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Enzymes in oil processing: a search for milder, more sustainable, and economical solutions
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The world of biotech manufacturing is comprised of start-ups and strongholds. Companies that have manufactured consumer goods for over 200 years innovate alongside businesses that popped-up just over two years ago. Since 2014, investment in biotech has risen (http://tinyurl.com/y5fythht), and the industry is poised to breach the boundaries of its initial success in the pharmaceutical sector and enter a new realm.

• A diverse set of products is entering the consumer goods market thanks to the successful application of biotechnology.

• Manufacturers are routinely editing genes to investigate the viability of synthesizing a cellular product that will improve or replace the components used in automobiles, food, textiles, personal care, home care, and other products.

• The technology has become economically competitive by developing high-throughput testing and finding opportunity in the products consumers use every day.

“In the healthcare segment, people have seen the value and the power of a technology that can solve and cure diseases. That could never have been done before,” says Michael Arbige, vice president of research and development at DuPont Industrial Biosciences in Wilmington, Delaware, USA. “What we are doing is just an extension of those same tools and those same capabilities applied to very important industrial problems, problems that affect the world every day.”

Having reined in the lengthy product development process that once plagued progress, biotechnology has become economically viable for application in everyday products. High-throughput screening of microbial alterations enables researchers to tackle reverse-engineering problems in short order. Ironing out sequencing wrinkles quickly frees up time for optimizing full-scale manufacturing processes. For some products, the improved efficiency of biotech manufacturing coupled with the ability of biotech to fine-tune a molecule’s specifications gives them a competitive edge in the market. Many consumers and product developers are also making naturally derived materials a priority, and it is obvious that the current market is ripe for the success of biotech in consumer goods. This article looks at how two very different companies use this technology to find success in the market.

A SIZE-DEPENDENT APPROACH

When DuPont established its Industrial Biosciences division in 2011, the company had already spent 30 years in the research and development of marketable biotech products (http://biosciences.dupont.com/). During that time, the company also acquired many of the early biotech start-ups, gaining manufacturing facilities around the world. Having teams of scientists hone the biotech manufacturing process over decades has advantages.

Arbige says DuPont Industrial Biosciences commercializes over 50 products a year. They work on problems in animal nutrition, food and beverages, personal care, fabric and home care, and textile processing,
to name a few of the industries they serve. “We have a reputation that is very powerful out there and allows us to get into partnerships and gain access to different customers. It opens lots of doors,” Arbige says.

He acknowledges that having the capital backing provided by a large corporation means researchers can gamble on new ideas that may fail. He says that by attempting new things over the years and quickly recovering from disappointment, DuPont has built a turn-key biomanufacturing system. According to Arbige, DuPont has put a lot of science into developing a fast, robust process to go from finding a new molecule to manufacturing it at production scale. And, he says, this can be done for any of their product areas.

“One of the things which is foundational to the biotech effort at DuPont is to manipulate microbes to make products better, cheaper, and more efficiently across a range of hosts—and for a range of products,” Arbige says.

Despite millions in investment dollars flowing into new biotech start-ups, they do not have the infrastructure to support this type of approach to product development (http://tinyurl.com/y5fythht). That means small companies have the best chance at success with a product that fills a niche.

“When you are a small company like us, you can’t explore too many things at one time,” says Scott Franklin, co-founder and chief scientific officer at Checkerspot, a materials biotech start-up in Berkeley, California, USA. As a former vice president of Solazyme, which filed for bankruptcy in 2017, after unsuccessful attempts at economical production of first biofuels then food oils, Franklin says he has learned from the mistakes of the early days.

Checkerspot’s objective is to manufacture products with improved performance and better environmental sustainability than those that are petrochemically derived. They have partnered with textile and outdoor gear companies to help them create moisture-wicking coatings for T-shirts, and they are working on polyurethane composites for a variety of applications, like skis and surfboards. They address these different applications by optimizing triglycerides for conversion to chemically versatile starting materials, such as polyols.

“We are very deliberate about developing a polyol, getting it into a material, and getting that material into our brand to animate what we can do with our technology,” Franklin says. Bioengineered microalgae are central to this process.

**MICROALGAE, SEEDS OF CHANGE**

Algae have been considered a potential source of oleochemicals for a long time due to their ability to produce large amounts of oil on fewer acres than typical oilseed crops. That is especially true for certain types of microalgae that can be raised on sugar inside a fermentation tank. Checkerspot further expands the organisms’ utility by genetically modifying their DNA so the microalgae bulge with triglycerides containing purposefully designed chemistry (Fig. 1, page 8).

Some seed oils, like castor, naturally produce a fatty acid with a hydroxyl group that when reacted with an isocyanate creates a urethane linkage (see structure of castor oil triglyceride, Fig. 2, page 8.). Other oil seeds contain fatty acids with points of unsaturation that can be converted into hydroxyl groups for the same purpose. Neither type of plant oil is ideal for synthesizing a polyurethane, according to Franklin, because the Natural Oil Polyols (NOPs) are all 18 carbons long with chemical functionality in a limited number of locations. When the urethane is polymerized, the fatty acyl dangles from the chain often imparting an undesirable elasticity to the final polyurethane material.
At Checkerspot, scientists are manipulating the cellular activity of the microalgae to produce fatty acids with a variety of chain lengths and with functionality in multiple locations. “When we think about engineering triglycerides—as it relates to making polyols and using those for polyurethanes—what we are trying to get to and explore is outside the narrow range that exists today,” Franklin says.

As is typical of biotech companies, nature inspires the innovations at Checkerspot. They searched for plants that produce seed oil containing shorter fatty acid chains. They found a plant that produces seed oil with fatty acid chains of 10 and 12 carbons that contain double bonds and hydroxyl groups throughout. By studying the biosynthesis of the oil, Franklin and his team can usurp the plant’s techniques and bioengineer those into their microalgae.

“He says they have successfully engineered the enzymes within their microalgae to install a double bond at a precise location in the carbon chain. These changes have a direct impact on the properties of the polyurethane that will ultimately be the final product. By this means, Checkerspot can make coatings, adhesives, foams, resins, or elastomers from a sustainable source that are nothing like what is available from oleochemicals currently on the market.

“When you start to combine all this technology, the universe of oleochemicals gets very, very big now,” says Franklin. He says Checkerspot is interested in making renewable lubricants and renewable dielectric fluids that have properties that are not possible with the currently available triglycerides.

While the company explores the capabilities of their bioengineered organisms to synthesize new triglycerides, they maintain a focus on the development and formulation of their current product for outdoor gear. Franklin says they have an organism that makes an oil at an impressively high percentage of the dry cell weight, meaning most of the cell is a triglyceride (Fig. 3). They have built a foundation of experience making polyols from that triglyceride and formulating it into polyurethanes designed for specific applications. He says as this product comes online, he looks forward to applying all Checkerspot has learned to developing their next product. “There is not enough time in my lifetime to explore all the possibilities of all the potential materials that we can make,” Franklin says.

ENZYMES, ENZYMES EVERYWHERE

Unlike a start-up, a company like DuPont has the liberty to explore multiple biotech product applications in tandem. In addition, established partnerships mean there is no shortage of problems to solve. “For example, we will go to Procter &..."
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Gamble (P&G) and ask, “What are your big issues that you see coming up?” Arbige says.

When DuPont posed that question in 2012, P&G responded that they were interested in offering customers a more environmentally sustainable way to wash clothes. They asked DuPont to help them create a cold-water detergent. Arbige enlisted the Industrial Biosciences enzyme group to address the task.

“We have a whole class of materials that we make called enzymes which affect different industries such as the food industry, the fabric and household care industry, the animal nutrition industry, the personal care industry,” says Arbige. “Enzymes can breakdown or synthesize a wide range of the natural materials that are out there: starches, fats and oils, proteins, and all the cellulosic materials.”

For a detergent to work in cold water, the DuPont scientists knew that they would need to find an enzyme that could break down stains at low temperatures. Like Checkerspot, they turned to nature to find an organism that was already performing this task. “We go to cold-climate countries or the tops of mountains, and we find new microbes in these environments,” Arbige says. “We find the enzymes that are in those microbes, and then we have a starting place.”

Arbige adds that since they were interested in breaking down protein stains, in this instance they looked for a cold-water protease. He says, typically once they find an enzyme it is rarely close to operating as a product. The enzyme must function and be stable alongside other chemistries that are in a detergent, such as surfactants, builders, and chlorinating compounds. In addition, the detergent needs to work in various

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**Biotech lipids for sun protection**

To solve a problem with biotechnology, researchers often turn to nature to find a living organism that functions within the specific parameters of the problem as part of its daily existence. One group of organisms, haptophyte microalgae, may hold the solution to prevent coral reefs from dying.

In 2018, the state of Hawaii issued a ban on sunscreens containing the organic ultraviolet (UV) filters, octinoxate and oxybenzone [https://www.capitol.hawaii.gov]. A year later the city of Key West, Florida, USA, did the same [https://tinyurl.com/ycmlx7r8]. Now, Miami, Florida is considering a similar measure [https://tinyurl.com/yyyr6qb9]. The ban of these sunscreen ingredients results from research showing that the compounds are causing coral reefs to sicken and die [https://doi.org/10.1289/ehp.10966]. The US National Parks Service reports that 4,000 to 6,000 tons of sunscreen is washed from human skin into the ocean yearly.

While saving coral reefs is a priority for many consumers, so is avoiding skin damage from the sun’s UV rays. Sunscreen formulators need alternative filters that satisfy both concerns. A team of researchers investigated a family of lipids known as alkenones and found them superior to the banded filters when blocking UV light [https://doi.org/10.3390/cosmetics6010011].

Gregory O’Neil, organic chemistry professor at Western Washington University in Bellingham, Washington, USA, says that alkenones are well-known compounds that still need in-depth study to understand how they can be used in personal care applications. “The history of these alkenones is really fascinating,” O’Neil says. “But nobody has really focused on bioengineering or even the physiological role of these compounds.”

In addition to other specialists, O’Neil teamed up with Gabriella Baki, assistant professor of pharmaceutics at the University of Toledo in Toledo, Ohio, USA. Baki formulated the lipids into sunscreen and lipstick. While the ingredient functioned well in these products, it did not thicken the sunscreen adequately enough for spreading.

According to O’Neil, their findings indicate that more research on these compounds could prove valuable, especially if biotechnology were applied. He says little is known about the biosynthesis of alkenones by algae. Learning more about how organisms make the compounds could guide future research to bioengineer alkenones to act as naturally derived UV filters. “It would be nice to know how you might tweak the organism to change the alkenone profile,” says O’Neil. “Or, in our case, we would be interested in learning how to increase alkenone production within the algae.”

**REFERENCES**


regions around the world and be effective in a range of water chemistry.  

“With all these parameters in mind, we use protein engineering to change amino acids in the enzyme to other amino acids that give it new functionality in the detergent,” Arbige says. “We will modify that protein and study it under the conditions that we have designed to see if it works under those conditions.” He says the enzyme evolves over months to years before they begin to determine if they can make it economically by producing it through fermentation in microbes. Finally, after formulating it to work in their customer’s product the enzyme is commercialized.

Arbige says DuPont makes enzymes that are a sustainably sourced from natural ingredients, which can also improve a food’s nutritional value and reduce food waste. Typical emulsification compounds, for example, do not fit the demands of a clean label. DuPont produced an enzyme that works in-situ as an emulsifier to stabilize the interface between oil and water. In addition, they have created enzymes that extend the shelf-life of bread by breaking down certain starches that would cause the bread to go stale. Their most recent bioengineered enzyme product was designed to be an ingredient in yogurt. The enzyme converts sugar in yogurt into a prebiotic fiber that helps with digestion.

To keep bringing new biotech products to market, Arbige says you must maintain the innovative mindset of a small start-up despite being a big company. Failure happens all the time, and you cannot be afraid of it, he says, recover and move on quickly.

THE HIGH-THROUGHPUT ADVANTAGE

New biotechnology tools help with that speedy turnaround. Arbige says that for many of DuPont’s products, these tools make it possible to go from concept to production in little over a year. Characterization tools such as genome sequencing, transcriptome analysis, and metabolic analysis, have become faster and cheaper. This means scientists can see the outcome of their reverse-engineering efforts within hours.

