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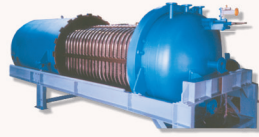
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January 2022

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2022

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Janet Cheney
Director of Membership
janet.cheney@aoacs.org



Victoria Santo
Program Manager, Membership
Recognition
victoria@aoacs.org



Bill Stanton
Manager, Member
Communities and Engagement
william.stanton@aoacs.org

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The new **bio-based** surfactant feedstock

Rebecca Guenard

Scaling and commercializing sustainable surfactants has been an arduous challenge manufacturers attempted to surmount starting in the early 2000s. Researchers continue to pursue a solution, since the ubiquity of surfactants for both home and industrial use implores sustainable sourcing. For example, analysts predict continued growth in the global cleaning products market alone will reach \$58.3 billion by 2024 (<https://tinyurl.com/6jb5hjvy>). Low-toxicity, natural alternatives to petroleum-based surfactants would guarantee the sustainable use of these compounds in household cleaning, as well as agriculture, bioremediation, and personal care applications, for decades to come.

- Conventional soybean oil is not common for making laundry surfactants since the long chain length of its fatty acids leads to molecules that lack the physical properties necessary for stain removal. Epoxides of conventional soybean oil can further lead to rapid side reactions causing undesirable by-products.
- Epoxidized high-oleic soybean oil (HOSO), however, offers the flexible chemistry necessary to adjust functional groups, bio-based content, and hydrophilic-lipophilic balance.
- Researchers claim they have developed 49 HOSO surfactant candidates, including cationic, anionic, nonionic, and amphoteric, with up to 100% biobased content that are stable over a range of pHs.
- New HOSO surfactants are poised to launch in a range of business areas.

Pursuit of naturally sourced surfactants has taken many routes. One way to get environmentally friendly products is to take advantage of the surfactants present in the roots, seeds or leaves of plants. Although a small number of compounds, like lecithin and saponins, have achieved some level of commercial success, most remain too expensive to process at a high enough yield to commercialize for a large market. Biosurfactants, excreted from a microorganism, do not require as much chemical processing, but do undergo extensive purification to isolate the desired compound from a stew of biochemical products. Rhamnolipid biosurfactants have crept into more household and personal care items, but so far cost of production has limited their wide-spread use.

Bio-based surfactants, made by replacing petroleum-sourced hydrophobic carbon chains with fatty acids from vegetable oils or animal fats, have proved to be the most economical way to produce a more sustainable ingredient. Coconut and palm kernel oils, containing up to 60% lauric acid (12:0), are currently the major feedstocks for the surfactant industry. Battelle Memorial Institute, a non-profit, technology development company in Columbus, Ohio, filed a patent last year introducing a new feedstock to compete against them.

"Since soybeans can be grown more sustainably than current feedstocks, we were interested in figuring out a way to make surfactants from C18 instead of C12," says Dan Garbark, lead materials scientist at Battelle (<http://www.battelle.org/>). Instead of lauric acid, he says they focused on oleic acid as the building block for their natural surfactants.

The development groups of two different ingredient companies have also recently announced their efforts to take



advantage of the more plentiful feedstock. Are soybeans likely to become a prominent bio-based feedstock?

WHY SOYBEAN?

According to industry groups like the United States Soybean Export Council, soybean oil is one of the most economical and sustainable bio-based feedstocks. Almost 100% of US farmers participate in the Soy Sustainable Assurance Protocol (SSAP) following a set of guidelines to ensure responsible farming. The US Department of Agriculture conducts audits on participating farms as an independent third party. In addition, they are held to the standards of the European Feed Association. While adhering to current benchmarks for protecting environmental resources, SSAP members commit to continuously improve their sustainability performance.

The reputation of soybean oil and meal as a petroleum replacement has grown in the past decade. Chemical and materials manufacturers are achieving the high-performance requirements for applications like asphalt and tires from soybean feedstocks in markets where petroleum-based products have been a holdout. In particular, high-oleic soybeans gained the attention of researchers interested in the development and commercialization of sustainable versions of performance-demanding products since its fatty acids remain stable in extreme conditions.

High-oleic oils contain concentrations of between 60 and 90% oleic acid content, with a corresponding reduction in the

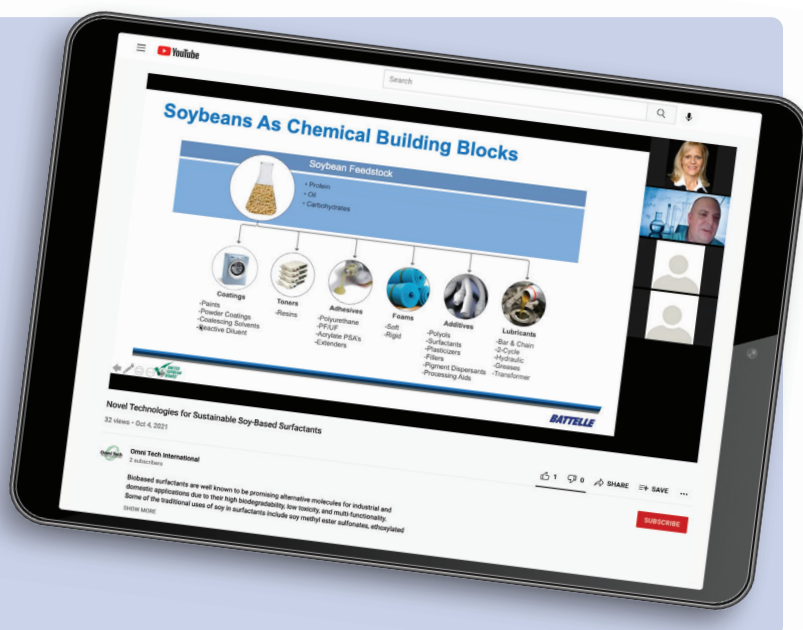
Soybean Oil Triglyceride	Linoleic Acid C18:2 Commodity soybean oil: 52-56% High-oleic soybean oil: 7%	<chem>CCCCCCCC=CCCCCCCC(=O)O</chem> $C_{18}H_{32}O_2$
	Oleic Acid C18:1 Commodity soybean oil: 23-25% High-oleic soybean oil: 72-75%	<chem>CCCCCCCCC=CCCCCCCC(=O)O</chem> $C_{18}H_{34}O_2$
	Palmitic Acid C16:0 Commodity soybean oil: 8-11% High-oleic soybean oil: 3-7%	<chem>CCCCCCCCCCCCCCCC(=O)O</chem> $C_{16}H_{32}O_2$
	Linolenic Acid C18:3 Commodity soybean oil: 4-8% High-oleic soybean oil: 8-17%	<chem>CCCCCCCC=CC=CC=CCCCCCCC(=O)O</chem> $C_{18}H_{30}O_2$
	Stearic Acid C18:0 Commodity soybean oil: 52-56% High-oleic soybean oil: 7%	<chem>CCCCCCCCCCCCCCCC(=O)O</chem> $C_{18}H_{36}O_2$

FIG 1. Comparison of fatty acid profile for commodity versus high-oleic soybean oil. Source: soynewuses.org/benefits/

amounts of linoleic and linolenic acids compared to conventional oils (Fig. 1). Varieties of high-oleic sunflower, safflower, and canola have been available for some time. By comparison, soybean is new to the high-oleic scene, but its unique chemistry makes it well-suited for surfactant development compared to other high-oleic crops.

HOSO Presentations

To find out more about HOSO surfactants, view two recent presentations outlining their development. During the 2021 AOCS Annual Meeting, Dwight Rust, commercialization manager for the consulting group Omni Tech International, introduced the new technology. A few months later, the consulting group hosted a webinar in which Dan Garbark described the bio-based chemistry in detail and Rob Comber discussed some of the soy-based surfactants Colonial Chemical has developed for personal care ingredients. Both innovations rely on high-oleic soybean oil as a platform for developing natural surfactants (<https://tinyurl.com/3y7b8x4j>).



