making remarkable advances in tissue replacement. Researchers in the dentistry school synthesized a jawbone graft out of a pig's stomach fat. After implanting the fat-based graft, it grew a new joint in the animal.

Most bones in the body are straight; jawbones are curved. For this reason, anyone suffering from a jawbone injury or congenital disease rarely undergoes any kind of jawbone transplant. The joint at the end of a jawbone is particularly difficult to replicate because of the cartilage that connects it to the rest of the skull. Bioengineered replacements have not yet been able to incorporate both bone and cartilage.

In 2010, Gordana Vunjak-Novakovic, a biomedical engineer at Columbia University, proposed that using a patient's own cells, she could construct a graft that could be integrated into a defective area to regrow bone (https://doi.org/10.1089=ten.tea.2009.0164). The accomplishment would require a source of osteogenic cells, the only bone cells that divide. There are several sources for these types of cells throughout the body, including, skin, lungs, and fat. Vunjak-Novakovic chose to use stem cells from body fat to grow the bone, because of its abundance and easy access through liposuction. Her group cultured the adipose-derived stem cells on small cylinders of cow bone, the size of a pencil eraser.

## *Information*

The design of poly(lactide-co-glycolide) nanocarriers for medical applications, Essa, D., *et al.*, *Front. Bioeng. Biotechnol. 8*: 48, 2020.

Tissue-engineered autologous cartilage-bone grafts for temporomandibular joint regeneration, Chen, D., *et al.*, *Sci. Transl. Med. 12*: 565, 2020.

Mechanically activated microcapsules for 'on-demand' drug delivery in dynamically loaded musculoskeletal tissues, Mohanraj, B., *et al.*, *Adv. Funct. Mater. 29*: 15, 2019.

Bone grafts engineered from human adipose-derived stem cells in perfusion bioreactor, culture, Fröhlich, M., *et al.*, *Tissue Eng. Part A. 16*: 1, 2010.

The bone was thoroughly washed, and all bone marrow was removed to act as a scaffold for the osteogenic cells. After five weeks in a bioreactor, the engineered bone had grown sufficiently enough to be implanted.

After a decade of work, a successful implant was reported in an article published in Science Translational Medicine (https://doi.org/10.1126/scitranslmed.abb6683) in October 2020. In this case, each cow bone scaffold was precision milled to match the jawbone that had been removed from the pig for the experiment. After five weeks of growth, the grafts were inserted into the pigs.

For six months, the pigs ate and functioned as normal. Then the researchers sacrificed the animals to examine if the grafts took. They found that a new jawbone had grown in place of the old one and was indistinguishable from the one the researchers had removed. The scaffolding made of cow bone had been absorbed by the pigs' bodies and was no longer visible.

A clinical trial has been scheduled with six human participants who suffer from a severe birth defect that results in a shortened face and an open bite. The trial involves elongating their faces using engineered bone. The Columbia University team is cautiously optimistic that they will be able to close the bite of these patients. Given the results of the animal experiments, they also believe the regrown bone will be able to withstand the pressure the jaw experiences during biting and chewing. In the future, the technique can hopefully be applied to other bones and joints making the need for metal prosthetics obsolete.

Both recent breakthroughs indicate that in the future the degradation of active joints will cease to be an expectation of advancing age. Arthritis and discomfort after a healed injury will be a thing of the past.

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

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# Dutch web tool screens chemicals for potential SVHCs

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

A new web-based tool could quickly and simply screen for potential substances of very high concern (SVHCs), according to a team from the Dutch National Institute for Public Health and the Environment (RIVM).

The process of officially identifying chemicals as SVHCs under REACH is lengthy, and therefore it helps to prioritize which chemicals to consider for identification from the vast numbers on the market.

The team from RIVM and the Institute of Environmental Sciences (CML) used a Dutch list of SVHCs to develop models to assess whether chemicals are structurally similar to known SVHCs. The models compare chemicals with known hazardous properties:

- carcinogenicity, mutagenicity or reprotoxicity (CMR);
- endocrine disruption (ED); and
- persistent, bioaccumulative and toxic (PBT) properties.

The developers set a similarity threshold, above which a substance can be considered to be sufficiently structurally similar to an SVHC to suggest comparable toxicological effects.