The improved speed of computing means that Arbige and Franklin can search large databases of gene sequences for the natural species they model their engineered organisms on. This allows them to get to the starting line faster. Once there, they can go through a rapid succession of fine-tuning the organism and testing its metabolic pathways.

“We can expend a lot of effort doing molecular genetics and molecular biology on the organism to start to generate molecules that will be interesting,” Franklin says. “On the front end, as we are doing the work in the organism, the cost is incremental.” He adds that if they have an idea about engineering unsaturation into a triglyceride, they can test if their

![Diagram of Conagen's integrated manufacturing chain](Credit: Conagen Inc.)

**FIG. 4.** Conagen’s integrated manufacturing chain. (Credit: Conagen Inc.)

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**References**


idea was successful without having to ferment a large volume of microalgae.

Biotech companies have more options for product development as new businesses launch to serve a role once available only through government-funded programs. The Advance Biofuels and Bioproducts, Process Development Unit (http://abpdu.lbl.gov/about-us) is a US Department of Energy-funded laboratory operating as part of Lawrence Berkeley National Labs in Emeryville, California, USA. The facility opened in 2012 to assist biotechnology researchers who were interested in developing a microbe to produce at full-scale. Privately run companies such as Conagen, Inc. (http://conagen-inc.com) and Cultural Biosciences (https://www.culturebiosciences.com) are now establishing themselves to serve the same purpose (Fig. 4). With the number of synthetic biology start-ups increasing, fermentation space is at a premium, and these companies offer start-ups a chance to prove themselves to investors.

As more support industries become part of the infrastructure of biotechnology, the number of naturally derived consumer goods will grow. The industry has expanded beyond its original objective of replacing fossil fuels and into lower-volume, higher-value products that are more likely to bring economic success as a component of consumer goods. Everything from pet food to anti-aging cream to indoor carpet now has a biosynthesized option. Today, with a microbe, a gene-editing tool, and a computer algorithm, scientists can make any product they can imagine.

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In edible oil processing, refineries must comply with stringent environmental rules, while improving the efficiency of operational costs and delivery of good quality products. Current trends are to reduce chemical and solvent use, energy consumption, and environmental impact; improve safety; operate under milder conditions; and optimize the nutritional properties of end products. Enzymatic processes help achieve these objectives, and novel or improved enzyme production methods allow enzymes to be used in a more economical and user-friendly way. Crude edible oils resulting from seed or fruit extraction are further chemically or physically refined by applying successive degumming, neutralization, bleaching, winterizing, deodorization, and deacidification operations; these treatments generate side-streams and valuable co-products. Besides, downstream modification processes (interesterification, fractionation, and hydrogenation) make it possible to extend the use of vegetable oils in food products. Some enzymatic processes are proven, some are under development, and others are ready to be industrially implemented.

**ENZYME-ASSISTED EXTRACTION**

Seed oil extraction is generally carried out with a solvent; (iso-)hexane is preferred for efficiency reasons. Many alternative solvents have been reported in the literature, but most have not reached industrial implementation. While in the past, the use of enzymes was related to safety issues, interest in aqueous enzymatic extraction processes has been
revived due to increasing environmental and health concerns. Various proteases, cellulases and pectinases can be used, but most are expensive and have limited commercial availability. Enzymatic extraction avoids damage to proteins which can be classified as food-grade and, in general, oil extracted this way is lower in phospholipids and free fatty acids. The main drawback is a low oil yield due to emulsification problems. On the other hand, palm oil is extracted from the fresh fruit bunches by screw pressing after sterilization, stripping and digestion. However, the oil yield using this method is low compared to the oil content in the palm bunch. A process was recently developed that allows the stripped fruits to be enzymatically treated throughout the digester, which maximizes the oil yield after pressing; it also results in a substantial increase in the oil extraction rate (OER) and produces less effluent. Cellulolytic enzymes or specific cocktails are commercially available for this process, which is being prepared for implementation in palm oil mills.

ENZYMATIC DEGUMMING

In physical refining, degumming is sometimes difficult, with high oil losses and poor degumming efficiency. Phospholipids are classified as hydratable or non-hydratable. Hydratable phospholipids are easily removed by water-washing, although the non-hydratable ones require more severe chemical treatment. Cost-efficient and stable phospholipases, such as PLA₁, PLA₂, PLC, and PI-PLC (Fig. 1), are commercially available, allowing for significant improvement in degumming efficiency. Enzyme-assisted water degumming is already applied industrially on phospholipid-rich crude oils like soybean oil, using PLC, PLC/PLA₂, or PLC/PLA₂/PI-PLC cocktails. PLC convert the phospholipids into diglycerides, which contribute to oil yield increases, and phosphate esters, which result in a less neutral oil entrained in the residual gums.

PLC efficiency can be improved by adding a PLA or by incorporating a PI-PLC. The yield increase depends on the oil type and enzymes used. With the PLC/PLA₂/PI-PLC cocktail, an impressive 2.5% yield increase can be expected for some crude soybean oils; however, use of PLA causes the release of some free fatty acids. A more robust PLC that results in a significant increase in neutral oil yield was recently brought to market. In general, satisfactory phosphorus contents < 150–200 ppm can be obtained after enzyme-assisted water degumming.

Deep enzymatic degumming is applied on both crude or water degummed oils and typically uses PLA enzymes. In this case, all phospholipids can be converted into hydrolyzed phospholipids (lyso-lecithin) with release of free fatty acids, the latter being valorized in the deodorizer distillate, resulting in less neutral oil entrained in fewer gums. Reaching phosphorus contents below 5–10 ppm is possible, provided some phosphoric or citric acid is added upfront to condition the gums, and the pH is later adjusted using caustic soda before enzyme addition (Table 1).

Today, more robust and efficient PLA₁ and PLA₂ enzymes for full degumming that can operate at higher temperatures and do not require a pH adjustment after acid preconditioning are available. These improve the enzyme activity and reduce the risk of soap formation. Adding amylases during water degumming degrades the polysaccharides and slightly improves the oil yield, without having a significant effect on phospholipid removal; deep degumming combining PLA₁ and amylase is also possible but with very low benefit compared to

![FIG. 1. Specific activity of the different commercial phospholipases used for enzymatic degumming or for enzymatic gums de-oiling](image-url)
PLA₂ alone. Adding an acyltransferase capable of transferring a fatty acid from a phospholipid to a free sterol forming a sterol ester, may allow some oil yield increase during enzymatic water degumming with a PLC/PI-PLC cocktail.

Finally, treating soybean or rapeseed oils with chlorophyllases during water degumming helps chlorophyll split into porphyrin (water soluble) and phytol (oil soluble); these enzymes can degrade chlorophyll components to very low residual levels (< 50 ppb), which reduces bleaching earth consumption; this process was successfully tested on pilot scale. In alkali elts (< 50 ppb), which reduces bleaching earth consumption; can degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual levels; these enzymes degrade chlorophyll components to very low residual

**ENZYMATIC TREATMENT OF GUMS**

Wet gums produced during water degumming are either added back to the meal for animal feed or turned into food-grade lecithin. Today, the market for food-grade lecithin especially from soybean oil is not big enough to absorb all gums from water degumming. Hence, a large part is sold as such or blended back into the meal at reduced price. Several processes have been developed to better valorize these wet gums and enzymes play an important role in that.

**Enzymatic modification of lecithin**

Enzyme-catalyzed reactions are carried out to improve the properties of natural lecithin; the use of enzymes in these reactions offers possibilities of greater selectivity and products that cannot be made by chemical methods. Physical properties of lecithin are modified by phospholipase or another lipase. PLA₁ cannot be used as it fully hydrolyses the lecithin suppressing its emulsifying capacity; however, PLA₂ converts lecithin into lyso-lecithin while improving its oil/water (o/w) surfactant properties. Certain lipases can selectively hydrolyze the sn-1 fatty acid of PC which opens the door to the manufacture of new types of phospholipids.

**Enzymatic gums de-oiling**

An alternative to enzymatic degumming is enzymatic de-oiling of the wet gums (Fig. 2). It is applied on a side-stream with no impact on the refining process, and, globally, the enzyme consumption is 3–5 times lower compared to enzymatic degumming. Enzymatic gums de-oiling involves a phospholipase-assisted neutral oil recovery from gums obtained by water or acid degumming. PLA₂, PLA₃ and PLC or their cocktails can be used, the wet gums being split into lyso-lecithin or phosphate ester and recovered oil. When applied to soy gums, de-oiling with PLA₂ or PLA₃ allows 1.1–1.2% oil yield increase (calculated on crude oil), as seen in Table 1. De-oiling with PLC cocktails (PLC, PLC-PI, PLA₂) allows up to 1.4–1.5% oil yield increase; higher values may be expected if using combination of PLC cocktails and PLA. The main advantage of using PLC cocktails is that the recovered oil is low in free fatty acids; fortunately, the high DAG content results in only small increase when recycled back to the crude oil. Higher free fatty acid content in the recovered oil when using PLA₂ or PLA₃ makes it less attractive for food but does not pose a problem for biodiesel.

**ENZYMATIC SOAP STOCK SPLITTING**

Soap stock is the main co-product from the alkali refining; it consists of soaps, gums, and entrained neutral oil. Usually, alkaline soap stock is converted into fatty acids by treatment with strong acids like sulfuric acid. Processes that allow enzymatic conversion of the entrained glycerides into fatty acids are described; the enzymes typically used are lipases, which can be added before (alkaline detergent lipase) or after (non-regioselective lipase) the strong acid. The principal advantage is an easier phase separation due to less emulsifying effects by partial glycerides and phospholipids, allowing significant fatty acids yield increase.

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**TABLE 1. Deep enzymatic degumming of crude rapeseed oil and enzymatic de-oiling of soy gums**

| Table 1. Deep enzymatic degumming of crude rapeseed oil and enzymatic de-oiling of soy gums |
|-----------------------------------------------|-------------------------|----------------------|----------------------|
| **Deep Enzymatic Degumming**                  | **Enzymatic Gums De-oiling** |
| Crude rapeseed oil                            | Soy gums from water degumming |
| Crude oil                                     | PLA₂                   | PLC cocktail        |
| **Oil conditioning:**                         | **Enzyme reaction:**   | **Oil quality:**    |
| pH adjustment                                 | pH adjustment          | FFA (% as oleic acid) |
| **Enzyme reaction:**                         |                        | P (ppm by ICP)      |
| Time (hours)                                  | 2–3                    | DAG (%)             |
| Enzyme dosage (ppm)                          | 50                     | P (ppm by ICP)      |
| **Oil quality:**                              | 1.4                    | Oil yield increase (%) |
| FFA (% as oleic acid)                        | 1.8                    | 1.1–1.2             |
| P (ppm by ICP)                                | 477                    | 5–0                 |
| Ca + Mg (ppm by ICP)                         | 231                    | ~1.5                |
| **Enzyme reaction:**                         |                        | < 100               |
| Enzyme dosage (ppm)                          | 500                    | < 40                |
| **Oil quality:**                              | 4–20                   | 500                 |
| FFA (% as oleic acid)                        | 9–15                   | 2000                |
| DAG (%)                                      | ~1.5                   |                     |
| P (ppm by ICP)                                | < 100                  |                     |
| Oil yield increase (%)                       | 1.1–1.2                |                     |
ENZYMATIC REMEDIATION
Some crude oils (like palm or rice bran oils) may suffer from enzymatic degradation if they are not properly stored or processed after harvest. This leads to high free fatty acid and diglyceride contents and correspondingly high refining losses. Enzymatic remediation is defined as the use of specific lipases (esterase) to reform triglycerides by catalysing the condensation of free fatty acids on the partial glycerides present in the oil, with the water generated being removed by working at reduced pressure. For technical applications, glycerol can be added to assist the condensation reaction. Also, fatty acid distillates from physical refining can be converted into a mixture of mono-, di-, and triglycerides by reacting with glycerol in presence of an esterase, the re-synthetized oils being possibly used for biodiesel production.

ENZYMATIC FAT SPLITTING
The primary process to split glycerides into fatty acids requires stringent operating conditions that allow almost full conversion to be achieved in a short time. On the other side, enzymatic fat splitting with lipases can be performed at normal pressure and relatively low temperature; however, due to high enzyme consumption, very long splitting times, and low efficiency, it has not yet become an industrial alternative. Inroads for improved performance are being made, and some potential interests have been identified (enzymatic pre-treatment prior to thermal splitting or fatty acid distillate polishing).