Garbark says his group used an epoxidation ring-opening reaction to produce surfactants with different hydrophilic-lipophilic balances. This versatile industrial reaction involves forming ether groups by adding alcohols onto epoxides at the site of unsaturation. The chain length and functionality of the hydrophilic portion of the resulting surface-active molecule can be modified for specific performance needs.

The single unsaturated bond in oleic acid simplifies the ring-opening epoxide reaction. Multiple unsaturated bonds, as in linoleic and linolenic acid, can lead to cross-linking during the ring opening reactions. These reactions decrease water miscibility and cause sporadic gelation of the surfactant when attempting to formulate for laundry applications. By contrast, functional groups can be added to the epoxidized oleic acid without interference from nearby epoxides leading to undesirable properties.

Finally, surfactants made from high-oleic soybean oil cost less to produce at commercial scale than other plant-based materials since epoxidizes can be formed at low temperatures using enzymes. Also, since there are fewer reaction products, minimal purification is needed to isolate the desired surfactant.

FORMULATING FOR DETERGENTS

Currently, only about one percent of conventional soybean oil is used for surfactant production. Garbark says a highly reactive hydroxyl can be used effectively to functionalize the fatty acids in epoxidized conventional soybean oil for surfactant synthesis. However, new high-oleic varieties of soybeans, predicted to increase production to 600 billion pounds in the next two years, will bring a welcome expansion to current bio-based options.

According to Garbark, the detergents market in the United States will require an estimated 14.3 billion pounds of surfactants in 2022. Currently, only a third of the surfactants in clean-

ing applications are made from bio-based sources. Therefore, his organization chose to first target this growing market when developing high-oleic soybean surfactants. He says his research group synthesized molecules with a range of functionalities using plant-sourced chemicals, and then evaluated their effectiveness on stain removal.

Of the 49 surfactants Battelle developed, they created more HOSO equivalents for nonionic surfactants than for anionic surfactants. However, they synthesized HOSO replacements for cationic, anionic, nonionic, and amphoteric surfac-

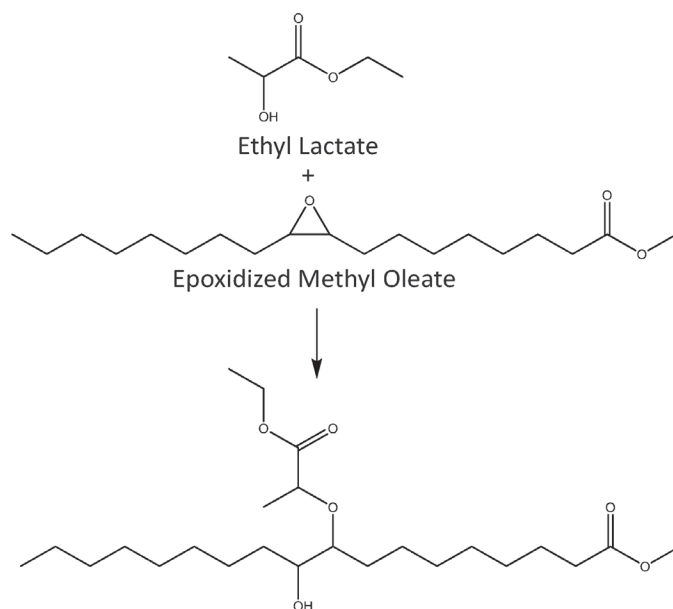


FIG 2. An example of a structure created by reacting a lactate with epoxidized oleic acid. After saponification, the molecule becomes a bio-based surfactant. Source: Battelle

tants typically using 60% soybean oil by weight; although, they could go as high as 100% bio-based.

In developing their product range, Garbark says they started by making ethoxylates and propoxylates of hydroxy-acids, such as citric, lactic, and malic (Fig. 2). The alkoxylates of those acids create a more reactive hydroxyl group which could lead to future production benefits. In addition to these initial structures, they created dozens of other soy-based surfactants with functionalities corresponding to popular surfactants currently on the market. Then the researchers tested those surfactants.

PERFORMANCE AND PRICE

In early testing, the soy-based structure formed by a reaction with citric acid resulted in a surfactant with improved stain removal when replacing the nonionic surfactant in a standard laundry formulation. The standard formulations contained sodium dodecylbenzene sulfonate, sodium lauryl sulfate, cocamidopropylbetaine, and other surfactants that are common in name brands. Successful stain removal compared to the standards encouraged the researchers to test against off-the-shelf detergents (Table 1).

“A mix of all soy surfactants, with a small amount of sodium xylene sulfonate to maintain solubility, surprisingly resulted in equivalent cleaning to off-the-shelf detergents,” said Garbark.

The formulation, composed of nearly 90% bio-based content, achieved soil removal for common stains even when scaled up to 30 gallons. In addition, other newly developed surfactants showed potential for applications in oil recovery and hard surface cleaning. However, he says his group has not yet considered personal care surfactants.

Garbark said his group evaluated the costs of producing the surfactant candidates that performed stain removal best. Taking

into consideration both capital expenditures and operating expenses, they found that several of their soy-based products were cost equivalent to surfactants currently on the market.

HOSO IN PERSONAL CARE

Rob Comber, vice president of research and development at Colonial Chemical, in South Pittsburg, Tennessee (<https://colonialchem.com/>), also says that for certain products high-oleic soybean surfactants can bring down cost. For several years, his specialty chemical company has been evaluating where the longer fatty acid chains in soybean oil could be advantageous as personal care ingredients.

“The shorter chain lengths you get from palm and coconut oils are traditionally more popular in a surfactant company because they provide more detergency and foaming,” Comber says. “But soy-based products provide different benefits, like thickening and shine, that are important in the personal care space.”

Comber says, for customers interested in developing sustainable, palm-free surfactants, his group at Colonial has expanded their soy-based product line to include HOSO surfactants. Among other compounds, they have a hair conditioning ingredient proven to lower combing forces, and a deodorizer that enhances odor removal. He says his group also developed a high-oleic soybean oil equivalent to replace olive oil in product formulations. The HOSO surfactant significantly reduces the price to manufacture personal care products that contain olive oil.

“So far, the personal care side of the business has been more involved with finding uses for what we have developed,” Comber says. “With time, I think other business areas will find applications for some of these candidates.” He says the utility of soy should not be considered for personal care alone; it can be used for a broad range of industries.

TABLE 1. Comparing stain-lifting ability of surfactants synthesized from high-oleic (soy mix 1, candidate 43) and conventional (candidate 48) soybean oil with name brand products currently on the market. Source: Battelle

Soils on cotton	Off-shelf Tide	Off-shelf 7 th Generation	Off-shelf Purex	Soy Mix 1	Candidate 43 (30-gal scale)	Candidate 48
Clay	63.8	60.0	53.7	57.6	71.8	69.0
Coffee	54.7	51.4	46.0	64.1	48.1	49.5
Dust Sebum	63.9	60.1	53.8	60.6	69.2	65.5
EMPA 101 (olive oil)	8.7	8.2	7.3	9.9	8.7	7.8
EMPA 112 (cocoa)	2.6	2.4	2.2	11	5.1	6.0
EMPA 116 (blood, milk, ink)	8.8	8.3	7.4	15.6	10.4	10.6
Grass	55.8	52.5	47.0	45.4	59.9	53.6
Make up	59.6	56.0	50.1	54.4	61.1	55.2
Red wine	30.8	28.9	25.9	39.1	27.5	25.5
Spaghetti	86.0	80.9	72.3	84.7	80.6	78.7
Overall soil removed	434.7	408.8	365.7	442.2	442.4	421.4

ENHANCING BIOSURFACTANT PROCESSING

In addition to the synthesis of new bio-based surfactants from HOSO, biotech manufacturer Jeneil based in Saukville, Wisconsin, is evaluating HOSO's potential as a substrate for rhamnolipid production (<https://www.jeneilbiotech.com/>). Researchers compared the fermentation of different oils to see how they affected rhamnolipid yields. They found that more of the biosurfactant formed in the presence of high-oleic oils than those containing a greater proportion of linoleic acid.