"In comparison to many other screening models, which focus on the presence or absence of specific functional fragments or a mechanistic relation between chemical properties and specific effects, the current models focus on total chemical similarity," lead author, Pim Wassenaar, from RIVM, told *Chemical Watch*.

"When two chemicals are structurally very similar, they are likely to have similar chemical functionalities, and therefore comparable properties and effects. A high resemblance in chemical structure between a chemical with unknown properties and an SVHC could therefore be a trigger for further evaluation," he added.

By comparing chemicals to known SVHCs, the method has the advantage that it flags up specific concerns, such as mutagenicity, therefore guiding follow-up analysis. Structural similarity could also be used as a first step in defining substance groups for read-across approaches, Wassenaar suggested.

The researchers tested the structural similarity models using the "broader universe" of chemicals registered under REACH.

A group of chemists and toxicologists then assessed model predictions for 256 pairs consisting of substances with unknown SVHC properties and their SVHC matches. The experts agreed that 102 of the 256 substances could indeed be considered potential SVHCs.

The results suggest the models work well for finding potential CMR (carcinogenic, mutagenic or toxic to reproduction) and ED (endocrine disruptor) substances but is less predictive for PBT (Persistent Bioaccumulative Toxic) substances. One reason for this could be that the model doesn't currently take into account the type of halogen in potential PBT substances, although future adjustments could change this.

In general, the structural similarity models show "great potential" for screening and prioritization purposes, the team reports in the journal *Regulatory Toxicology and Pharmacology* (open access at https://doi.org/10.1016/j.yrtph.2020.104834).

"By enhancing attention on chemicals of potential SVHC concern as early as possible— both within regulatory frameworks, product development and chemical synthesis—this methodology can help in avoiding regrettable substitution, and contribute in the transition towards a non-toxic environment," Wassenaar said.

The web tool containing the models is freely available online at https://rvszoeksysteem.rivm.nl/ZzsSimilarityTool.

# Meet Hans Christian Holm

*Member Spotlight is a slice of life that helps AOCS members get to know each other on a more personal level.*


**Hans Christian Holm and his wife, Ninna, are pictured in July 2020 during a seven-day, 1,075-kilometer (670-mile) charity bike ride around Denmark (www.team-rynkeby.com). Their team collected 100,000 euros for critically ill children through their efforts.**

## Fast facts

| | |
|---|---|
| **Name** | Hans Christian Holm |
| **Joined AOCS** | 1999 |
| **Education** | M.Sci. from Danish Technical University, followed by a bachelor's degree in commerce from Copenhagen Business School. |
| **Job title** | Science Manager |
| **Employer** | Novozymes A/S (Denmark) |
| **Current AOCS involvement** | Session chair and presenter, past vice chair of the Processing Division, and member-at-large of the Biotechnology Division |

## PROFESSIONAL

*My favorite part of my job is…*

My favorite part of my job is challenging the status quo. In meetings with customers and partners, the idea that "the customer is always right" is often pure nonsense. I really love the challenge of understanding why the customer expresses a certain desire and find that it is often possible to do much better after merging/sparring over our respective competencies.

*Is there an achievement or contribution you are most proud of? Why?*

Professionally, three major achievements come to mind. First, in 2005, ADM and Novozymes received a US Green Chemistry Challenge Award. Then, in 2008, I was awarded the Euro Fed Lipid Technology Award. Both awards were given for the use of enzymes to create margarine and shortening free of trans fatty acids. Finally, as president of Euro Fed Lipid (2018–2019) I had the pleasure of seeing restructuring efforts result in sustainable economics for our federation. Such achievements are always the result of the work of a great team, and it is the teamwork I appreciate.

*What event, person, or life experience has had the most influence on the direction of your life?*

Watching my four kids grow has been so rewarding. My wife Ninna and I are as far from being a soccer mom/soccer dad as you can get, but we still managed to induce proper values and inspire them to pursue proper developmental paths. In fact, our oldest son became a medical doctor in 2020; our oldest daughter received a master's degree in chemical engineering; our next son began studying biotechnology at Danish Technical University; and our youngest daughter decided to become a pediatrician. (University is still four years in the future for her. It is free of charge in Denmark; we just pay a lot of taxes.)