Today, the key reason producers want to move toward enzymatic fat splitting is that the process operates at a much lower temperature, which delivers better product quality for both fatty acids and glycerin.

ENZYMATIC BIODIESEL
A lipase-assisted process can convert fatty acid distillates, used cooking oils, or other poor-grade oils into biodiesel. It consists of a combined hydrolysis and esterification reaction as both glycerides and free fatty acids are converted into methyl esters using methanol and some water.

At the end of the reaction, the light phase, still high in free fatty acids and bound glycerides, requires some caustic polishing to get biodiesel that meets international specs (EN 14214 or ASTM 6751). The enzymatic process is today implemented in various industrial plants, the resulting biodiesel being sold to the same trade specification as biodiesel created through traditional chemical processing.

ENZYMATIC INTERESTERIFICATION
Among other modification technologies involving fractionation and hydrogenation, enzymatic interesterification is a milder and cost-efficient alternative to the chemical process and is mostly applied to fully refined oils. The process uses lipases for random or specific rearrangement of the fatty acids on the glycerol modifying physical properties of the fat or yielding novel products.
Bulk fats for margarines and shortenings
Interest in this process increased when trans fatty acids were being phased out of foods for health concerns. In enzymatic interesterification, the oil flows continuously through interconnected packed-bed reactors filled with immobilized lipases (Fig. 3); it is particularly suitable for large quantities of bulk fat with minimal cross contamination. Besides reduced oil losses, the major advantage of the enzymatic process lies in the quality of the finished product: better oxidative stability, no color reversion, and no induced contaminants. Enzymatic interesterification is generally completed by a mild deodorization. Today, multiple plants with capacities ranging from 20 to 200 tpd are in operation worldwide, replacing chemical interesterification slowly but steadily.

Structured lipids
Sn-1,3 specific immobilized lipases are used in the production of high-value products, such as structured lipids for cocoa butter equivalents (CBE) or human milk fat substitutes (HMFS). For CBE, the enzymatic reaction leads to products containing high amounts of symmetrical SOS and POS, which when mixed with high POP palm mid fractions, allows to formulate CBE without using exotic fats. Today, several producers manufacture CBE from OOO enriched fats which are either high-oleic sunflower oils or prepared by enzymatic condensation of oleic acid on glycerol using an esterase. These OOO enriched fats will specifically exchange their fatty acids in sn-1,3 positions with stearic acid, preferably ethyl stearate or optionally high stearic triglyceride oil. A HMFS is particularly enriched in OPO and often produced from palm super stearin and oleic acid; this acidolysis reaction aims to replace palmitic acid by oleic acid at sn-1 and 3 positions. In both cases (CBE and HMFS), the resulting triglycerides are mixtures of original and new fats, which are further purified using short path distillation to preserve the stereo-specificity, and often dry or solvent fractionation to fine-tune the targeted composition.

OMEGA-3 CONCENTRATES FROM FISH OIL
The EPA/DHA content in most fish oils is lower than desired for efficient omega-3 supplementation. Strategies combining physicochemical processes and enzymes have been developed making it possible to increase this content. The first stage is a chemical or enzymatic esterification to produce ethyl esters followed by short path distillation to properly concentrate the desired EPA/DHA. The triacylglycerol form being better absorbed, enzymatic reaction using esterase enables, under vacuum, these EPA/DHA ethyl esters to be condensed on glycerol. This process is now practiced in several fish oil processing plants around the world.

Today, enzymes can be applied at various stages of oil processing. They are greener, cleaner, and more sustainable, use less energy, increase oil yields and quality, reduce or even completely replace harsh chemicals, and improve product properties.

Time will tell how quick and how far enzyme processes will replace current chemical processes in oil processing. But one thing is sure, if an enzymatic solution is cost competitive with its chemical alternative, the choice is clear: enzyme.

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Years ago, I was sitting in the office when I got a call from an engineer who was starting up a small deodorizer in a remote location. We were expecting an operating pressure of about 5 mbar. By today’s standards this is high, but due to a fault in the vacuum system the pressure would not go lower than 15 mbar. The customer was anxious to make some deodorized oil. So, on the grounds that it is better to do something than nothing, I suggested that he tried to run the deodorizer despite the unpromising conditions. To our surprise, the oil came out reasonably well.

Vacuum systems for deodorizers: the “ideal” deodorizing pressure

A look at the history of deodorizing reveals that much higher pressures than are customary today were once commonly used until the early part of the 20th century. Even deodorizing oil at atmospheric pressure was practiced—although the running costs were very high. (Lee and King, October 1937).

Modern deodorizers are usually designed to operate at about 2 to 3 mbar. When the Votator deodorizer was very popular in the 1950s, 60s, and 70s it was normal to deodorize oil at about 8 mbar (6 mm Hg), and when I started working in the edible oil industry in the 1980s, 5 mbar was regarded as a perfectly good pressure. So, what is the reason for operating at much lower pressures today, and how is it possible to deodorize oil at 15 mbar or even atmospheric pressure?

Deodorizers operate at much lower pressures today than they did in the past.

Is there an ideal deodorizing pressure and, if so, what is it?

This article takes a look at the economics of deodorizing at different pressures.

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FIG. 1. In a deodorizer, steam mixes with oil via perforated pipes and/or gas lift pumps to carry away volatile impurities, even when the vapor pressure of those impurities is far below the pressure in the head space.
The key to this conundrum is that injecting or “sparging” steam into oil can carry away volatile impurities even if the vapor pressure of those components is much less than the pressure in the head space of the deodorizer (Fig. 1.).

Triacylglycerol, also known as triglyceride, the main component of vegetable oil, has virtually no vapor pressure and is not distilled during deodorization. According to Raoult’s Law the vapor pressure of any volatile component dissolved in the oil is proportional to the vapor pressure of the pure substance multiplied by the molar concentration. For example, when deodorizing neutralized soybean oil, it is common to reduce the free fatty acid (FFA) level from about 0.1% to 0.03%. At 0.03% by weight, oleic acid, which makes up most of the fatty acid present, has a vapor pressure, according to Raoult’s law, of about 0.04 mbar at a temperature of 250°C. Even glycidyl esters, which in a deodorizer have a vapor pressure of $10^{-4}$ mbar or less, can be removed by contact with steam at a pressure of 2 mbar.

Aldehydes and ketones, which are the main components giving rise to taste and odor in oil, are much more volatile than either fatty acids or glycidyl esters, but they are usually present in such low concentrations that their vapor pressure is also well below the deodorizing pressure. For example, if we heat oil to 250°C at a pressure of 2 mbar and do not inject any steam, then it will not be deodorized, because the substances we are trying to remove from the oil will not boil away.

According to the theory of steam stripping, at a constant temperature, the amount of volatile matter removed depends on the volume of steam (x) used so that x kg/h of steam at 2 mbar has the same effect as 2x kg/h at 4 mbar, even when the vapor pressure of the volatile components of the oil are several orders of magnitude below the operating pressure. Of course, some deviation from ideal behavior is always possible, but experience shows that the theory is broadly correct. Zendher and Mcmichael (JAOCS, October 1967) and Gavin (JAOCS, November 1977) agree that steam volume is the main factor in steam stripping. Zendher et al. also say that some processors believe that lower absolute pressures are desirable. We still find this idea in the industry today. Zehnder describes it as a moot point that must be examined by each processor based upon specific requirements and beliefs. At very low pressure, it becomes difficult to condense volatile organic matter in the vapor scrubbers of deodorizers, and the cost of creating the vacuum starts to rise steeply as the pressure gets lower.

A classical multi-stage steam ejector vacuum set cooled by cooling tower water uses about 8 times the amount of steam coming from the deodorizer (Fig. 2). This ratio rises as the pressure decreases.
sure is reduced while the amount of steam required to deodorize the oil falls. There is a balance between these two effects when the lowest steam consumption occurs at about 2 mbar at the vacuum set suction, giving about 3 mbar in the deodorizer, although there is little variation in steam consumption at about 1 mbar on either side of those pressures.

If we replace the cooling tower water with chilled water at about 5°C, then the steam required for the ejectors falls by about two thirds depending on the conditions. Some electricity is required to cool the water, but this is usually much less than the cost of the steam saved, unless steam is very cheap and/or electricity is very expensive.

A further reduction in energy can be achieved by freezing the deodorizer vapors so that the vacuum ejectors only have to handle a small amount of air and associated water vapor (Fig. 3, page 19).

The overall energy requirement of sublimators is much lower than classical barometric or chilled water systems as shown in the graph below calculated for a 1000 T/day deodorizer including a packed column for the removal of fatty acid with a 1 mbar pressure drop between the suction of the vacuum ejector set and the top of the deodorizer (Fig. 4).

As we have seen already, the pressure with the lowest running cost when using cooling water is about 3 mbar in the deodorizer. The same applies to chilled water. But for sublimator systems, the lowest steam consumption is at a pressure of around 2 mbar. The power consumption for the chilled water and sublimator refrigeration systems is shown at the bottom on the same scale. A kWh of electricity usually costs more than a kg/h of steam and this varies from place to place, but in most cases refrigerated systems save so much in energy costs that the extra investment is quickly repaid.

The cost of water, pumping, and effluent treatment are not accounted for in the graph, and these become lower as the steam consumption is reduced. The energy required to heat the oil is also not shown here, and it is not linked to the energy required for steam sparging and generating the vacuum.

For sublimators, steam ejector sets can be replaced by dry vacuum pumps which make the running costs, even at less than 1.5 mbar, very low. But below 1.5 mbar in the deodorizer, even small reductions in operating pressure add significantly to the capital cost. The sizing of a vacuum set after a sublimator is mainly determined by the air leakage rate into the deodorizer. At a lower pressure, less steam is required for deodorizing but the mass flow of air remains the same and occupies an increasing volume as the pressure goes down.

So, to answer my original question: What is the ideal deodorizing pressure? From an economic perspective, the answer is approximately 2 to 3 mbar in the deodorizer, depending on the type of vacuum system installed. However, varying the pressure slightly either way does not have a very big effect on the energy consumption.

I have noticed throughout my career that there has been a steady fall in the pressures commonly used in deodorizers. This is partly due to economics and partly to the continuing preference in the industry for lower pressures that was observed by Zehnder in 1967, nearly 20 years before I started to take an interest in these things. Sublimator systems are very economical to operate, which is a major reason for investing in them. Their ability to operate economically at a lower pressure than other systems is also an attractive feature.

What of the future? It seems likely that the trend toward lower energy use and lower pressure will continue, although there is less room for improvement these days than there used to be. Refrigeration systems and the use of new types of refrigerants is another area where developments are being made. The requirements for processing oil can change at any time, so we should always expect the unexpected.

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Transitioning from the **Protein Digestibility Corrected Amino Acid Score (PDCAAS)** to the **Digestible Indispensable Amino Acid Score (DIAAS)**

to evaluate protein quality of human foods

Hannah M. Bailey and Hans H. Stein

The nutritional value of protein is commonly assessed using the PDCAAS methodology, which is federally approved and has been in use for over 25 years. However, many limitations were identified with this methodology. In 2011, a Food and Agriculture Organization (FAO) Expert Consultation was held and ultimately named DIAAS as the successor to PDCAAS for evaluating protein quality of human foods.

Prior to approval by the U.S. Food and Drug Administration and implementation of DIAAS into the food system, a database of DIAAS values for a sufficient number of human foods needs to be developed.

- The DIAAS methodology measures the protein quality of a food item by determining its amino acid (AA) concentration and AA digestibility at the end of the small intestine of humans, pigs, or rats, and comparing those values with human AA reference protein patterns established by the Food and Agriculture Organization (FAO) of the United Nations.

- Cereal grains and plant proteins, with the exception of soy proteins, are generally low-quality proteins with DIAAS values below 75, whereas, milk and meat proteins are “good” or “excellent” quality proteins that can complement low-quality proteins in mixed meals.

- Processing may negatively affect protein quality and decrease DIAAS values; however, moderate heating and curing increases the DIAAS value of meat products.
PROTEIN DIGESTIBILITY CORRECTED AMINO ACID SCORE

The PDCAAS method utilizes the growing rat as a model for humans to determine protein content, amino acid (AA) concentration, and protein digestibility of a food item. The PDCAAS value is expressed as the AA in least concentration in the test protein relative to the same AA in a reference protein pattern, and then corrected for the digestibility of protein in the test protein. The reference protein pattern used for PDCAAS is based on AA requirements for preschool children who are 2 to 5 years old. The protein digestibility is determined by feeding a diet containing a test protein as the sole source of crude protein (CP) and AA to a rat and measuring nitrogen in the feces.