Tom Overbeck, research scientist at Jeneil, says the company hopes to optimize its established soybean oil fermentation processes using high-oleic varieties. If the switch successfully increases rhamnolipid yields, the cost of producing the biosurfactants would decrease, lowering a barrier for bringing more of these surfactants to the market place.

THE GMO HURDLE

While high-oleic soybean oil has great potential to revolutionize the plant-based surfactants industry, opposition to genetically modified (GM) organisms could limit its potential. In 2018, the Court of Justice of the European Union in Luxembourg ruled that gene-edited crops mimicked conventional genetically modified organisms enough that they should be regulated according to the same strict standards. While the law is meant to impose restrictions for developing GM crops for food, business developers generally avoid any GM application to sidestep regulatory procedures and negative consumer perceptions.

The most efficient way to produce high-oleic soybean oil is through genetic mutation. By altering the genes that produce the enzyme responsible for removing hydrogen atoms and creating double bonds (known as fatty acid desaturase), scientists restrict how much linoleic and linolenic acids a soybean plant produces. Food-grade high-oleic soybean oil is gaining popularity in the United States as the product proves its value through longer frying times in restaurants and greater stability of baked goods. As industrial uses also gain success, the acreage of gene-edited soybean in the United States is likely to increase (<https://tinyurl.com/eawptxb7>).

Growers will have to wait and see if EU companies adopt high-oleic soybean oil for industrial use. So far, they remain hesitant. Garbark noted that Battelle has developed a handful of surfactants using commodity soybean oil for customers interested in ingredients that have not come from gene-edited plants. Comber says his company has also developed products from conventional soy. Regardless of the soybean oil's source, these plant-based surfactants have proven their potential for manufacturing new products.

"You would not normally think of getting a detergent or surfactant from a molecule with a C18 chain length," Comber says. "Battelle found a clever way to get into laundry by doing that. In the next ten years, we are going to see soy-based products like this in a lot more business areas."

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

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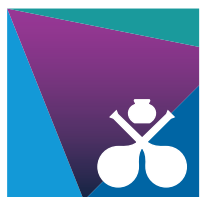
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Unlocking the potential of food-derived bioactive ingredients for preventing *Helicobacter pylori* infection

Xiaohong Sun and Chibuike Udenigwe

- *H. pylori* has infected approximately 50% to 80% of the world population and is categorized as a Class I carcinogen by the World Health Organization. Antibiotics are the most widely used therapy to combat *H. pylori* infection, but they have major drawbacks—particularly the emergence of antibiotic-resistant strains.
- Anti-adhesive therapy against *H. pylori* infection is considered an alternative approach to antibiotic treatments. Some anti-adhesive components against *H. pylori* have been identified from food sources such as human and bovine milk, plant proteins, and cranberry, most of which were demonstrated to function as receptor analogs.
- Although food ingredient-based anti-adhesive therapy has promising potential for preventing *H. pylori* infection in the future, this research field is still in its infancy. Future research is needed to identify more anti-adhesive components from food sources (e.g., bovine milk, egg white, plant proteins) that are safe, cost-effective, readily available, and have commercial potential.

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that can survive and grow in the human stomach. *H. pylori* has infected an estimated 50% to 80% of the world population, particularly in developing countries. *H. pylori* infection can cause a variety of gastric diseases, such as peptic ulcer disease, chronic gastritis, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. Notably, *H. pylori* has been categorized as a Class I carcinogen by the World Health Organization (WHO).

Antibiotics are the most widely used therapy against *H. pylori* infection, resulting in eradication rates in the range of 60% to 90%. However, antibiotics have several major drawbacks, such as the emergence of antibiotic-resistant strains, low biostability and bioavailability, and side effects (Sun, *et al.*, 2020a). Nowadays, people consume food not only to obtain essential nutrients, but also to enhance human health and prevent chronic diseases. Many food-derived compounds have shown various health-promoting properties, such as anti-hypertensive and anti-inflammatory activities. Can food-derived bioactive ingredients prevent *H. pylori* infection? To address this question, it is important to understand the pathogenesis of *H. pylori* infection.

HOW DOES *H. PYLORI* INFECT THE HUMAN STOMACH?

When entering the stomach lumen, *H. pylori* utilizes urease to neutralize the acidic condition and thus can survive in

the harsh environment of the stomach. Also, the viscosity of mucus layers decreases due to the increased environmental pH, which facilitates *H. pylori* penetration of the mucus layers and movement toward the epithelial cells. As illustrated in Figure 1, *H. pylori* adhesins (the outer membrane proteins) specifically recognize the host receptors on the surface of epithelial cells, achieving the *H. pylori* adhesion. Once adhered to the gastric epithelium, *H. pylori* can resist the hostile environmental conditions, such as the forces exerted by gastric peristalsis and emptying, obtain nutrients for growth, deliver toxins to damage the host cells, and subsequently cause gastric infection.

WHAT IS ANTI-ADHESIVE THERAPY?

H. pylori adhesion to the gastric epithelium is an essential step in the establishment of an infection. Thus, the infection should be prevented if bacterial adhesion to the epithelium can be inhibited by anti-adhesive components. This approach is called anti-adhesive therapy (Sun, *et al.*, 2017 and 2019). Anti-adhesive therapy against infectious diseases is considered an alternative approach to antibiotic treatments. Compared to antibiotic regimens, anti-adhesive therapy is moderate and safe since it does not exert an environmental pressure (e.g., kill pathogens or inhibit their growth) and is less likely to produce resistant strains. Although resistant strains may also develop in anti-adhesive therapy, they would be diluted by the sensitive strains, resulting in a significantly lower rate of producing the drug-resistant strains.

WHY DO FOOD-DERIVED BIOACTIVE INGREDIENTS HAVE ANTI-ADHESIVE ACTIVITY AGAINST *H. PYLORI*?

The major function of anti-adhesive components is to interfere with the interactions between bacterial adhesins and host receptors. Generally, anti-adhesive components can be classified as adhesin analogs and receptor analogs (Sun and Wu, 2017).

Specifically, adhesin analogs (e.g., plant lectins) would competitively bind to the host receptors, making the binding sites for bacterial adhesins less available. On the other hand, anti-adhesive components as receptor analogs competitively bind to *H. pylori* via adhesins, such that *H. pylori* is not able to adhere to host cells (Fig. 2). Based on current research, most of the identified food-derived bioactive ingredients with anti-adhesive activity against *H. pylori* function as receptor analogs (Sun, *et al.*, 2020b).