## PERSONAL

*How do you relax after a hard day of work?*

I enjoy listening to books—mostly thrillers—but because our house is an old brick house from 1891, quite some house repair is needed. Really relaxing involves taking the kids to a soccer match or watching "Sunday Night Football." (I have a hard time being a Giants fan.)

*What is the most impressive thing you know how to do?*

Cooking for a large group of people (100+ people from infants to grandparents who spend a week every year in the small Kingdom of Elleore—www.elleore.dk). I once grilled 75 pheasants and then prepared a stew by mixing the meat with 12 bottles of port wine and 20 liters of cream, with some veggies and rice on the side.

*What skill would you like to master?*

I would love to master patience, but I never had enough patience to get there.

# PATENTS

## Chocolate, method for producing chocolate-covered food product coated by the same, and method for preventing increase in viscosity of chocolate for coating

Oonishi, K., *et al.*, The Nisshin OilliO Group, Ltd., US10716316, July 21, 2020

Features of the present disclosure provide a chocolate with good heat-resistance, bloom resistance, and melt-in-the-mouth property, disclose a production method by which a chocolate-covered food product coated with the chocolate is produced, and disclose a method for preventing an increase in the viscosity of chocolate mix. Disclosed is a method for producing a chocolate comprising an addition step for adding a seeding agent containing at least .beta.-StOSt crystal to chocolate mix in a melted state, wherein the fat content of the chocolate mix is 26 to 70 mass % StOSt (where StOSt is 1,3-distearoyl-2-oleylglycerol).

## Non-winterized, standardized marine source oil products and methods of making thereof

Martinsen, B.R., *et al.*, Ambo Innovations, LLC, US10722542, July 28, 2020

Disclosed is a liquid medicament/supplement composition including a non-winterized marine source oil (e.g., oil derived from fish, krill, and/or squid), a food grade or pharmaceutically acceptable form of vitamin D.sub.3 or a derivative thereof admixed in the non-winterized marine source oil; a food grade or pharmaceutically acceptable form of vitamin A or a derivative thereof admixed in the non-winterized marine source oil; optionally a food grade or pharmaceutically acceptable form of CoQ10 or a derivative thereof admixed in the non-winterized marine source oil; a food grade or pharmaceutically acceptable form of concentrated eicosapentaenoic acid and docosahexaenoic acid or ethyl ester; glyceride ester or salt of the acid and polyphenol rich vegetable oil admixed in the non-winterized marine source oil; and optionally a food grade or pharmaceutically acceptable form of melatonin or a derivative thereof admixed in the non-winterized marine source oil. The liquid medicament/supplement composition preferably has an oxidation amount measured as totox of less than 5, and the liquid medicament/supplement composition preferably has an overall eicosapentaenoic acid to docosahexaenoic acid ratio (DHA:EPA) ranging from 2:1 to 1:2 at a concentration ranging from 15 to 35 wt % of total liquid weight calculated as weight % of the corresponding free acid.

## Process for manufacturing purified glycerol

Visser, D., *et al.*, Purac Biochem B.V., US10723681, July 28, 2020

A process for manufacturing purified glycerol including the steps of providing a starting glycerol fraction comprising glycerol, water, and fatty acid methyl esters; subjecting the glycerol fraction to a partial evaporation to form an evaporated fraction including glycerol, water, and fatty acid methyl esters, and a remainder fraction including glycerol; condensing the evaporated fraction to form a liquid; subjecting the liquid evaporated fraction including glycerol, water, and fatty acid methyl esters to a liquid-liquid separation step, resulting in the formation of a fatty acid methyl ester fraction and a glycerol-based fraction including glycerol and water. The process makes it possible to efficiently separate the fatty acid methyl esters from glycerol, without the need for complete glycerol distillation. Also provides glycerol fractions suitable for use as carbon source in fermentation processes, without problems in down-stream processing, and without the need for cost-intensive purification steps for the glycerol.