The PDCAAS methodology has been critically reviewed by many authors and several limitations have been identified. First, proteins are made up of individual amino acids that are digested and absorbed entirely in the small intestine and at different rates. Therefore, the assumption that all AA have the same digestibility as protein is inaccurate, and each AA should be corrected for its own digestibility. In addition, fecal contents contain microbial proteins synthesized in the hindgut, and therefore inaccurately represent undigested protein coming from a food item. Another significant limitation of PDCAAS is the use of the growing rat as a model for humans. Published data verify the use of the pig as a more appropriate animal model to evaluate protein quality for humans. Values for PDCAAS are also required to be truncated to 100%, which eliminates the identification of complementary proteins. Because of these inaccuracies in the PDCAAS methodology, it has been concluded that PDCAAS values for low-quality proteins are generally overestimated, whereas values for high-quality proteins are underestimated by the PDCAAS method.

DIGESTIBLE INDISPENSABLE AMINO ACID SCORE

The DIAAS methodology addresses the limitations of PDCAAS evaluation system. Protein quality of a food item determined by DIAAS is best estimated by measuring the digestibility of each AA at the end of the small intestine of humans, but if it is not possible to use humans the growing pig is an appropriate model. Although the growing rat may also be used to generate DIAAS values, the pig is recognized as a better model according to the FAO. DIAAS values are based on the digestible indispensable AA in least concentration in a food item compared with the same AA in 1 of 3 reference protein patterns. Therefore, DIAAS corrects each AA by its own digestibility as determined at the end of the small intestine.

The reference protein patterns have also been refined and expanded into 3 groups: 1) birth to 6 months, 2) children from...
6 months to 3 years, and 3) children older than 3 years, adolescents, and adults (FAO, 2013). In addition, values for DIAAS are not truncated to 100%, enabling the recognition of high-quality proteins to complement low-quality proteins to produce a mixed diet that is balanced in all indispensable AA. For regulatory purposes, claims on a food items protein quality can be made using the DIAAS methodology. Food items with a DIAAS value greater than 100% or between 75% and 100% can be considered “excellent” or “good” quality proteins, respectively, and food items with a DIAAS value below 75% cannot have a claim made on the basis of protein quality.

**DIAAS OF PROTEINS**

Since the introduction of DIAAS 6 years ago, several proteins have been assigned DIAAS values using the growing pig or the growing rat as models because FAO specifies that when new food items are being assayed in vivo studies should be conducted to determine AA digestibility at the end of the small intestine (Fig. 1, page 23). Values for DIAAS have been determined for several cereal grains including, barley, buckwheat, corn, millet, oats, rice, rye, sorghum, and wheat, and DIAAS values in these cereal grains ranges from 7% to 77%. Oats is the only cereal grain assigned a DIAAS value greater than 75%; therefore it can be considered a “good” quality protein. However, when oats were cooked or processed to oat protein concentrate the DIAAS value fell below 75%. The limiting AA in cereal grains is usually lysine, although the sulfur containing AA are first limiting in buckwheat.

Plant proteins that have been assigned DIAAS values include kidney beans, peas, peanuts, and soy, and values range from 43% to 105%. Soy flour is the only protein with a DIAAS value greater than 100% meaning it can be considered an “excellent” protein; however, when soy was processed into soy protein isolate the DIAAS value decreased. The limiting AA in many plant proteins are sulfur AA.

Several animal proteins have been assigned DIAAS values. Dairy proteins, including milk and whey, are limiting in sulfur AA and histidine, respectively, and generally have DIAAS values greater than 100%. DIAAS values of several meat products have been determined using the growing pig. Meat products from beef generally have DIAAS values greater than 100%, and valine is the limiting AA for intact meat. Meat products from pork also have DIAAS values greater than 100%, and valine is generally the limiting AA in pork proteins, although leucine and histidine may be limiting in some pork proteins. Meat almost always undergoes processing prior to human consumption, and it was observed that curing and moderate heating to 63°C to 64°C may increase DIAAS values, whereas grinding meat prior to some processing methods and overcooking may reduce the DIAAS value. Overall, meat and dairy products are generally considered “good” and “excellent” quality proteins for persons older than 6 months.

**FUTURE RESEARCH**

Prior to approval of DIAAS by the U.S. Food and Drug Administration and its implementation into the human nutrition sector, a peer-reviewed database with foods commonly consumed by humans around the world needs to be established. Specifically, there is a need to add data for fish, egg, and poultry proteins. Also, many locally grown proteins, particularly those in developing countries, need to be included in the DIAAS database. In addition to single-ingredient foods, DIAAS also needs to be determined for mixed meals, and additivity of DIAAS values from individual ingredients needs to be confirmed in mixed meals.

**Further reading**


Hannah Bailey has degrees in Animal Science from Iowa State University (B.S.) and the University of Illinois (M.S.). Her Master’s thesis was focused on determining DIAAS values for meat products. She is currently a Ph.D. research assistant at the University of Illinois, working under Hans H. Stein.

Hans H. Stein is a professor of Animal Science at the University of Illinois, Urbana-Champaign, where he conducts research and provides outreach programs in the area of intestinal physiology and feed and food ingredient evaluation. He obtained a Ph.D. degree in monogastric nutrition from the University of Illinois, and he and his graduate students have determined DIAAS values in more than 60 food proteins. He can be contacted at hstein@illinois.edu.
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Is there an affordable way to make microbial biosurfactants?

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

Rebecca Guenard

Over the past three decades, an accumulation of studies show that biosurfactants are less damaging to the environment and to human health than chemical surfactants (https://doi.org/10.1007/978-978-981-10-7434-9_10). Research in recent years indicates that biosurfactants are antibacterial (https://doi.org/10.1007/s13205-016-0478-7), antifungal (https://doi.org/10.1007/s00253-017-8554-4), and antiviral (https://doi.org/10.1186/s13568-017-0363-8).

In addition, compared to some chemical surfactants, biosurfactants prove to be superior emulsifiers at lower concentrations. Though established as a more sustainable, beneficial type of surfactant, a nagging flaw continues to keep biosurfactants from widespread use. Manufacturing biosurfactants is too expensive for them to be competitive against their petrochemical counterparts. What will it take for more of these compounds to reach consumers?

Richard Gross, professor of chemistry and biology at Rensselaer Polytechnic Institute in Troy, New York, USA, has spent his career looking for green chemistry solutions to environmental problems. Gross says that since surfactants are used in a lot of different applications it is important that they are safe. Their use in foods, cosmetics, and pesticides means that humans routinely interact with and dispose of the compounds. Hence, many chemical surfactants have been phased out of use because they are toxic or do not easily biodegrade. Meanwhile, the industry has waited decades to replace them with biosurfactants, but the field is mired in confusing labels and complicated biotechnology.

“‘Biosurfactants’ is a fairly loose term that is not just used for surfactants that are made by microbes.” Gross, explains. The label currently refers to any amphiphilic compound made up of different combinations of carbohydrates, lipids, and proteins, with some portion—but not necessarily the whole molecule—being naturally sourced. For clarity, the industry categorizes biosurfactants as first- or second-generation based on how they are synthesized. First-generation is the moniker used for a biosurfactant that is chemically synthesized from a renewable resource, while second-generation surfactants are biosynthesized through the fermentation of a microorganism.

Unfortunately, these subcategories are often left out of surfactant reports, making it difficult to predict a compounds’ potential for commercial development.

Gross says that microbial biosurfactants represent fewer than 5% of the total amount of biosurfactants being produced. “If we really want to make a distinction, we need to call these surfactants bio-based surfactants versus natural surfactants,” says Gross.
Take the most popular commercial biosurfactants, alkyl polyglucosides (APGs), as an example. APGs are non-ionic compounds, which makes them versatile in a variety of applications, including household detergents, industrial cleaners, personal care, and agricultural chemicals. This diverse set of applications stems from the favorable properties of APGs, such as dermatological and ocular safety, biodegradability, wettability, foam production, and cleaning ability. Europe is currently the largest producer and consumer of APGs, but the Asia Pacific market is expected to overtake Europe in the next five years. Due to the potential of APGs to have an impact in the surfactant market, major producers are increasing capacity.

APGs are polymeric acetals formed by combining a vegetable oil with glucose. Researchers have investigated the potential of a range of sugar and lipid-enriched industrial residues including whey, molasses, animal fat, and tallow as feedstocks for the opposing polar ends of the acetal chain. Although these surfactants are bio-based and not made from petroleum, the methods used to breakdown natural feedstocks often entail expensive catalysts and environmentally risky solvents. Consequently, many researchers, like Gross, are working toward developing biosurfactants that are produced by microbes, with minimal chemical synthesis.

Biotechnology has proven effective in the manufacture of a range of specialty chemicals in the textile, food, and lubricant industries, for example. Compounds with both hydrophilic and hydrophobic groups grow naturally in bacteria, yeasts, and fungi, and could be used as surfactants, but these compounds are not a major cell component as is the case for the products of industries successfully employing biotech. Microbial surfactants are found on cell surfaces or as extracellular excretions. Gross says part of the difficulty of microbial surfactant production is the low yield that results after trying to separate the surfactant from an organism or its growth medium. “Even then, microbially produced surfactants are generally mixtures. They are not usually a single structure, and that complicates the downstream processes.” He says one way current manufacturers can lower costs is by selling their product as a liquid mixture instead of in the typical dried form. There is also opportunity to lower costs by evaluating the performance of natural surfactants in different applications. Doing so could result in determining that less of the surfactant is needed to achieve the same product quality. Nevertheless, until natural surfactants can stand on their own without the co-additives currently needed, their incorporation into formulations will be limited. Gross says that the performance of most natural surfactants needs to improve before they can be used independently as a formulation ingredient.

Many natural biosurfactants fall under the classification, glycolipids, with sophorolipids being one type with commercial potential. Gross has focused his research on sophorolipids, because they are produced in higher yields by microbes that are less pathogenic than those that produce other types of glycolipids. His research group has identified a way to improve performance by modifying the esters of sophorolipids using green chemistry. “We can improve the performance by 10-fold. Then, the product cost comes down because you can use less.” Gross says. “There is not enough attention being paid to those kinds of strategies.”

If anyone understands the difficulty of commercializing a natural biosurfactant, it is Gross. In 2008, he established the company SyntheZyme with the intention of producing a naturally derived sophorolipid. Unfortunately, investors were difficult to find, and those that did invest did not have the patience to wait for the technology to be perfected, Gross says. Biotech has come a long way in the past decade, and Gross hopes someday to revive the project. “We are still holding the patents,” he says. “We are still hoping that there will be a realization that what we were doing and the patents we filed have real value.”

Product developers’ eagerness to replace petrochemically derived surfactants creates an internal challenge in the biosurfactant market between bio-based and natural surfactants. “The current price of alkyl polyglucosides is about a dollar a pound,” Gross says. “Right now, the sophorolipids are probably the lowest-priced biosurfactants that are microbially produced. They are in the $5 a pound range. So how are you going to compete?”

In 2019, Gross was the recipient of the American Chemistry Society’s Affordable Green Chemistry award—in part, because of his research on sophorolipids. He is aware of the obstacles that block natural surfactants from making their way from research labs into manufacturing plants. There is a lot of work that still needs to be done, Gross says. “These are a new class of surfactants, and not enough time and money have been put into commercially developing them yet. The only way to get the cost to performance value up is to put in that time and effort.”

Investment forecasts predict that greater interest in green chemicals may drive innovation toward lower production costs for lipid-based surfactants. If this prediction proves true, microbial surfactants may finally receive the research and development dollars needed to eventually become cost effective.

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

References


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Russian-led bloc pushing ahead on Eurasia-REACH

When the Eurasian countries—Armenia, Belarus, Kazakhstan, Kyrgyzstan and Russia—missed a December 2018 deadline to pass secondary legislation to implement Eurasia-REACH, the target dates to harmonize and modernize classification, labelling, and chemical registration requirements in the region were at risk.

The proposed second-tier documents, expected to be approved by mid-2019, are likely to put efforts back on track however, requiring companies to pre-register chemicals in 2020. If all goes as planned, the law authorizing REACH-like chemical registration, EAEU TR 041/2017 (TR 41), should come into force in 2021.