RESEARCH PROGRESS ON THE FOOD INGREDIENT-BASED ANTI-ADHESIVE THERAPY AGAINST *H. PYLORI* INFECTION

Some anti-adhesive components against *H. pylori* infection identified from food sources are summarized in Table 1 (Sun and Wu, 2017; Sun, *et al.*, 2020b). The anti-adhesive activity of these food ingredients was established based on *in vitro* models, animal studies, and human clinical trials. For example,

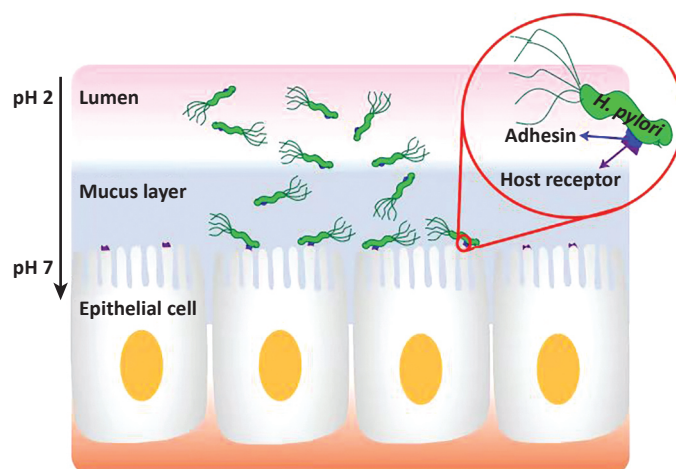


FIG. 1. *H. pylori* adhesion to the gastric epithelium is an essential step in the infection process.

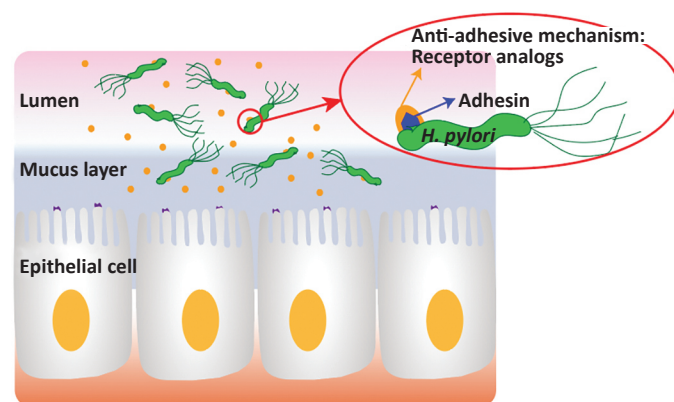


FIG. 2. Anti-adhesive mechanism of food-derived bioactive ingredients against *H. pylori*

caseins, which account for almost half of the protein contents in human milk, showed anti-adhesive activity against *H. pylori* *in vitro*. As a possible bioactivity mechanism, the fucose-containing glycan moieties in κ -casein functioned as receptor analogs and adhered to *H. pylori*.

In vitro studies also indicated that cranberry is rich in anti-adhesive components against *H. pylori*. A high molecular weight, non-dialyzable material (NDM), and polyphenols were identified to be responsible for the anti-adhesive activity of cranberry, although their anti-adhesive mechanisms seemed different. The anti-adhesive activity of NDM from cranberry arose from its ability to block the sialyllactose-specific adhesin of *H. pylori*, whereas polyphenols from cranberry inhibited *H. pylori* growth by changing *H. pylori* into a coccoid form. Recently, our group (Sun, *et al.*, 2020a) identified several peptides with potent anti-adhesive activity against *H. pylori*, from hydrolysates of wheat germ (main byproducts of wheat milling industries), and the possible structure-activity relationship is currently being established.

Lactoferrin and fat globule membrane fractions from bovine milk showed moderate capacity to inhibit *H. pylori* infection in a mouse model, and the highest healing

TABLE 1. Identified food-derived anti-adhesive components against *H. pylori*

Anti-adhesive component	Food sources	Phase of clinical research	Underlying mechanism	Year
κ-casein	Human milk	<i>In vitro</i>	Fucose-containing glycan moieties as receptor analogs to bind with <i>H. pylori</i>	1995
Non-dialyzable material (NDM)	Cranberry	<i>In vitro</i>	NDM blocked the sialyllactose-specific adhesin of <i>H. pylori</i> .	2002, 2007
Polyphenols	Cranberry	<i>In vitro</i>	Inhibit <i>H. pylori</i> growth by changing <i>H. pylori</i> into a coccoid form	2008
Peptides	Wheat germ protein	<i>In vitro</i>	Peptides act as receptor analogs.	2020
Lactoferrin, fat globule membrane fractions	Bovine milk	Mouse model	Sialylated glycoconjugates acting as receptor analogs	2001
3'-Sialyllactose sodium salt (3'-SL)	Human and bovine milk	<i>In vitro</i> , animal study, clinical trial	3'-SL acts as receptor analog and binds to sialic acid-binding adhesin (SabA) of <i>H. pylori</i> .	1997, 1999, 2003

rate was 30%. Moreover, 3'-Sialyllactose sodium salt (3'-SL), an oligosaccharide naturally occurring in human and bovine milk, is the most well-studied anti-adhesive component. Its anti-adhesive activity against *H. pylori* has been evaluated using *in vitro* models, animal studies, and human clinical trials. The safety of 3'-SL-mediated anti-adhesive therapy was proved using rhesus monkeys; however, its efficacy for eradication of *H. pylori* was not conclusive. Specifically, two out of six monkeys were cured permanently, and one was cleared transiently after treatment with 3'-SL. The other three monkeys remained persistently colonized by *H. pylori* after the treatment. The safety of anti-adhesive therapy based on 3'-SL was further validated in humans in a double-blind, placebo-controlled clinical study, but 3'-SL did not reduce or eradicate gastric colonization by *H. pylori*.

In conclusion, although food ingredient-based anti-adhesive therapy holds promising future for preventing *H. pylori* infection, this research topic is still in its infancy. Most of the anti-adhesive studies were conducted *in vitro*, and the anti-adhesive efficacy of food ingredients have not been validated using animal studies and human clinical trials. Besides, the molecular mechanisms of the anti-adhesive components and structure-activity relationship need to be further elucidated. Future research is needed to identify more anti-adhesive components from food sources (e.g., bovine milk, egg white, plant proteins) that are safe, cost-effective, readily available, and have commercial potential.

Xiaohong Sun is currently working as a postdoctoral research fellow at the School of Nutrition Sciences, University of Ottawa, Canada, and also as an Associate Professor in the College of Food and Biological Engineering, Qiqihar University, China. She obtained her Ph.D. degree in Food Science and Technology at the University of Alberta in 2017. Her main research interests include food chemistry, food processing, food matrix interaction,

and value addition to agro-food byproducts. Sun has published 26 peer-reviewed papers in high-impact journals related to food research and three book chapters. She can be contacted at xs7@ualberta.ca and xsun5@uottawa.ca.

Chibuike Udenigwe is Professor & University Research Chair, School of Nutrition Sciences, University of Ottawa.