## Methods and compositions for preparing triglycerides containing fatty acid vicinal diester functionality

Benecke, H.P., *et al.*, Battelle Memorial Institute, US10731105, August 4, 2020

A method for producing a triglyceride including fatty acids with vicinal diesters: (a) providing a triglyceride including fatty acids with epoxide groups; (b) reacting the epoxide groups with carboxylic acid salts under basic conditions to produce a triglyceride including fatty acids with vicinal ester/alkoxides; (c) protonating the vicinal ester/alkoxides to produce a triglyceride including fatty acids with vicinal ester/alcohols; and (d) reacting the vicinal ester/alcohols with carboxylic acids under acidic conditions to produce a triglyceride including fatty acids with vicinal diesters.



## Dough with a lipolytic enzyme and/or xylanase and a monooxygenase

Lundkvist, H., *et al.*, Novozymes A/S, US10743551, August 18, 2020

A method for preparing a dough or a baked product prepared from the dough which method comprises incorporating into the dough a lipolytic enzyme and/or a xylanase and an X143 polypeptide, wherein the X143 polypeptide is a monooxygenase.

*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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## JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY


- Thermal degradation of p-hydroxybenzoic acid in macadamia nut oil, olive oil, and corn oil, Castada, H., *et al.*, *J. Am. Oil Chem.' Soc. 97*: 289, 2020, http://dx.doi.org/10.1002/aocs.12331.
- Formation kinetics of monomeric cyclic fatty acid methyl esters of alpha-linolenic acid: effects of mono cis/trans isomers, Angers, P., *et al.*, *J. Am. Oil Chem'. Soc. 97*: 615, 2020, http://dx.doi.org/10.1002/aocs.12348.
- Rapid nondestructive analysis of intact canola seeds using a handheld near-infrared spectrometer, Barthet, V., *et al.*, *J. Am. Oil Chem.' Soc. 97*: 577, 2020, http://dx.doi.org/10.1002/aocs.12335.
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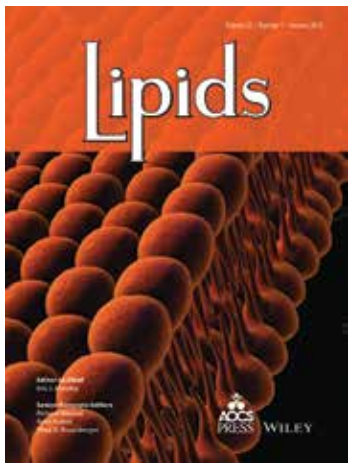
## LIPIDS




- Lipid profiles of five essential phospholipid preparations for the treatment of nonalcoholic fatty liver disease: a comparative study, Fricker, G., *et al.*, *Lipids 55*: 271, 2020, http://dx.doi.org/10.1002/lipd.12236.
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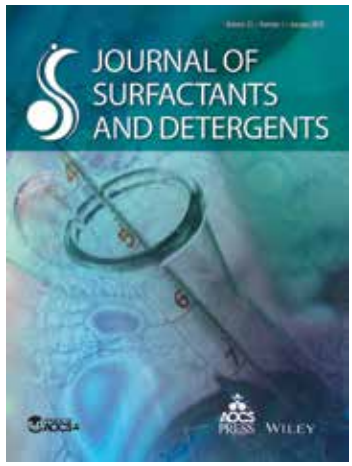
- The fifth WS/DGAT enzyme of the bacterium *Marinobacter aquaeolei* VT8, Feussner, I., *et al.*, *Lipids 55*: 579, 2020, http://dx.doi.org/10.1002/lipd.12250..
- Quantification of fatty acids and their regioisomeric distribution in triacylglycerols from porcine and bovine sources using 13C NMR spectroscopy, Kildahl-Andersen, G., *et al.*, *Lipids*, online September 2020, http://dx.doi.org/10.1002/lipd.12277.
- Cholesteryl ester transfer protein impairs triglyceride clearance via androgen receptor in male mice, Stafford, J., *et al.*, *Lipids*, online August 2020, http://dx.doi.org/10.1002/lipd.12271.

## JOURNAL OF SURFACTANTS AND DETERGENTS




- A new method to evaluate the antimicrobial efficacy of domestic laundry detergents, Bockmuehl, D., *et al.*, *J. Surfact. Deterg. 23*: 629, 2020, http://dx.doi.org/10.1002/jsde.12401.
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- The application of a nuclease enzyme to clean stubborn soils and odors in laundry, Morales-Garcia, A., *et al.*, *J. Surfact. Deterg. 23*: 797, 2020, http://dx.doi.org/10.1002/jsde.12398.