Drafts of the secondary legislation are in a “high degree” of readiness, says Batima Ismadieva, chief of division for international cooperation at the Eurasian Economic Commission (EEC). The documents are in legal review with approval anticipated by the end of the second quarter.

“At the same time, the register of chemicals and mixtures of the Eurasian Economic Union will begin to be formed,” Ismadieva says.

The bigger problems—the timeline for implementation and a split between Russia and its neighbors—may not be so easily resolved, however.

THE DECADE-LONG STRUGGLE

Russia, Belarus, and Kazakhstan founded the Eurasian Customs Union in 2010, and it was extended to Armenia and Kyrgyzstan and led to the five-country Eurasian Economic Union (EAEU).

Implementation of Globally Harmonized System (GHS) and Eurasia-REACH were approved in primary legislation TR 41, contingent upon finalizing secondary legislation. The five nations had difficulty reaching a consensus, however.

Russia split with the group last year, adopting its own version of REACH, which is scheduled to enter into force in July 2021. Industry fears that the adoption of national standards by Russia could leave them saddled with one set of rules for Russia and another for Armenia, Belarus, Kazakhstan, and Kyrgyzstan.

Russia would not be in a position to maintain its own national standard if the Eurasian standard is implemented, however. The EEC will only approve national standards in the absence of international and/or interstate standards, Ismadieva says.

Olesia Popcharska, Finland-based senior consulting manager for REACHLaw, says Russia is pushing hard to agree on the necessary legislation for Eurasia-REACH.

“A single regulation, covering the customs union, will be good for industry,” Popcharska says. “Multiple potential registrations may easily result in extra work and also costs. Additionally, industry would have to comply with potentially different requirements of national laws.”

If Eurasia member states do agree on the way forward, Russia-REACH could be revoked by a separate decree, making Eurasia-REACH the single chemicals regulation for the whole...
market: “At the moment nothing is certain, but all the efforts are being made for TR 41 to see daylight,” Popcharska says.

**EURASIA-REACH VS EU-REACH**

Eurasia-REACH is expected to include provisions that could be burdensome to industry, especially to small- and medium-size enterprises. Cefic has submitted comments on:

- requirements to register substances <1 ton/year;
- inclusion of mixtures in the registration scheme, also with no minimum volume exclusion;
- extensive information requirements, with no reduced testing for lower volume bands; and
- criteria for identification of substances of high concern include GHS aquatic chronic category 1 classification instead of persistence, bioaccumulation, and toxicity (PBT and vPvB).

On the positive side, although there is no de minimus tonnage volume, TR 41 states that substances present in mixtures at <0.1% are exempt from identification as new chemical substances and therefore from chemical registration.

The plan is for national registers in each EAEU country to hold extensive chemical information on substances and mixtures registered in that member state. A unified register will have information on hazard classifications, impact on health, first aid measures, and so on.

Russia’s existing Federal Register of Potentially Hazardous Chemical and Biological Substances will likely be a significant starting point for the EAEU inventory. The site lists 10,921 chemicals which can be searched at no cost. After the initial inventory phase closes, any chemical not included in the unified register will be considered “new” and subject to a web-based notification procedure.

### Belarus: National preparations for Eurasia-REACH

Belarus, a landlocked country of 9.5 million people lying east of Poland and southwest of Moscow, exemplifies the preparations being undertaken by Russia’s neighbors to support Eurasia-REACH.

Belarus’ chemical industry constitutes 5% of GDP with 378 companies involved in chemical production, but the country has only started its three-year plan to prepare for Eurasia-REACH requirements.

The Ministry of Health will have overall responsibility for the national register while the Scientific and Practical Centre for Hygiene will maintain the national part of the register of chemical substances and mixtures as well as conduct preliminary work establishing expertise, testing, and research capacity.

Two international projects support the preparations:

- The World Health Organization sponsors a team working on key elements of the national systems for Belarus and Kazakhstan; and the UN Environment’s Special Programme is leading a project aimed at adoption of the Rotterdam Convention in Belarus.

  The WHO’s Johann F Moltmann says industry may benefit from efforts to offer on-line registration, but the project is not expected to be finished until March 2021, more than a year after the deadline for the initial inventory of chemicals.

  The UN project also runs through 2021, with plans to regulate the transboundary movement of hazardous substances and prevent illegal imports.

The situational analyses undertaken in these projects has led to investments in chemical management infrastructure in Belarus, including training and the expansion of laboratory facilities.

A new laboratory building with an animal research facility is being built by the Ministry of Health and modelled after GLP vivariums operating in Russia and Ukraine.
If the unified register is implemented, and TR 41 takes effect, GHS also becomes mandatory. This will succeed to modernize and harmonize safety data sheets (SDSs) and labels in the five countries, which currently have only a mutual recognition agreement for hazard classifications.

The GHS provisions differ from those applied in the EU and US, however. For example, all building blocks are adopted and the mixture classification thresholds for some hazard classes are lower than those adopted in the EU.

**INDUSTRY REPRESENTATIVES ADVOCATE LESS ONEROUS PROVISIONS**

Cefic and the Association of European Businesses in the Russian Federation have submitted comments to the working group of authorities drafting the Eurasia-REACH rules.

Daria Hammacher, senior manager for innovation and ingredients registration at Switzerland’s Firmenich SA, says industry has been working to convince the national authorities to simplify the inventory listing procedure, implement a low volume exemption, clarify the testing data required versus tonnage band, exempt mixtures, and extend the registration deadlines.

“There has been quite a lot of discussion,” Hammacher said.

The draft Eurasia-REACH implementing rules contain a deadline of January 1, 2020 for finalizing the inventory of existing chemicals in all EAEU countries, but this deadline is expected to be extended. It is unclear when the draft deadline will be implemented in the five countries, although Hammacher says Kazakhstan started its inventory listing procedure in February with a deadline at the end of 2019.

**INVENTORY LISTINGS**

There are general indications about how the inventory listings will work in practice. Popcharska says that if a business misses an inventory listing, but other companies list the same substance, the business will not be penalized. If, however, a specific substance is not listed in the inventory, companies will have to make a new substance notification, similar to a lead registrant type of dossier in EU-REACH.

Companies may also nominate substances up until June 2, 2023, if they can prove these were on the market on or before June 2, 2021, but the details of how to do this are still unclear, she adds.

The requirement to register substances and mixtures <1 ton/year has not been amended in the draft secondary legislation, but the text has been amended to require the national authorities to evaluate the feasibility of this provision and advise the Eurasian Commission by June 2, 2028, before registration of such low volumes would take effect.

Christine Lepisto is a reporter for Chemical Watch.

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I recently learned that Chile produces award-winning extra virgin olive oil (EVOO), and I was curious about Chile and EVOO production. Here is what I found out in a Q&A format.

Q: How did olive oil production develop in Chile?

A: When Christopher Columbus came to America in 1492, he introduced European plant specimens into the local ecosystem. Originally, olive trees went from Seville (Spain) to the Caribbean region, then spread from there to the rest of the continent. By 1560, there were olive plantations in Argentina, Chile, Mexico, Peru, and the United States (California). In Chile, José Canepa Vaccarezza brought Italian technology into his company. By 1952, he had uncovered the olive oil potential in Chile, but it took approximately 40 years for larger investors to enable production leading to exports [1]. By 1995, olive oil production in Chile became more competitive, with higher yields due to the introduction of cultivation per hectare, irrigation, and better selection of plant types leading to better oil quality. By the year 2007, the olive plantation surface was 12,000 hectares, and by the year 2015 it was 25,000 hectares [2]. Current olive oil production yields award-winning oils, such as those selected as “The Best by Flos Olei,” a designation that recognizes the best 20 olive oils worldwide. These include the “Olivos Ruta del Sol S.A.,” awarded “The Best 20–Extra virgin olive oil blend/ lightly fruity (2018)” [3,4], and “Agrícola Pobeña S.A.,” awarded “The Best 20 Extra virgin olive oil quality/quantity (2019)” [4].

Q: What is the general process for obtaining EVOO?

The process starts by harvesting the olives, transporting them to the processing plant for their wash, and then crushing. The resulting paste undergoes a kneading process to break the emulsion and separate a continuous lipid phase. A centrifuge or decanter is used to do a final extraction, yielding three components: solids, water phase, and oil (some of these three components may be on the same phase initially, depending how the extraction is performed). The resulting oils is then centrifuged to eliminate residual water, and it is later filtered to ensure that no solids remain. From this point, it is packaged [5].

Also related to olive oil production in Chile, is the “Acuerdo de Producción Limpia (APL)- (Agreement of Clean Production), a

Latin America Update is a regular Inform column that features information about fats, oils, and related materials in that region.
voluntary agreement between ChileOliva and the “Agencia de Sustentabilidad y Cambio Climático” (Sustainability and Climate Change Agency), funded by the Ministry of Economy. (Note that ChileOliva is the Association of Olive Oil Producers in Chile). The overall objective of this agreement is to use clean production practices and technology that not only support the environment, hydric resources, and sustainability, but yield competitive product of high quality [1].

Q: What exactly is EVOO?
A: According to the International Olive Council, olive oil is the oil extracted from the olive fruit (*Olea europaea* L.), excluding the oils obtained using solvents, re-esterification, and/or mixing with other oils. Virgin olive oils are obtained by physical methods from the fruit of the olive tree, and these methods are not to modify the oil. In other words, virgin olive oil is minimally treated (washing, decantation, centrifugation, and filtration) at mild temperatures that preserve the innate oil qualities of the oil. Virgin olive oils (extra virgin olive oil, virgin olive oil, and ordinary virgin olive oil) are ready for consumption as soon as they are obtained. Extra virgin olive oil has a free acidity of not more than 0.8 grams per 100 grams (expressed as oleic acid), while virgin olive oil and ordinary virgin olive oil have a free acidity of not more than 2 grams per 100 grams and 3.3 grams per 100 grams, respectively. Lampante virgin olive oil is not fit for consumption as extracted and has free acidity above 3.3 grams per 100 grams [6].

Q: How does the olive oil production, consumption, import, and export in Chile compare to other South American Countries (Argentina, Brazil, Uruguay)?
A: A comparison table (Table 1) was constructed from information distributed by the International Olive Council, November 2018 [6]. All units are 1,000 metric tons and refer to the “olive crop year,” which runs from 1 October to 30 September.

<table>
<thead>
<tr>
<th></th>
<th>2017/18 (prov)</th>
<th>2018/19 (prev.)</th>
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<td>Consumption</td>
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<td>Argentina</td>
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<td>8.0</td>
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<tr>
<td>Brazil</td>
<td>-</td>
<td>76.5</td>
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<tr>
<td>Chile</td>
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</tr>
<tr>
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</table>

Latin America Update is produced by Leslie Kleiner, a senior research scientist and contributing editor of *Inform*.

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**References**

A commitment to education and AOCS

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

Three characteristics that sum up Eric Cochran are commitment, a passion for research and education, and a habit of saying, “Yes, I will!”

As a professor of chemical engineering and director of graduate education at Iowa State University (ISU; Ames, Iowa, USA), Cochran’s primary role is to teach and mentor both graduate and undergraduate students—a role he has performed for the past 13 years. “Graduate education is research-intensive,” he notes, adding that his research group at ISU specializes in novel biobased plastics and heterogeneous polymeric materials.

Within AOCS, Cochran has been vice chair of the Industrial Oil Products (IOP) Division since 2018. As a divisional vice chair, he also participates in the AOCS Annual Meeting Program Committee. “Most of the time, my involvement takes only a few hours a month,” he says, “primarily through staying in contact with session chairs and AOCS program staff. We also have three teleconferences per year. In addition, I review award applications and hot topics.”

When asked how he became involved, the answer is one that many AOCS volunteers provide for that question. “There was a need,” he says. “One of my colleagues recommended joining AOCS and also asked me to chair a session.” In the midst of organizing the session, the vice chair position opened up, and Cochran was asked if he would consider stepping into that role.

“It was an easy thing to say ‘yes’ to,” he notes. “For one thing, it didn’t sound like it would take an inordinate amount of time. Plus, the best way to get involved in a new community is to volunteer.”

Cochran explains that the main challenge AOCS is addressing as a whole is declining membership. “We’ve seen it in the division,” he says. “Which means we have to make sure the value of the division is well-advertised and that we keep programming up to date and relevant.”