Further reading

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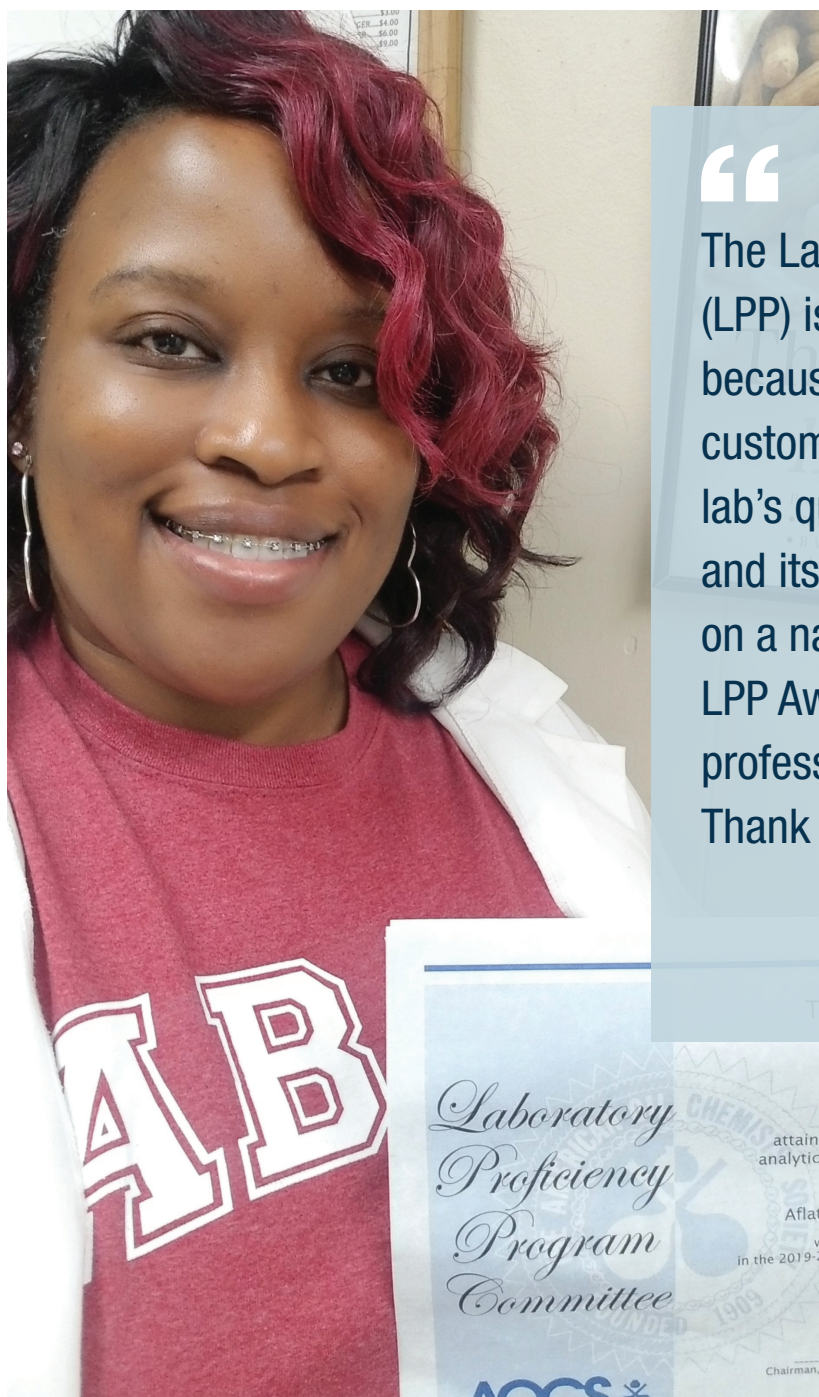
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Dendrimers for enhancement of lipase activity

Adeeb Hayyan, Amal A.M. Elgharbawy, Maan Hayyan, Andrew T.H. Yeow, Wan Jeffrey Basirun, Sharifah Shahira Syed Putra, and Mohamed E.S. Mirghani

- Lipases are prominent and versatile enzymes used in biochemical reactions and organic synthesis.
- Dendrimers can be used to immobilize lipase enzymes and overcome their inherent drawbacks.
- Dendrimers have many merits as immobilization carriers; they have high biocompatibility, are simple to modify, and are highly tailorable for specific applications.
- Our research group has used PAMAM dendrimers to enhance the enzymatic activity *Rhizopus niveus* lipase (RNL).

Lipases are enzymes that promote biochemical reactions in food, beverage, textile, leather, oleochemical, and pharmaceutical production, as well as organic synthesis (Fig. 1).

The lipase triacylglycerol ester hydrolase, EC 3.1.1.3, for example, catalyzes the kinetic resolutions of a broad spectrum of substrates in various bioconversion reactions, such as ester hydrolysis and synthesis transesterification, interesterification, and other organic reactions, due to its hydrolytic properties (de Miranda, *et al.*, 2015).

Lipases have been effectively applied as enzymatic catalysts in a variety of industrial applications, from the synthesis of flavors and fragrances that enhance food and beverage products to the transesterification of oils and fats in biodiesel production. Additionally, lipases are extensively deployed in wastewater treatment in anaerobic digesters

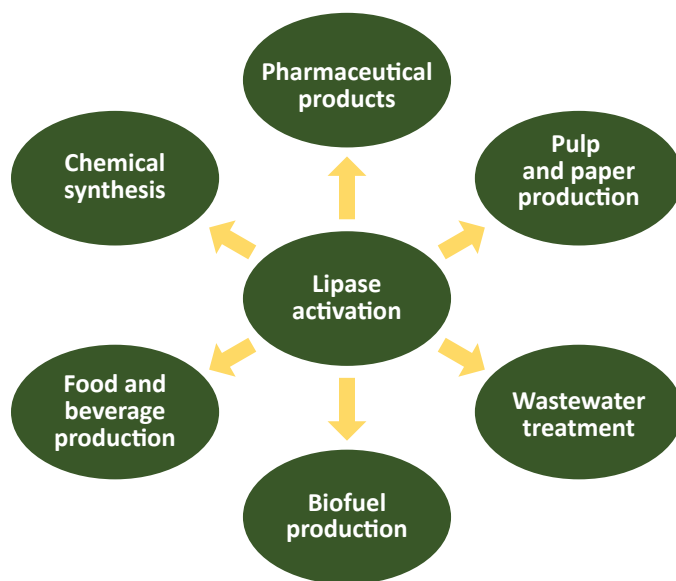


FIG. 1. Versatile uses of activated lipase for various industrial applications



that degrade lipids as a pre-treatment step during wastewater treatment of industrial effluents (Chandra, *et al.*, 2020). However, industrial uses of lipases often lead to denaturation due to their low stability, and the reusability and recovery of free lipases is poor due to their solubility in aqueous solutions, leading to highly challenging processes and high costs (Elgharbawy, *et al.*, 2020).

The immobilization of lipase enzymes can be used to overcome these drawbacks while maintaining their enzymatic activity or specificity and increasing their reusability. Various methods for enzyme immobilization have been explored on an industrial scale, with the most common methods being physical (through adsorption or entrapment) and chemical (through crosslinking or chemical bonding). Particularly in the case of chemically immobilizing enzymes, nanomaterials, such as carbon nanotubes and magnetic nanoparticles, have been explored to immobilize lipase enzymes for various applications (Zhao, *et al.*, 2016).

Among them, dendrimer-modified nanomaterials offer promising prospects in enzyme immobilization due to their high compatibility with biomolecules. Dendrimers are three-dimensional, synthetic polymeric macromolecules that are hyperbranched in structure (Yetim and Sarı, 2019). Structurally, dendrimer molecules start from a central core consisting of multiple reactive sites that further grow into branches symmetrically. The number of branching reactions grown from its

center point is classified into generations, and each generational increase causes a change in size (radius), shape, flexibility, and active sites (Kurbatov, *et al.*, 2020).

Polyamidoamine dendrimers (PAMAM) are particularly interesting due to a structure containing large quantities of amino functional groups that can enable the binding of biomolecules. Additionally, the highly branched structure of dendrimers provides a larger surface area, which contributes to protecting the conformational structure of lipase, thus increasing the contact area between the active site of lipase and the substrates (Li, *et al.*, 2018).

PAMAM dendrimers have been demonstrated as immobilization carriers for lipase enzymes. Zhao, *et al.* (2016) reported on the immobilization of *Candida rugosa* lipase (CRL) onto PAMAM-grafted magnetic carriers. CRL immobilized on the fourth generation ($G_{4.0}$) amine-grafted dendritic magnetic carriers exhibited superior performance compared to free lipases, with increased tolerances in operating pH and temperatures. Wan, *et al.* (2019) successfully immobilized porcine pancreatic lipase (PPL) onto $G_{3.0}$ PAMAM dendrimer-grafted hollow magnetic fibrous silica nanoparticles, resulting in the immobilized PPL maintaining nearly 90% of its initial activity after eight recycling runs (Fig. 2, page 18).