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PCP Protein and Co-Products
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## Original Articles

### IOP PRO Effect of additive structure on the performance of biodiesel fuel winterization

Abe, M., *et al.*, *Fuel*, online December 4, 2020, 119747, https://doi.org/10.1016/j.fuel.2020.119747.

The effects of additive structure on the separation of saturated fatty acid methyl esters (FAMEs) from FAME mixtures by winterization were investigated. Six sorbitan derivatives, seven palmitate derivatives, and sorbitan monopalmitate, which is known to improve the low-temperature separation of FAME mixtures, were studied. A model FAME mixture was prepared by blending saturated FAME (methyl palmitate) and unsaturated FAME (methyl oleate). Sorbitan derivatives that had fatty-acid groups with the same carbon chain length as the main saturated FAME in the FAME mixture improved the separation significantly. Shorter chain lengths and unsaturated fatty acid groups did not promote the winterization of FAME mixtures because their interactions with the main saturated FAME were too weak. Cyclic structures and hydroxy (OH) groups in the ester groups of the palmitate derivatives were found to be essential for preventing crystal growth and liquid contamination of the recovered solid phase. Cyclic structures affected the appearance of the FAME mixture during winterization, and the presence of OH groups affected the separation factor and the cloud point (CP) of the recovered liquid FAME. Span40, which has palmitate and sorbitan groups, was found to be the most effective additive for mixtures containing approximately 50 wt% methyl palmitate. The CP of the recovered liquid decreased 4–9 K, and the separation factors were between 3.6 and 7.5. These results will be useful for the separation and purification of saturated rich-FAME mixture such as palm, lard, mahua, neem, and jatropha biodiesels.

### EAT H&N Functional characterization and sensory evaluation of a novel symbiotic okara beverage

Voss, G.B., *et al.*, *Food Chem. 340*: 127793, 2021, https://doi.org/10.1016/j.foodchem.2020.127793.

This study aimed to produce four different beverages from okara (soybean by-product) previously hydrolyzed by *Cynara cardunculus* enzymes and fermented by probiotic bacteria or unfermented beverage. The probiotic viable cells, the isoflavones profile, and organic acids were evaluated. In addition, total phenolic content, antioxidant, and ACE inhibitory activities of all beverages were evaluated at storage time and during *in-vitro* gastrointestinal digestion. The probiotic was viable throughout storage. Significant bioconversion of the isoflavone glycosides into their corresponding bioactive aglycones was observed. Furthermore, the beverages showed good ACE inhibitory activity. After passing through the gastrointestinal model, all beverages showed an increase in antioxidant and ACE inhibitory activities. These findings indicate that use of okara in multifunctional beverages could be a promising strategy for preventing disease prevention and contributing to a zero waste approach in the food industry.

### LOQ H&N Bioactive compounds and antioxidant capacity of grape pomace flours

Monteiro, G.C., *et al.*, *LWT–Food Sci. Technol. 135*:110053, 2021, https://doi.org/10.1016/j.lwt.2020.110053.

Grape pomace is the main residue from juice and wine industries, and their derived products, such as flours, can be a rich source of phenolic compounds and antioxidants. The overall objective of this study was to assess the phenolic compounds, antioxidant capacity, biogenic amines, and amino acids in these flours. Four cultivars (Niagara Rosada, Bordo, BRS Violeta, and IAC 138-22 Máximo) were used, isolated, or used as blends for juice production; the resulting pomace was used to produce flours. All flours assessed, independently of the cultivar or blend, showed valuable potential as additives or supplements to improve the antioxidant and bioactive content of food, cosmetic, and pharmaceutical products. The lowest levels of phenolic compounds and antioxidant capacity were observed in the flour produced entirely from cultivar Niagara Rosada. However, when this cultivar was incorporated in blends with other cultivars, flours with increased levels of phenolic compounds and antioxidant capacity were obtained.

## LOQ H&N Valorization of sunflower by-product using microwave-assisted extraction to obtain a rich protein flour: recovery of chlorogenic acid, phenolic content, and antioxidant capacity

Náthia-Neves, G. and E. Alonso, *Food Bioprod. Process.125*: 57–67, 2021, https://doi.org/10.1016/j.fbp.2020.10.008.