Part of the value to Cochran of his volunteer efforts is in getting international exposure. “I’m able to leverage my connections to increase attention to my research projects, to meet potential employers for my students, and to hear what everyone else is doing to make polymers from fats. Meaningful connections with people really happen at AOCS meetings because the size of the meeting makes it easier to have one-on-one time with people,” he concludes. “I appreciate that.”

**Fast facts**

<table>
<thead>
<tr>
<th>Name</th>
<th>Eric W. Cochran</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joined AOCS</td>
<td>2017</td>
</tr>
<tr>
<td>Education</td>
<td>Ph.D., chemical engineering, University of Minnesota, Twin Cities (2004)</td>
</tr>
<tr>
<td>Job title</td>
<td>Professor and Director of Graduate Education</td>
</tr>
<tr>
<td>Employer</td>
<td>Iowa State University, Ames, Iowa, USA</td>
</tr>
<tr>
<td>Role in AOCS</td>
<td>Industrial Oil Products Division Executive Steering Committee and Annual Meeting Program Committee</td>
</tr>
<tr>
<td>High-fat Indulgence</td>
<td>Cheesecake</td>
</tr>
<tr>
<td>Favorite Social Media</td>
<td>Linkedin</td>
</tr>
<tr>
<td>Most memorable AOCS experience</td>
<td>The 2018 Fun Run in Minneapolis: “It’s a great way to get out of the building and interact with everybody in a nontechnical way.”</td>
</tr>
<tr>
<td>Other involvement</td>
<td>Annual Meeting session chair, Division Council</td>
</tr>
</tbody>
</table>
Soybean transformation method

The present disclosure relates in part to a method for identifying a soybean germline transformant from a population of soybean transformants by incorporating a selection agent within rooting medium used in tissue culture during the soybean transformation process. The soybean germline transformants are selected from a population of soybean transformants which are comprised of a combination of non-germline and germline soybean transformants. The soybean non-germline transformants are identified and eliminated early in the transformation process. The soybean germline transformants are identified and selected for culturing into mature soybean plants. The method is readily applicable for screening and obtaining a soybean germline transformant at an early stage in the soybean transformation process.

Methods and compositions for X-ray induced release from pH sensitive liposomes
Fologea, D., et al., University of Arkansas, US10220000, March 5, 2019

Compositions including pH sensitive lipid vesicles comprised of a lipid layer, an agent, and an organic halogen such that the agent is released from the vesicles after exposure to ionizing radiation. Methods of delivering the agent to a target in a subject using the compositions provided herein are also described. The methods allow for controlled release of the agent. The timing of release of the agent from the lipid vesicle may be controlled as well as the location of release by timing and localizing the exposure to ionizing radiation exposure.

Controlled drug release liposome compositions and methods thereof
Kan, P., et al., Taiwan Liposome Co., Ltd., US10220095, March 5, 2019

The present invention relates to pharmaceutical compositions comprising at least one liposome, at least one polyvalent counterion donor or a pharmaceutically acceptable salt thereof, at least one monovalent counterion donor or a pharmaceutically acceptable salt thereof, and an amphipathic therapeutic agent or a derivative or pharmaceutically acceptable salt thereof. The present invention also relates to methods of inhibiting cancer cell growth while reducing toxicity, comprising administering the pharmaceutical composition described herein.

Methods for making ultrasound contrast agents
Robinson; S.P., et al., Lantheus Medical Imaging, Inc., US10220104, March 5, 2019

Provided herein are improved methods for preparing phospholipid formulations including phospholipid UCA formulations.

Method of inhibiting corrosion using a corrosion inhibitor derived from vegetable oils
Aguilar, B., et al., Instituto Mexicano de L Petroleo, US 10221368, March 5, 2019

The present invention is directed to a formulation of corrosion inhibitors for corrosion control of low carbon steel piping, carrying different fuels products, obtained in refineries or petrochemical processes. The composition comprises active imidazoline inhibitors derived from vegetable oil selected from the group consisting of sunflower, canola, soybean, safflower, corn, and mixtures thereof that are reacted with a polylkylated polyamines such as diethylenetriamines (DETA), triethylenetetramine (TETA) and tetraethylenepentamine (TEPA). The imidazoline is reacted with 1 to 3 moles of a carboxylic acid having 2 to 6 carbon atoms. The product obtained is formulated with 50 to 60% weight of an aromatic solvent and 10 to 20 wt % of an alcohol. The compositions were evaluated in sour environments under the NACE TM0172 and ASTM G 185 method, and both, sour and sweet environments in the NACE ID182 method, so they are fit to pass the tests as indicated by the NRF-005-PEMEX-2009 standard for application in pipelines.

Lubricant compositions
Mitrovich, M.J., et al., MPL Innovations, Inc., US10221373, March 5, 2019

Lubricant composition formulations comprising at least one biodegradable plastic, such as polyhydroxyalkanoate and/or polybutylene succinate, and at least one lubricant, such as a vegetable oil.
Process for refining glyceride oil comprising a basic quaternary ammonium salt treatment
Fedor, G., et al., Evonik Degussa GmbH, US10221374, March 5, 2019

The present invention relates to a process for refining glyceride oil comprising the steps of: (i) contacting glyceride oil with a liquid comprising a basic quaternary ammonium salt to form a treated glyceride oil; wherein the quaternary ammonium salt comprises a basic anion selected from hydroxide, alkoxide, alkylcarbonate, hydrogen carbonate, carbonate, serinate, prolinate, histidinate, threoninate, valinate, asparaginate, taurinate, and lysinate; and a quaternary ammonium cation; (ii) separating the treated glyceride oil from a salt comprising the quaternary ammonium cation; and (iii) subjecting the treated glyceride oil after the separation step to at least one further refining step; and to the use of contacting a glyceride oil with the basic quaternary ammonium salt for preventing or reducing the formation of fatty acid esters of chloropropanols and/or glycidol upon heating of the glyceride oil.

Protease enzyme and uses thereof
Valtakari, L., et al., AB Enzymes Oy, US10221377, March 5, 2019

The present invention is related to a fungal serine protease enzyme, which said enzyme has serine protease activity and comprises an amino acid sequence of Malbranchea ALKO4122 mature protease as defined in SEQ ID NO:18 or an amino acid sequence having at least 66% identity to the amino acid sequence of SEQ ID NO:18. Also disclosed is an isolated nucleic acid molecule, comprising a polynucleotide sequence which encodes a fungal serine protease enzyme, nucleic acid sequences encoding said protease, a host cell and a process of producing a polypeptide having serine protease activity. Said protease is useful as an enzyme preparation applicable in detergent compositions and for treating fibers, wool, hair, leather, or silk, for treating food or feed, or for any applications involving modification, degradation or removal of proteinaceous material.

Thickened or structured liquid detergent compositions
Detroch, L.M.C., et al., The Procter & Gamble Co., US10221379, March 5, 2019

Liquid detergent compositions can be stably structured using amides of an aliphatic polyamine with two, three or four molecules of fully saturated hydroxyl alkyl acids, even in the presence of hydrolyzing detergent ingredients such as lipase enzyme.

Method for modifying characteristics of a lipase
Reiser, A.V., et al., Novozymes A/S, US10221404, March 5, 2019

The present invention relates to a method for modifying one or more characteristics of a lipase, comprising the step of associating a peptide to the lipase, wherein the peptide has at least 50% sequence identity with the amino acid sequence of at least one major lipase contact zone of the propeptide of said lipase.

Process for producing dark brown natural cocoa
De Muijnck, L., Olam International Ltd., US10226059, March 12, 2019

Natural, dark brown cocoa products are disclosed. Processes for producing such natural, dark brown cocoa products are also disclosed, as well as foods including such cocoa products.

Dyeing composition comprising a fatty substance, a non-ionic guar gum, an amphoteric surfactant and a non-ionic or anionic surfactant, and an oxidizing agent, dyeing process and suitable device
Charrier, D., et al., L’Oreal, US 10226411, March 12, 2019

The subject of the present invention is a composition for dyeing human keratin fibers such as the hair, comprising: (a) at least one oxidation dye precursor; (b) at least one first amphoteric or zwitterionic surfactant; (c) at least one second non-ionic or anionic surfactant; (d) one or more fatty substances; (e) at least one cationic polymer; (f) at least one non-ionic guar gum; (g) at least one oxidizing agent other than atmospheric oxygen. The present invention also relates to a process for dying human keratin fibers, in which this composition is applied to said fibers, and also to a suitable multicompartiment device.

Tissue repair of the nasal mucosa and treatment of rhinitis with alphatocopherol compositions

Composition for topical application, for use in increasing the trophism of nasal mucosa, comprising an ester of vitamin E with a carboxylic acid of formula R–COOH, in which R is an alkyl radical having 1 to 19 carbon atoms, or an alkenyl or alkynyl radical having 2 to 19 carbon atoms, and an oily vehicle; such composition can be used for the treatment of chronic atrophic rhinitis and for obtaining tissue repair of the nasal mucosa following nasal and sinus surgery.
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http://dx.doi.org/10.1002/lipd.12123

8. Docosahexaenoic acid (22:6n-3) ameliorated the onset and severity of experimental autoimmune encephalomyelitis in mice
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9. Resolvin E1 improves mitochondrial function in human alveolar epithelial cells during severe inflammation
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Diesel precursors via catalytic hydrothermal deoxygenation of aqueous canola oil emulsion


Aqueous extraction for protein isolation from oilseeds is a promising alternative to the conventional hexane-based solvent extraction widely used in the industry. However, during aqueous extraction, a stable oil-in-water emulsion is produced that results in decreased oil yield. We demonstrated the conversion of this aqueous extract into renewable hydrocarbons on 20%w/w Ni/C at 315°C and an initial hydrogen headspace pressure of 1.95 MPa. Moderate yield (>50%) and selectivity (~70%) of hydrocarbons within the diesel range were obtained within 12 hours of reaction without additional external hydrogen input. It was also shown that a prolonged experimental run at 305°C can result in near-complete conversion of triacylglycerol oil into diesel-range hydrocarbons (70%) and oxygenates (9%) with selectivity of ~80%. Although the study demonstrates for the first time the possibility of integrating aqueous extraction of protein with renewable diesel production in a hydrothermal medium, the limitations and challenges experienced during this initial study justify additional work that is presently underway.

Economics of plant oil recovery: a review


Plant oil is a major agricultural commodity used in food, feed, and chemicals. Presently plant oil is produced from oil seeds either using mechanical pressing or solvent extraction. These technologies have steadily improved for increased oil recovery; however, production cost is especially important for a commodity. Herein three technologies and their costs are reviewed for on-farm pressing, industrial mechanical pressing, and solvent extraction. Solvent extraction is the dominant technology because it offers high oil recovery and low production cost. In contrast, industrial mechanical pressing has the highest production cost because of its low oil yield; nevertheless, the simple process results in the lowest fixed capital investment. For on-farm pressing, lower material cost results in lower production cost than industrial mechanical pressing. Additionally, credits from co-products play an important role in determining total revenues, especially for mechanical pressing. Therefore, broadening the applications and values of the co-product is also critical for profitability for the plant oil industry.

Fourth generation biofuel: a review on risks and mitigation strategies


Fourth generation biofuel (FGB) uses genetically modified (GM) algae to enhance biofuel production. Although GM algae biofuel is a well-known alternative to fossil fuels, the potential environmental and health-related risks are still of great concern. An evaluation of these concerns and accordingly devising appropriate mitigation strategies to deal with them are important to a successful commercialized production of FGB. While extensive research has been carried out on genetic modification and other technologies that aim to increase the productivity of algae strains, only a handful of them deal with the legislative limitations imposed on exploiting and processing GM algae. This paper examines this legislation and the mitigation strategies to meet potential risks associated with the exploitation and processing of FGB. Open-pond system is an economic solution for large-scale cultivation of microalgae; however, the concern regarding the health and environmental risk of cultivating GM algae and the associated stringent regulations is considered as the main barrier of FGB production. Disposal of the residue is another important issue that should be considered in FGB production. The by-products obtained from energy extraction step and residual water from the harvesting process may contain plasmid or chromosomal DNA that may cause the risk of lateral gene transfer. Hence an appropriate mitigation practices should be used for replacement of the hazardous water residue and by-products with more environmentally friendly alternatives. The results obtained from several field testing projects for open-environment exploitation of GM algae show that under the various conditions used, there was no apparent proof to support possible horizontal gene transfer in release of GM algae.