In addition to lipase enzyme immobilization, the role of PAMAM dendrimers as protein and enzyme activators have been reported by our group (Elgharbawy, *et al.*, 2020). When

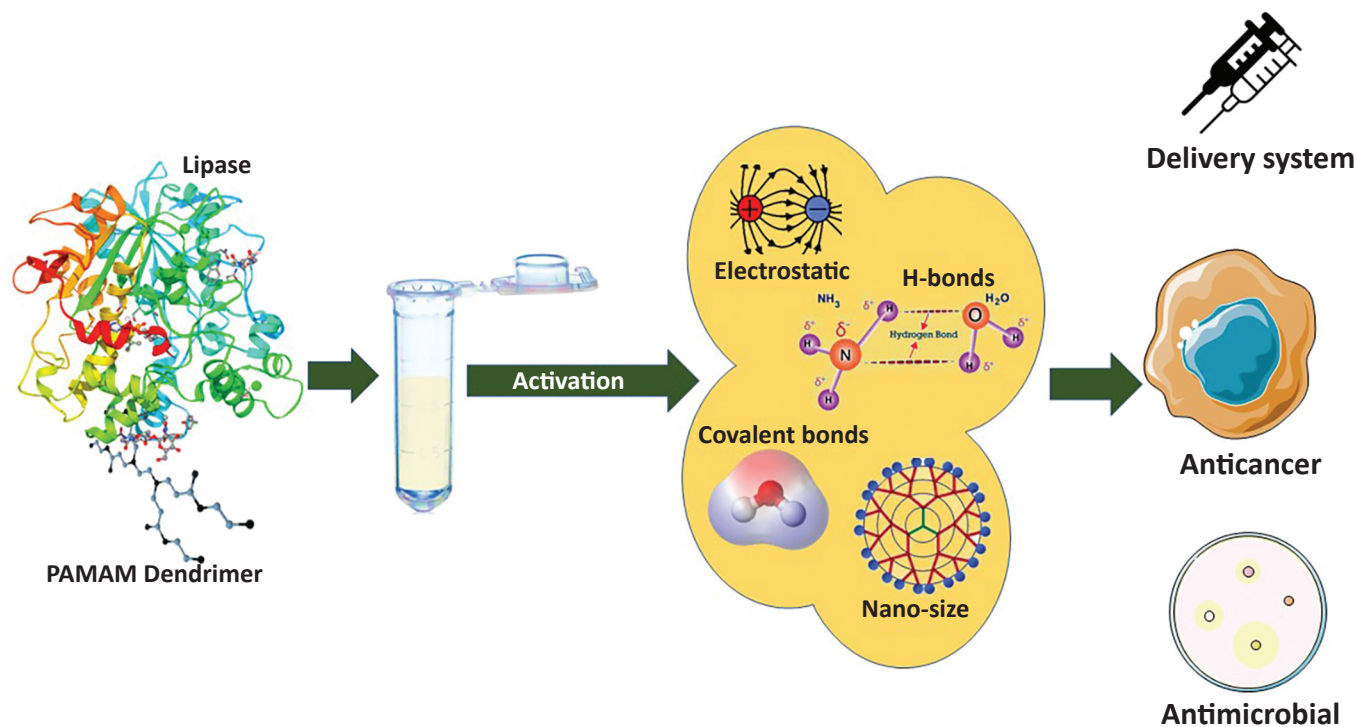


FIG. 2. Applications of lipase activation using dendrimers

PAMAM dendrimers were dispersed in the lipase enzyme buffer solution, an overall increase in the relative activity for the hydrolysis of para-nitrophenyl palmitate (pNPP) was observed as depicted in Fig. 3. The addition of G_{0.0} PAMAM dendrimers was observed to enhance the enzymatic activity of RNL by eight-fold and can be attributed to the hyperbranched nature of PAMAM that provides multiple attachment sites through the terminal amino groups for the lipase enzyme.

As the G_{0.0} PAMAM dendrimers were observed to exhibit the highest enzymatic activation correlated to the high relative activity of RNL against the control group, further optimization

of several operating parameters, including enzyme coupling time, incubation temperature, and pH of environment were investigated. The optimal incubation parameters were identified at 40°C for 2 h at a neutral to mildly alkaline pH environment of 7.0 to 8.0, which aligns well with previously reported works on lipase immobilization with dendrimers (Wan, *et al.*, 2019; Yemul and Imae, 2005). Additionally, the half-life of lipase incubated at the optimal parameters were studied with and without the G_{0.0} PAMAM dendrimers in the aqueous solutions. The half-life of the lipase in the dendrimer-dispersed solution was determined to be 6.4 times higher than the buf-

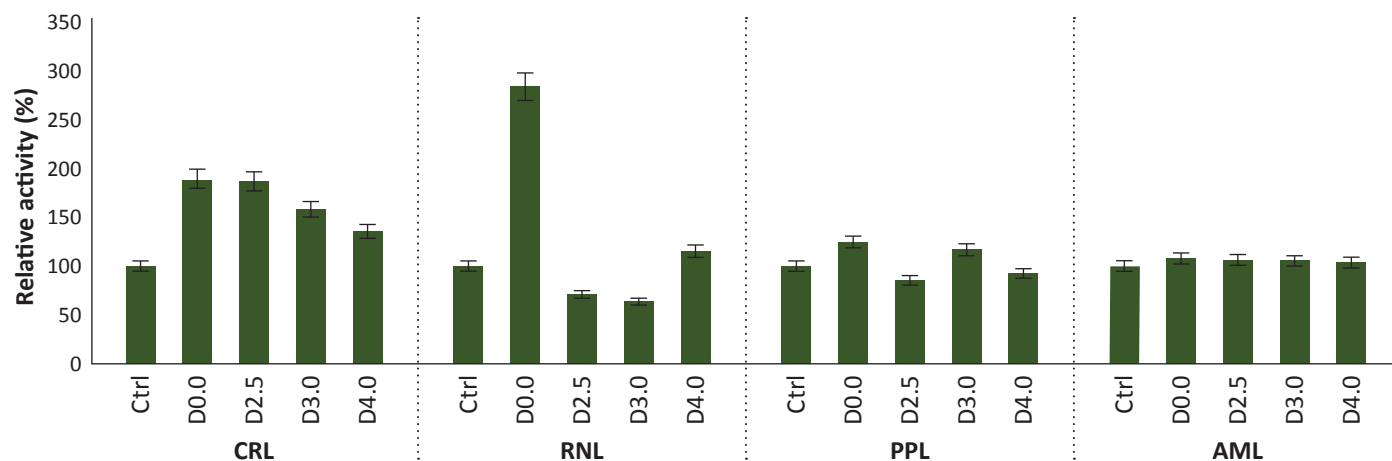


FIG. 3. Comparison of lipase activity in the presence of PAMAM dendrimers. (Abbreviations: *Candida rugosa* lipase (CRL), *Rhizopus niveus* lipase (RNL), Porcine pancreas lipase (PPL), Amano lipase PS (AML). Republished with permission from *Materials Today Communications*, 25, 101492. doi:10.1016/j.mtcomm.2020.101492.

fer solution lipase, indicating that the G_{0,0} PAMAM dendrimers enhanced the stability and maintained the activity of RNL (Elgharbawy, *et al.*, 2020).

The unique physicochemical properties of dendrimers have been investigated for a broad range of applications. Due to their well-defined molecular structure, abundance, and high functionality, dendrimers are viable nanomaterials that can be used for enzyme immobilization through chemical modifications as well as enzyme activators. As a dispersed nanomaterial, dendrimers exhibited lipase activity and stability enhancements. Their relatively mild optimum operating parameters and high enzyme stability make them attractive candidates for further investigations and possible scale up for lipase-catalysed industrial processes.