The sunflower cake, a by-product from sunflower oil refining, is a rich source of chlorogenic acid (CGA), a phenolic compound that must be removed before the oil can be used for human consumption. This work studied the extraction of CGA from this by-product using microwave-assisted extraction (MAE). We began by identifying conditions of solvent and solvent-to-feed ratio (S/F) that maximized the CGA extraction. The highest CGA yield was obtained using 70% ethanol, but varying the S/F did not have a significant effect on CGA yield. We then evaluated the effects of power (100, 200, and 300 W) and extraction time (30, 60, 90, and 120 seconds) on the extraction of CGA with 70% ethanol and an S/F of 10. MAE processes were evaluated in terms of global and CGA extraction yields, total phenolic content (TPC), and antioxidant capacity. The process allowed recovery of 8.4 ± 0.1 mg CGA/g of raw material in just 30 seconds. ORAC assay revealed that the extracts presented antioxidant capacity. FTIR spectra exhibited no significant differences with respect to the analyzed samples, and SEM images showed that the sunflower by-product structure was affected by the irradiation power. The MAE process proved to be a fast and efficient method to obtain CGA-rich extracts and a residual solid with a high content of protein (26%) and essential amino acids that allows its usage in products for human nutrition.

## LOQ H&N An efficiency strategy for extraction and recovery of ellagic acid from waste chestnut shell and its biological activity evaluation

An, J-Y., *et al.*, *Microchem. J. 160*: 105616, 2021, https://doi.org/10.1016/j.microc.2020.105616.

In this study, an efficient and green approach was established to extract and recover the highly valued phenolic compound, ellagic acid, from waste chestnut shells with choline chloride-based deep eutectic solvents (DESs). By utilizing optimized ultrasonic-assisted extraction conditions with choline chloride/n-propanol DES, solid–liquid ratio of 40 mg/mL, extraction time of 70 min and extraction power of 200 W, a highly efficient extraction value for ellagic acid of 4.64 mg/g was obtained. Meanwhile, ellagic acid was recovered easily with a recovery percentage of 94.9% by anti-solvent precipitation method through the denaturation of DES with water. The purity of ellagic acid in the extracts reached 85.6%, which is more than two times that via ethanol extraction methods (36.4%). In addition, the recovered ellagic acid exhibited strong antioxidant activity with IC50 (0.309 mg/mL) and significant antibacterial activity with minimal inhibitory concentration (MIC) (250 mm) and diameter of inhibition zone (DIZ) (17.60 microg/mL). The anti-solvent precipitation method denatured DES, led to higher extraction and recovery efficiency, and produced high purity ellagic acid simultaneously in one step. The novel method could add value to chestnut production by providing a valuable use for waste chestnut shells. This green, pollution-free alternative process could potentially be used to extract and recovery phenolic components from other natural resources for pharmaceutical and biochemical applications.

## LOQ H&N Valorization of black mulberry and grape seeds: chemical characterization and bioactive potential

Gómez-Mejía, E., *et al.*, *Food Chem. 337*: 127998, 2021, https://doi.org/10.1016/j.foodchem.2020.127998.

Grape (*Vitis vinifera* L. var. Albariño) and mulberry (*Morus nigra* L.) seed pomace were characterized in terms of tocopherols, organic acids, phenolic compounds, and bioactive properties. Higher contents of tocopherols (28 ± 1 mg/100 g fw) were obtained in mulberry, while grape seeds were richer in organic acids (79 ± 4 mg/100 g fw). The phenolic analysis of hydroethano-

lic extracts characterized grape seeds by catechin oligomers (36.0 ± 0.3 mg/g) and mulberry seeds by ellagic acid derivatives (3.14 ± 0.02 mg/g). Both exhibited high antimicrobial activity against multiresistant *Staphylococcus aureus* MIC = 5 mg/mL) and no cytotoxicity against carcinogenic and non-tumour primary liver (PLP) cells. Mulberry seeds revealed the strongest inhibition ($p < 0.05$) against thiobarbituric reactive substances (IC50 = 23 ± 2 µg/mL) and oxidative haemolysis (IC50 at 60 min = 46.0 ± 0.8 microgram/mL).