Thermo-sonic assisted enzymatic pre-treatment of sludge biomass as potential feedstock for oleaginous yeast cultivation to produce biodiesel


Solubilization of activated sludge is a crucial process before its use as an appropriate renewable feedstock for biofuel generation which could be a legitimate alternative arrangement for contem-
porary concerns on fuel crisis, climate change and food security. The present study investigates the thermo-sonic assisted enzymatic pre-digestion of municipal waste activated sludge (MWAS) to cultivate oleaginous yeast *Naganishia liquefaciens* NITTS2 to produce lipids for biodiesel production. The maximum suspended solids reduction and sCOD observed were 36.42 ± 0.7 and 41.35 ± 0.5%, respectively at optimum conditions. The pre-digested sludge was used as a nutritional medium for yeast cultivation and the obtained maximum biomass and lipid content were 17.85 ± 0.64 g/L and 65.43 ± 1.60%, respectively. The consumption of nutrients present in the medium was analyzed before and after the batch cultivation. Lipid extraction was optimized using ultrasonication at different temperature and its characteristic profile was analyzed by GC-MS. Fatty Acid Methyl Esters (FAMEs) was produced (88.45 ± 1.2%) through enzymatic transesterification and further confirmed by 1H NMR spectroscopy. Thus, the combined pre-digestion would help to improve the solids reduction in the MWAS and the solubilized sludge could be used as a renewable substrate for biodiesel production.

**Prenylated diresorcinols inhibit bacterial quorum sensing**


The ability to control bacterial quorum sensing is a new filed of research with huge potential for developing a completely new line of products benefitting human health and wellness. This is one piece of evidence suggesting that natural products control bacterial colonies, which in turn control health outcomes for their host.

Current treatment options for bacterial infections are dependent on antibiotics that inhibit microbial growth and viability. These approaches result in the evolution of drug-resistant strains of bacteria. An anti-infective strategy that is less likely to lead to the development of resistance is the disruption of quorum sensing mechanisms, which are involved in promoting virulence. The goal of this study was to identify fungal metabolites effective as quorum sensing inhibitors. Three new prenylated diresorcinols (1–3), along with two known compounds, (4R)-regiolone and decahydroxytrinone, were isolated from a freshwater fungus (*Helotiales* sp.) from North Carolina. Their structures were assigned based on HRESIMS and NMR experiments. The structure of compound 1 was confirmed via X-ray diffraction analysis, and its absolute configuration was established by TDDFT-ECD and optical rotation calculations. Compounds 1–3 suppressed quorum sensing in a clinical isolate of methicillin-resistant *Staphylococcus aureus* (MRSA), with IC50 values ranging from 0.3 to 12.5 micrometers. These compounds represent potential leads in the development of antivirulence therapeutics.

**Structure-based *in silico* screening identifies a potent ebolavirus inhibitor from a traditional Chinese medicine library**


Traditional medicine practices have been used for hundreds of years in almost every part of the world. However, the mechanism and science behind traditional medicines is still not clear. The efficacy of a traditional formula is most likely to be assigned to many ingredients working potentially in synergy and seldom assigned to a single molecule with nano molar potency. The current study suggests that with advanced computing power and new modelling techniques, it may be possible to reveal the hidden secrets behind many traditional medicine formulas.

Potent Ebolavirus (EBOV) inhibitors will help to curtail outbreaks such as that which occurred in 2014–16 in West Africa. EBOV has on its surface a single glycoprotein (GP) critical for viral entry and membrane fusion. Recent high-resolution complexes of EBOV GP with a variety of approved drugs revealed that binding to a common cavity prevented fusion of the virus and endosomal membranes, inhibiting virus infection. We performed docking experiments, screening a database of natural compounds to identify those likely to bind at this site. Using both inhibition assays of HIV-1-derived pseudovirus cell entry and structural analyses of the complexes of the compounds with GP, we show here that two of these compounds attach in the common binding cavity, out of eight tested. In both cases, two molecules bind in the cavity. The two compounds are chemically similar, but the tighter binder has an additional chlorine atom that forms good halogen bonds to the protein and achieves an IC50 of 50 nM, making it the most potent GP-binding EBOV inhibitor yet identified, validating our screening approach for the discovery of novel antiviral compounds.

**Spent coffee grounds extract, rich in mannooligosaccharides, promotes a healthier gut microbial community in a dose-dependent manner**


Drink the coffee and eat the spent beans may be a new health slogan for coffee houses. This study is leading to a very important question: Do we understand what part of food and biomass is good for health and wellness beyond the sensory feelings of the food or the drink? The answer is not straightforward and warrants a thorough analysis of the spent materials in food supply chains from farm to waste.

Coffee is one of the most consumed beverages around the world. Consequently, spent coffee grounds are a massively produced residue that is causing environmental problems. Reusing
them is a major focus of interest presently. We extracted mannooligosaccharides (MOS) from spent coffee grounds and submitted them to an *in vitro* fermentation with human feces. Results obtained suggest that MOS can exert a prebiotic effect on gut microbiota by stimulating the growth of some beneficial genera, such as *Barnesiella*, *Odoribacter*, *Coprococcus*, *Butyricoccus*, *Intestinimonas*, *Pseudoflavonifractor*, and *Veillonella*. Moreover, short-chain fatty acids (SCFA) production also increased in a dose-dependent manner. However, we observed that S-(hydroxymethyl)furfural, furfural, and polyphenols (which are either produced or released from the spent coffee grounds matrix during hydrolysis) could have an inhibitory effect on other beneficial genera, such as *Faecalibacterium*, *Ruminococcus*, *Blautia*, *Butyricimonas*, *Dialister*, *Collinsella*, and *Anaerostipes*, which could negatively affect the prebiotic activity of MOS.

Quality-driven design of sponge cake: insights into reactivity, furan mitigation and consumer liking


This work highlights the importance of considering reactivity in the quality-driven design of heat-treated foods, which should cover the mitigation of process-induced contaminants and the improvement of the sensory properties of the foodstuff. The joint effects of formulation and baking conditions on reactivity and several quality aspects (i.e., volatile generation, physical properties, sensory, and consumer tests), followed by product optimization (i.e., consumer liking and furan mitigation) were studied. While key markers are affected by all factors and their interactions, the effect of sugar and whole egg are the clearest. Furan would be predominantly generated from glucose via caramelization and/or Maillard reaction, whereas the formation of Strecker aldehydes and lipid oxidation products would be favored by precursors in whole egg. Formulations with a low glucose content, baked at low temperatures/short times, lead to optimal products. Egg-based ingredient content may be set according to preference or by applying different optimization approaches.

The use of next-generation sequencing for improving food safety: translation into practice


Next-generation sequencing (NGS) combined with powerful bioinformatic approaches are revolutionizing food microbiology. Whole genome sequencing (WGS) of single isolates allows the most detailed comparison possible hitherto of individual strains. The two principle approaches for strain discrimination, single nucleotide polymorphism (SNP) analysis, and genomic multi-locus sequence typing (MLST) are showing concordant
2020 AOCs Awards Call for

Society Awards

Nomination deadline: August 1, 2019

A.R. Baldwin Distinguished Service
Recognizes long-term, distinguished service to AOCS in positions of significant responsibility. The Society’s highest service award. Sponsored by Cargill.
$2,000 honorarium, $1,500 travel allowance and a plaque

AOCS Award of Merit
Recognizes an AOCS Member who has displayed leadership in administrative activities, meritorious service on AOCS committees or performed an outstanding activity or service.
Plaque and recognition during the AOCS Annual Meeting

AOCS Fellow
Recognizes achievements in science or extraordinary service to the Society. Fellow membership status, a plaque and custom medal

Scientific Awards

Nomination deadline: August 1, 2019

Supelco AOCS Research
Recognizes outstanding original research in fats, oils, lipid chemistry or biochemistry. Sponsored by MilliporeSigma, a subsidiary of Sigma-Aldrich Corp.
$10,000 honorarium, $1,500 travel allowance and a plaque

Stephen S. Chang
Recognizes a scientist, technologist or engineer who has made decisive accomplishments in research for the improvement or development of products related to lipids. Provided by the Stephen and Lucy Chang endowed fund.
$1,500 honorarium and a jade horse

AOCS Young Scientist Research
Recognizes a young scientist who has made a significant and substantial research contribution in one of the areas represented by the Divisions of AOCS. Sponsored by the International Food Science Centre A/S.
$1,000 honorarium, $1,500 travel allowance and a plaque

Alton E. Bailey
Recognizes outstanding research and/or exceptional service in the field of lipids and associated products.
$750 honorarium and a plaque

Division Awards

Nomination deadline: August 1, 2019

ANA Division Herbert J. Dutton
Recognizes an individual who has made significant contributions to the analysis of fats, oils and related products.
$1,000 honorarium, $1,000 travel allowance and a plaque

BIO Division Ching Hou Biotechnology
Recognizes a scientist, technologist or leader who has made contributions to the advancement of the Biotechnology Division’s area of interest.
$1,000 honorarium and a plaque

EAT Division Timothy L. Mounts
Recognizes research related to the science and technology of edible oils or derivatives in food products, which may be basic or applied in nature.
$750 honorarium and a plaque

EAT Division Outstanding Achievement
Recognizes a scientist, technologist or leader who has made significant contributions to the Division’s area of interest or to the advancement of edible oils.
$500 honorarium and a plaque

H&N Division Ralph Holman Lifetime Achievement
Recognizes an individual who has made significant contributions to the Division’s area of interest, or whose work has resulted in major advances in health and nutrition.
$500 honorarium, $1,000 travel allowance, a signed orchid print and plaque

H&N Division New Investigator Research
Recognizes a young scientist who is making significant and substantial research contributions in one of the areas represented by the Health and Nutrition Division of AOCS.
$1,000 honorarium and a plaque

IOP Division ACI/NBB Glycerine Innovation
Recognizes outstanding achievement for research in new applications for glycerine with emphasis on commercial viability. Sponsored by the American Cleaning Institute (ACI) and the National Biodiesel Board (NBB).
$5,000 honorarium and a plaque

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

The award recipient must agree to attend the AOCS Annual Meeting & Expo and present an award address. The 2020 AOCS Annual Meeting will be held in Montréal, Québec, Canada, from April 26–29, 2020.
Nominations

**PCP Division Lifetime Attachment**
Recognizes significant contributions to the advancement of protein and co-products through research and applications.
$1,000 travel allowance and a plaque

**PRO Division Distinguished Service**
Recognizes and honors outstanding and meritorious service to the oilseed processing industry.
$1,000 travel allowance and a certificate

**S&D Division Samuel Rosen Memorial**
Recognizes a surfactant chemist for significant advancement or application of surfactant chemistry principles. Initiated by Milton Rosen and this Division.
$2,000 honorarium and a plaque

**S&D Division Distinguished Service**
Recognizes outstanding and commendable service to the surfactants, detergents and soaps industry.
Plaque

**Student Awards**
**NOMINATION DEADLINE** OCTOBER 1, 2019

**Honored Student**
Recognizes graduate students in any area of fats and lipids. To receive the award, a candidate must remain a registered graduate student and must not have received a graduate degree or have begun career employment before the Society’s Annual Meeting.
$500 travel allowance, complimentary AOCS Annual Meeting registration and lodging, and a certificate

**Hans Kaunitz**
Recognizes a student conducting research related to fats, oils and detergent technology.
$1,000 honorarium, $500 travel allowance and a certificate

**Lipid Chemistry and Nutrition**
Recognizes outstanding performance and achievement of a graduate student conducting research in lipid chemistry and nutrition. Sponsored by Seawit Co., Inc.
$1,000 honorarium, $500 travel allowance and a plaque

**Lipid Processing and Biotechnology**
Recognizes outstanding performance and achievement of a graduate student conducting research in lipid processing and biotechnology. Sponsored by Myande Group Co., Inc.
$1,000 honorarium, $500 travel allowance and a plaque

**Ralph H. Potts Memorial Fellowship**
Recognizes a graduate student conducting research related to fatty acids and their derivatives, such as long-chain alcohols, amines and other nitrogen compounds. Sponsored by AkzoNobel, Inc.
$2,000 honorarium, $500 travel allowance and a plaque

**AOCS Division Student Awards**
Recognizes over 20 students from any institution of higher learning, who are studying and doing research towards an advanced degree in fats, oils and related materials.
Awards range from $50 to $1,000 and a certificate

How to nominate
1. Read the full award description and nomination requirements at aocs.org/awards. (Self-nominations are welcome.)
2. Email the nomination materials in PDF format to awards@aocs.org.
3. Questions along the way? Email awards@aocs.org.