Corresponding author Adeeb Hayyan is a Senior Lecturer in the Department of Chemical Engineering, Faculty of Engineering, University of Malaya, Malaysia. He has received several international awards, including the Young Chemical Engineer in Academia award from IChemE in 2014, the Manuchehr Eijadi Award from AOCS in 2015, the Laureate MAB Young Scientist Award from UNESCO in 2017, and 2nd runner up for the 2015 ProSPER.Net-Scopus Young Scientist Award in Sustainable Development. In 2020, he made Stanford University's list of the top 2% of Scientists in Engineering in the category of chemical and biotechnology engineering. His main interests include biodiesel production from low grade oils, FFA treatment from low grade oils, deep eutectic solvents, and catalysis. Recently, he

developed a novel technique for converting FFA to useful products and he welcome any collaboration with industrial sector in this field. He can be contacted at adeeb.hayyan@yahoo.com.

Amal A. M. Elgharbawy is an Assistant Professor at the International Institute for Halal Research and Training (INHART), where her research focuses on ionic liquids and deep eutectic solvents and enzymology. Elgharbawy has published several articles and invited reviews related to ionic liquids, enzymology, and nanomaterials in SCOPUS and ISI indexed journals, book chapters, and national and international conferences. She has served as a reviewer for several conferences and well-reputed journals and can be contacted at amal.elgharbawy@gmail.com.

Maan Hayyan is a Lecturer in the Faculty of Engineering and Technology, Muscat University in Oman. Hayyan has been recognized as one of the pioneering researchers in the fields of deep eutectic solvents and superoxide ions. Hayyan has invited from Chem Rev (IF 60.62) to publish the first review article on superoxide ions and their applications. He was awarded many gold medals from international exhibitions for research and innovation. His main interests are the generation and reactions of superoxide ions, in addition to applications pertaining to ionic liquids and deep eutectic solvents. He can be contacted at maan_hayyan@um.edu.my

Other authors are with the University of Malaya and Prof. Mirghani is from INHART, IIUM.

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Continuing the debate: shelf life VS accelerated storage for evaluating antioxidant effectiveness

Natalie Faubion, J. David Pinkston,
Karen Schaich, Sonia Calligaris,
David Bolliet, and Rick DellaPorta

- Developing improved formulations and processing for food stability requires methods that accurately report lipid oxidation in the shortest time possible, but these two goals are not always compatible.
- Accelerated storage methods at elevated temperatures provide rapid answers but can reflect different chemistry than that which occurs over long times at room temperature.
- The online 2021 AOCS Annual Meeting & Expo featured a Lipid Oxidation & Quality/Analytical joint technical session (archived at <https://tinyurl.com/b6zf9h9p>) in which experts discussed the advantages and disadvantages of these two approaches.
- Here is a short summary.

Faubion: The importance of determining product shelf life is well understood. However, divergent techniques for determining or predicting shelf life highlight constraints of this type of testing and are perhaps revealing of the priorities of various research groups. Can you speak to these constraints and explain what is the basis for the 'debate' surrounding ambient shelf-life testing and accelerated storage?

Schaich: Real-time testing provides most accurate measures but requires long times. For most oils and foods, this can take months to more than a year.

DellaPorta: The average product time to market is usually less than the shelf life for many CPG (Consumer Packaged Goods) once a final product formulation has been decided. Running a 3–6 month shelf life is not feasible, so we rely on the ASLT conditions that most closely target the critical parameter (e.g., lipid oxidation).

Calligaris: ASLT (accelerated shelf-life testing)/shelf-life prediction is often based on sample storage under environmental conditions that promote more rapid degradation than experienced by the product on the store shelves.

Bolliet: Food items normally kept under refrigerated conditions might be stored closer to ambient temperatures (ca. 20°C), whereas for items typically stored or displayed at ambient, an ASLT temperature would be higher (30°C, 40°C).

Schaich: Times required here are typically a few days to a few weeks. ASLT is more expedient, but accuracy of information obtained becomes a limiting issue. The problem for ASLT testing is that lipid oxidation is a collection of many different reactions, some occurring simultaneously and some in specific sequences.

Calligaris: Different reaction pathways can occur when chosen storage temperatures are too high for ASLT. Storage temperatures resulting in altered water migration or lipid phase transitions affect oxidation reac-



tion rates. In these cases, an abrupt change in the temperature dependence of the reaction rate can be observed causing positive or negative deviations from the Arrhenius behavior.

Schaich: Arrhenius kinetics have been applied almost universally in shelf-life studies, and Arrhenius kinetics do indeed describe some spoilage reactions. However, accurate use of the Arrhenius equation has two requirements that lipid oxidation does not meet: 1) Reactions and mechanisms must be the same at all temperatures, and 2) Reaction conditions (microenvironments) must remain constant as temperature changes.

Calligaris: The successful application of an ASLT procedure requires that food withstands the accelerating factor increase without changing the pathway of oxidation reactions or inducing [a change in] physical phenomena.

Schaich: Even in the simple free radical chain reaction scheme, reactions are not constant because initiation (formation of L^\bullet) mechanisms can change with temperature. The solubility of oxygen in oil phases and penetration through food matrices increases with heating, so more oxygen becomes available to drive or extend the reaction. The balance between hydroper-

oxide (LOOH) decomposition and new radical formation also changes with temperature. Above 40°C , LOOH decomposition to $LO^\bullet + \bullet OH$ increases and both LO^\bullet and $\bullet OH$ generate new L^\bullet at much faster rates than at low temperatures. We usually find LOOH decomposition dominant between 40 and 60°C , then formation increases faster than decomposition or transformation up to about 80°C . At higher temperatures, thermal scissions add to radical and LOOH formation and LOOH decomposition increases dramatically. The production of volatile lipid oxidation products increases with temperature, but competing processes also increase at the same time, leading to loss of products by volatilization, rapid transformation, or formation of alternate products.

Lipids do not oxidize in isolation. Heat increases molecular mobility and facilitates H abstractions, enhancing lipid radical transfer, e.g., to proteins. Heat also greatly increases Maillard reactions between protein amines and lipid carbonyl products, shifting the failure mode from off-flavors to browning. There is no product that consistently and accurately reflects the progress of lipid oxidation at all temperatures. Lipid oxidation measured by any product will be lower than what actually occurs, and extrapolation of this data will substantially overestimate room temperature shelf life.

Calligaris: Thus, the choice of the range of accelerated conditions to be used is crucial to avoid errors in shelf-life prediction, and an experimental validation is highly suggested to avoid dangerous over or under estimation of product shelf life. Moreover, beside temperature, light can also be regarded as an acceleration factor in ASLT. In this case, the ASLT is conducted by exposing the samples to increasing light intensity levels instead of different storage temperatures. To my knowledge, there are few examples of the use of light as accelerating factor and thus few predictive mathematical models.

Pinkston: What are the 'best practices' for predicting shelf life? What steps should we be sure to take?

Bolliet: Before starting an ASLT, there are many factors to consider, each bringing its own set of constraints and special considerations: the food matrix, packaging, temperature(s), sampling frequency, timeline, resources, data processing/statistics, and so on. Selecting multiple temperatures for an ASLT will result in a more robust shelf-life predictive model. Selecting the temperature where the food item or ingredient will be typically stored at, is a great way to validate the predictive model from the ASLT.

Calligaris: The most important open issues appear to be:

- the identification of acceptability limits—the quality level of products which are still acceptable for consumption. When regulatory limits are not available, the acceptability limit must be chosen according to quality standards and analytical limits;
- the reduction of the time needed for a shelf-life assessment without loss in predictive capability. The first critical choice is the selection of the most appropriate accelerating factor able to speed up change; and
- considering oxidation, both temperature and light could be considered. In contrast with temperature, no accepted mathematical models exist when dealing with light as an acceleration factor in ASLT. A mathematical model describing the light dependency of oxidation must be determined on a case-by-case experimental basis.