## PRO IOP Production of biolubricants from soybean oil: studies for an integrated process with the current biodiesel industry

Parente, E.J., *et al., Chem. Eng. Res. Des.*, online November 25, 2020, https://doi.org/10.1016/j.cherd.2020.11.012.

The competitiveness of the biodiesel industry may be improved by adding value through co-products and integration with the oleochemical industry, especially for novel products such as biolubricants, a product of increasing world demand. In this study, the synthesis of biolubricants from soybean oil was evaluated using transesterification, epoxidation, and oxirane ring opening reactions. Water, 2-ethylhexanol, and their mixtures were used to obtain hydroxyl-rich and/or ether-type branched molecules. All chemical modifications were monitored by Nuclear Magnetic Resonance (1H NMR) and evaluated through the physicochemical properties of the products. Several potential biolubricant samples were synthesized with viscosities at 40°C ranging from 26.6 to 99.6 cSt, viscosity index from 26 to 139, densities at 20 °C from 0.925 to 0.964 g/cm3, and pour points from -3 to −12°C. From these results, a proposal of a feasible industrial process to produce biolubricants from soybean oil is presented, consisting of 16 units, of which 15 may be integrated with an existing biodiesel plant.

## PRO IOP Sustainable diesel-range liquid fuel production via direct conversion of wet *Chlorella* sp. KR-1; critical impacts of water

Choi, I.-H., *et al.*, *Fuel*, online December 5, 2020, 119552, https://doi.org/10.1016/j.fuel.2020.119552.

Biodiesel production through direct conversion of wet algae has various advantages (economic feasibility, land use, emission of greenhouse gas, eco-friendly process, etc.). However, the effect of the water content of wet algae has not yet been clearly determined. The effect of water was thus investigated in the present study via the direct conversion of raw algae using sub- or supercritical methanol for sustainable liquid fuel production. To understand the reactions, gas-, liquid-, and solid-phase products were analyzed by various gas or liquid chromatography tools, including a high-voltage electron microscope and simulated distillate. In the direct conversion of raw *Chlorella* sp. KR-1, lipid extraction appeared to be the rate-determining step, and water contained in wet algae could improve the lipid extraction. It was found that 25 wt% water content of algae could increase the yields of fatty acid methyl esters at or below temperature of 250°C, which could be induced from enhanced interaction between methanol and algal lipids by water. Meanwhile, excessive water content (>25 wt%) caused undue hydrolysis, leading to lower yields of fatty acid methyl ester. Moreover, thermal decomposition and gasification progressed more in the reaction using wet algae than dried algae at higher temperatures (>250°C). Consequently, the critical impact of water could be optimized by those parameters toward maximizing the yield of fatty acid methyl esters.

## PRO IOP Production of renewable hydrocarbons from vegetable oil refining by-product/waste soapstock over selective sulfur-free high metal loading SiO2-Al2O3 supported Ni catalyst via hydrotreatment

Malins, K., *J. Clean Prod.*, online November 30, 2020, 125306, https://doi.org/10.1016/j.jclepro.2020.125306.

The performance, selectivity, recovery, and reusability of high metal loading commercial Ni66±5%/SiO2-Al2O3 catalyst were studied in renewable hydrocarbon synthesis from low-cost raw material pretreated soapstock (TS) by solvent-free one-pot hydrotreatment (HT). TS was extracted from rapeseed oil chemical refining by-product/waste soapstock (SS) by developed simple treatment method. The catalyst was characterized by FE-SEM, TEM, XRF, XRD, and N2 sorption analysis. Feedstock, liquid, and gaseous products were analyzed by FT-IR, GC-FID, GC-MS, GC-TCD, and CHNS elemental analysis. The effect of initial $H_2$ pressure (2–8 MPa), catalyst amount (3–9%), reaction temperature (280–340°C), and residence time (15–60 min) on hydrocarbon production were investigated. Combustible gases and pure marketable linear saturated hydrocarbons with high yield 79.1%, calorific value 47.22 MJ/kg, C/H ratio 5.6, and n-C17 content 82.7% were successfully produced from TS using minimum effective catalyst amount and reaction conditions: 5%, 6 MPa, 320°C, and 30 min.

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