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Who do you see on the stage?
Development of a robust HS-SPME-GC-MS method for the analysis of solid food samples. Analysis of volatile compounds in fresh raw beef of differing lipid oxidation degrees


This work presents a headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) method for the analysis of solid food samples in extended experiments. The final procedure was used to quantify 30 volatile compounds in fresh beef. The strategy adds robustness to the classic SPME methods for solid samples, by including a control solution that solves several challenges. The control solution contained one representative compound for each studied family of beef, and two internal standards. Response factors were calculated for each family and were subsequently applied to every compound belonging to the same family. This strategy allowed control of the quantification procedure even when the fiber, column, or control solution changed. Repeatability and reproducibility had relative standard deviation values below 17%, except for phenylacetaldehyde, (E)-2-nonenal, and (E,Z)-2,4-decadienal. Although the method described here was applied to animal products, it has also been successfully used to distinguish between samples from different lipid oxidation stabilities.

Oat and lipolysis: food matrix effect


Oat is rich in a wide range of phytochemicals with various physico-chemical, colloidal, and interfacial properties. These characteristics are likely to influence human lipid metabolism and the subsequent effect on health following oat consumption. The aim of this work was to investigate the impact of oat materials varying in complexity on the lipolysis process. The composition, structure, and digestibility of different lipid systems (emulsions, oil bodies, and oil enriched in phytosterols) were determined. The surface activities of phytosterols were examined using the pendant drop technique. Differences in lipid digestibility of the oat oil emulsions and the oil bodies were clearly seen. Also, the digestion of sunflower oil was reduced proportionally to the concentration of phytosterols present. This may be due to their interfacial properties as demonstrated by the pendant drop experiments. This work highlights the importance of considering the overall structure of the system studied and not only its composition.

A versatile shear cell for investigation of structure of food materials under shear


A versatile cell for X-ray and neutron scattering experiments on samples under shear has been designed. To our knowledge, it is the first shear cell which can be used for both SAXS and SANS in respectively synchrotron or reactor beamlines. The cell is mainly intended for scattering experiments in so-called “l-2 plane geometry,” but can also be modified into cone-plate and plate-plate rheological geometries, giving access to the 1-3 scattering plane. The latter two geometries, however, can only be used with neutron scattering. The final cell design is compact, which allows it to be used even with lab-based X-ray sources. A special thermostatic shell allows for the temperature control of the samples under investigation in the range from 5 up to 100°C. Several X-ray and neutron scattering experiments performed with the cell have helped in better understanding of the structuring under shear of food materials, such as: cellulosic suspensions, fat crystal networks, and milk proteins.

Renewable surfactants for biochemical applications and nanotechnology


Surfactants find applications in almost every chemical industry, such as household and industrial cleaning, paper, inks, agrochemicals, and personal care or pharmaceuticals. However, their production and use can have a negative impact on the environment and health. Increasing environmental concerns and the strong interest in renewable resources have led to the development of innovative and environmentally friendly surfactants produced by clean and/or sustainable technologies. The aim of this review is to explore the different types of surfactants and their architectures. Then, it will describe the two categories of renewable surfactants: biosurfactants obtained by fermentation, and bio-based surfactants containing either a bio-sourced polar head group or a bio-sourced hydrophobic tail. Finally, this review will focus on highly specialized applications of surfactants (protein crystallization, transfection, and nanotechnology), which are closely related to the ability of surfactants to organize themselves in supramolecular architectures.
Optimization of ultrasound-assisted extraction of biomass from olive trees using response surface methodology


Olive tree pruning biomass (OTP) and olive mill leaves (OML) are the main residual lignocellulosic biomasses generated from olive trees. They have been proposed as a source of value-added compounds and biofuels within the biorefinery concept. In this work, the optimization of an ultrasound-assisted extraction (UAE) process was performed to extract antioxidant compounds present in OTP and OML. The effect of the three parameters, ethanol/water ratio (20, 50, 80% of ethanol concentration), amplitude percentage (30, 50, 70%) and ultrasonication time (5, 10, 15 min), on the responses of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities (DPPH, ABTS and FRAP) were evaluated following a Box–Behnken experimental design. The optimal conditions obtained from the model, taking into account simultaneously the five responses, were quite similar for OTP and OML, with 70% amplitude and 15 min for both biomasses and a slight difference in the optimum concentration of ethanol (54.5% versus 51.3% for OTP and OML, respectively). When comparing the antioxidant activities obtained with OTP and OML, higher values were obtained for OML (about 40% more than for OTP). The antioxidant activities reached experimentally under the optimized conditions were 31.6 mg of TE/g of OTP and 42.5 mg of TE/g of OML with the DPPH method, 66.5 mg of TE/g of OTP and 95.9 mg of TE/g of OML with the ABTS method, and 36.4 mg of TE/g of OTP and 49.7 mg of TE/g of OML with the FRAP method. Both OTP and OML could be a potential source of natural antioxidants.

Antioxidant activity and mechanism of action of sesamol in triacylglycerols and fatty acid methyl esters of sesame, olive, and canola oils


The kinetics of the oxidation of triacylglycerols and fatty acid methyl esters of sesame, olive, and canola oils were concomitantly investigated in presence of different concentrations of sesamol at 60°C. In addition to the inhibition of peroxyl radicals, sesamol and its radical were likely to pro-oxidatively attack lipid hydroperoxides and substrates, respectively. The highest antioxidant activity of sesamol in triacylglycerols was found in the sesame oil from which sesamol naturally originates, followed by in the triacylglycerols of olive and canola oils. Sesamol was of higher antioxidant activity in the fatty acid methyl esters than in their triacylglycerols. Due to the destructured triacylglycerol backbones, sesamol exerted a lower antioxidant activity in fatty acid methyl esters of sesame oil than in those of canola and olive oils, respectively.

Buriti (*Mauritia flexuosa* L. f.) fruit by-products flours: evaluation as source of dietary fibers and natural antioxidants


Buriti by-products flours were evaluated as sources of dietary fibers and natural antioxidants. All flours presented chemical characteristics that allowed classification as high dietary fiber powders. Presence of pectic polysaccharides, arabinoxylans, and xyloglucans was inferred by the neutral monosaccharides profile. Peels and defatted pulp flours are highlighted as those with higher antioxidant potential (total extractable polyphenols and antioxidant activities by DPPH and FRAP) compared to endocarp and manually-produced bran flours. Carotenoids content were also higher in the peels flours. All produced flours showed expressive amounts of total non-extractable proanthocyanidins (NEPA). Buriti peels flours NEPA levels are among the highest values previously described in the literature. Blanching preserved the extractable polyphenols but not carotenoids or NEPA. Technological properties were influenced mainly by the size of the particles. Buriti by-products flours have potential to be used as sources of dietary fiber and natural antioxidants in food.

Evaluation of oxidative stability, fatty acid profile, and antioxidant properties of black cumin seed oil and extract


Black cumin is a strong aromatic seed which can be used as a nutraceutical or medicinal food. Our aim was to evaluate the fatty acid profile and oxidative stability of black cumin seed (BCS) oil as well as the antioxidant activity of water–methanol extract of BCS compared with BHT. Such evaluations would help us understand what kind of fatty acids exist in BCS oil and whether is it possible to apply BCS extract as a natural antioxidant or not. The physicochemical properties of BCS oil included an iodine value of 105.17 g/100 g oil, a PV of 11.88 meq/kg, an oxidative stability index of 16.48 h, viscosity of 21.3 mPa.s, and a refractive index of 1.45. BCS oil contained more than 79% unsaturated fatty acids. Its saturated fatty acids were composed mainly of palmitic acid (8.38%) and stearic acid (2.26%). The BCS extract contained 955.77 mg/kg total phenolics. In a DPPH assay, the IC50 of the BCS extract was measured as 104.76 mg/mL, while for BHT it was 8.06 mg/mL. In the incubation assay, the BCS extract inhibited the formation of oxidation primary products in raw soybean oil at a concentration of 100 mg/mL. BCS oil had a high content of polyunsaturated fatty acids and in spite of its high degree of unsaturation, the presence of phenolic compounds in BCS oil led to an increase in its relative oxidative stability. Also, at higher concentrations, BCS extract can compete with BHT in terms of antioxidant effects, and thus can be added into edible oils.
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influencers, directing the field and product.
Effects of olive leaf extract addition on fermentative and oxidative processes of table olives and their nutritional properties


An experimental investigation evaluated the possibility of increasing the nutritional value of fermented table olives by adding olive leaf extract (OLE). OLE was added to table olives fermented using indigenous bacteria and yeasts, and a commercial starter (Lactobacillus plantarum strain). Microbiological, physico-chemical, and sensory analyses showed that OLE addition resulted in fermented olives with higher levels of antioxidant, anti-inflammatory, and antimicrobial substances, but did not adversely affect their qualities. Moreover, OLE and the commercial starter functioned synergistically against spoilage microorganisms. In addition, fermented olives had higher values of hardness, total phenols, antioxidant activity, hydroxytyrosol, and verbascoside. Nonanal and ethanol contents were lower in fermented olives when Lactobacillus plantarum and OLE were used, indicating lower degrees of oxidation and fermentation. Finally, olives fermented with OLE had a less bitter taste.

Improving oxidative stability of flaxseed oil by encapsulation in electrospun flaxseed mucilage nanofiber


In this study, the potential of flaxseed mucilage nanofiber, as wall material, for encapsulation of flaxseed oil (FO) was investigated. Four series of O/W emulsions at different ratios of FO (0, 10, 20, and 40% w/w) were used to fabricate FO-loaded nanofibers and encapsulation efficiency (EE), loading capacity (LC), and the nanofiber morphology was investigated. Finally, the oxidative stability of entrapped oil as well as bulk FO was investigated during storage by measuring peroxide value (PV), thiobarbituric acid-reactive substances (TBARS), and totox value. The chemical structure and thermal properties of the nanofiber and oxidative stability of loaded FO were also evaluated on the selected best ratio. The highest LC (23.6%) was obtained in nanofiber containing 40% (w/w) FO which had uniform morphology with an average diameter of 332.9 nm. In regard to oxidation tests, PV of encapsulated FO increased from 8.1 to 25.5 meq O2/kg while for bulk oil, the value elevated from 1.4 to 25.5 meq O2/kg during 14 days. Moreover, TBA of protected FO increased to 78.49 mg/kg oil while in control sample, the index reached at 107.3 mg/kg oil. The results of oxidation test demonstrated that the nanofiber was successful to improve the oxidative stability of flaxseed oil.

Effect of acoustic cavitation phenomenon on bioactive compounds release from Eryngium caucasicum leaves


This study investigated the effect of acoustic cavitation phenomenon generated by ultrasonic waves on bioactive compounds release from Eryngium caucasicum leaves into the surrounding medium. Peleg’s model was implemented to provide a clearer insight into the kinetics modeling during 60 min sonication at different temperatures (30–60 °C) and ultrasonic power (50–150 W). The experimental data were successfully fitted employing Peleg’s model with the high coefficient of determination (0.95), low root mean square error (0.003%) and mean relative percentage deviation modulus (6.40%). Then, the optimal conditions, using response surface methodology (RSM), were determined as ultrasonic power of 112.10 W, temperature of 50.00°C and 33.53 min sonication time. Spectrophotometric analysis revealed that extract was a potential source of phenolics (64.00 ± 0.13 mg GAE g−1) with high scavenging ability of DPPH−, ABTS + and HO− (78.18 ± 0.12, 74.19 ± 0.14 and 49.38 ± 0.18%, respectively). The high-performance liquid chromatography (HPLC) revealed that gallic acid, chlorogenic acid, p-coumaric acid, ferulic acid, and apigenin were the main phenolics existed in the product. The process efficiency was enhanced significantly (p < 0.05) via performing preliminary static time (PST, 60 min). The quantity and quality of extracts improved using PST, where gallic acid had the highest concentration (24.59 ± 0.12 mg g−1). Scanning electron microscopy (SEM) images confirmed the dramatic effect of acoustic cavitation on cells structure.
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