DellaPorta: A critical point for best practices is to know the initial state of the product quality—moisture, color, texture, flavor, and oil quality. Have a control product with a known shelf-life history that is run in the ASLT. Samples can be either whole or crushed. The results can be both objective and subjective if whole product is used. All the testing should be run in standard air for the most rapid results. The objective data are the analytical results. The subjective results are derived from trained panelists. The end-result can be either a technical match or an 'equal liking' response depending on the overall expectations.

Schaich: If your goal is extrapolation of data from elevated temperature to predict shelf life at ambient temperature,

40°C should be maximum temperature for any oil or food when applying the Arrhenius equation. If your goal is to obtain rapid comparative information about lipid oxidation and antioxidant behavior, limit ASLT of complex foods to 40°C and include analyses for co-oxidation of relevant molecules. To model specific conditions that oils experience (e.g., frying) use higher temperatures (but not to determine ambient shelf life).

For all ASLT, monitor multiple lipid oxidation products to track how conditions alter lipid oxidation and co-oxidation and to catch shifts in mechanism and initiation, propagation, and termination progression. Include analyses of antioxidants to track stability, volatility, and degradation that affect product quality. Watch for products and changes that may correlate with sensory perceptions or specific problems. Use this detailed information to specifically modify formulations or processing and to design assays specifically focused on key problem or reporter molecules.

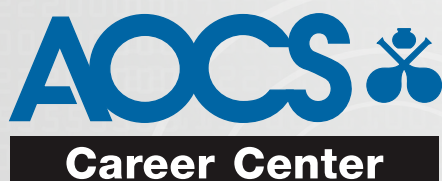
Calligaris: Based on these considerations, the generation of predictive models to assess shelf life is certainly an arduous task but it could provide valuable advantages allowing the reduction of time, cost, and labor required for estimation of shelf life of long-lasting products.

Bolliet: With the current data overload we are all experiencing, research teams across the globe have taken advantage of some of the tools offered by Artificial Intelligence (AI), e.g., artificial neural networks, support vector regression, and other algorithms. To help product development teams generate better and faster shelf-life predictions, we should embrace AI and the seemingly endless computing power it offers.

Schaich: Several papers in this session on the shelf-life testing debate addressed modelling in shelf-life testing. Modelling is essential for extrapolation from extreme to normal conditions, but the models used must accurately describe the data and not be force-fit to linear responses. At the same time, modelling reactions that are constantly changing is a distinct challenge, especially when only one product is analyzed and others are ignored. Multidimensional modelling of how lipid oxidation pathways shift with temperature (using the multiple product assays recommended above) could provide valuable maps to improve accuracy of shelf-life testing as well as efficiency of antioxidant approaches.

Natalie Faubion is Senior Scientist–Discovery at Kalsec, Inc., J. David Pinkston is Technical Services Manager at ADM, Karen Schaich is an Associate Professor in the Department of Food Science at Rutgers University, Sonia Calligaris is an Associate Professor in the Department of Agricultural, Food, Environmental, and Animal Sciences at the University of Udine (Italy), David Bolliet is Senior R&D Manager & Analytical Platform Lead at Kalsec, Inc., and Rick DellaPorta is a Senior Principal R&D Scientist at PepsiCo.

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Enzymatic degumming in oil processing:

a sustainable means of improving yield and reducing residual phosphorus levels

Ying Zha

- Degumming is a process step in the refining of vegetable oils. Its purpose is to remove phosphatides that can darken and reduce the quality of the final, refined oil.
- An alternative to the chemicals typically used in degumming is an enzymatic process based on the enzyme phospholipases, for instance phospholipase A1, and phospholipase C.
- The use of phospholipase A1 substantially reduces the overall phosphatide content in the degummed oil, resulting in an improved final yield (up to 2%), better quality, and reduced carbon footprint.

When crude vegetable oils are received from oil mills, they often still contain natural impurities and contaminants that must be removed during the refining process. The first stage of this process, called degumming, removes phospholipids, or gums, which can have a negative impact on the storage stability and the downstream processing of the oil. (Fig. 1).

Most of the gums are hydratable phosphatides and can be removed with water. Conventionally, phosphoric or citric acid is then added to the oil to convert non-hydratable phosphatides into hydratable phosphatides, which can then be removed by chemical or physical neutralization. Liquid vegetable oils, such as rapeseed oil (canola), sunflower oil, or soy oil, are usually treated with caustic soda, forming soapstock, during the chemical refining process.

Phospholipases were first used as a caustic soda alternative to remove phospholipid gums from vegetable oils as early as 1992. A porcine phospholipid A2 was used, although the results were unsatisfactory [Dijkstra, 1993]. A relatively new and much more successful development has been to use an enzymatic process based on phospholipase A1 and lyso-phospholipase.

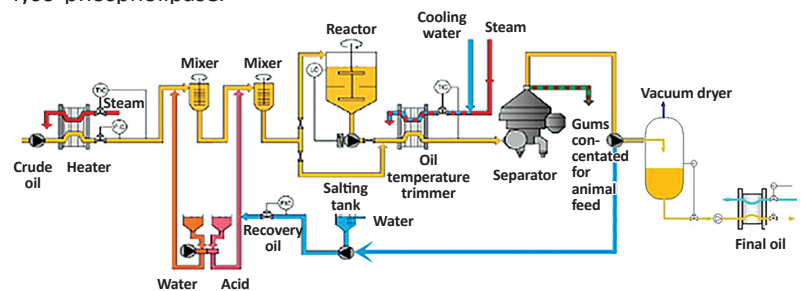


FIG.1 A schematic diagram of the conventional degumming of vegetable oils

Source: www.oil-expeller.net

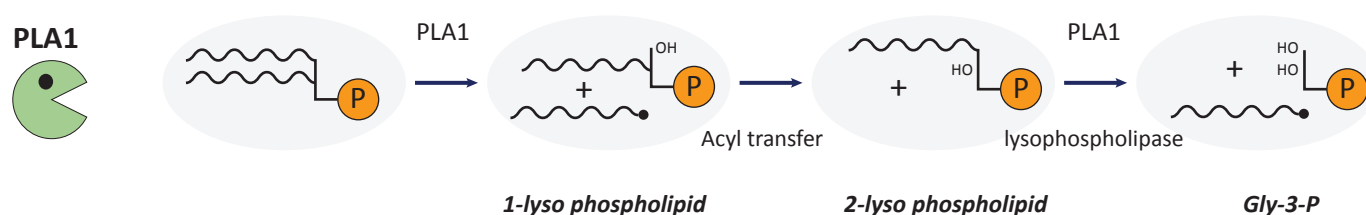


FIG. 2. Conversion of phosphatides into hydratable phosphatides using Purifine® PLA1

Degumming vegetable oils:

- improves quality by eliminating the phosphatides present in the crude oils;
- avoids high refining losses; and
- prevents darkening of the oil (oils with a high phosphatide content will darken, as they are thermally unstable).

A NEW ENZYME, PURIFINE® PLA1 FOR OPTIMAL DEGUMMING

In 2020, DSM developed a new phospholipase for enzymatic degumming under the trade name Purifine® PLA1. This enzyme converts phosphatides into lyso-phospholipids (LPL) and glycerol-phospholipids (GPL) (Fig 2).

In general, phospholipases hydrolyze the ester components of the phospholipid components of cell membranes to produce a range of simpler lipid materials, such as free fatty

acids, lyso-phospholipids, di-acyl glycerols, phosphotidates, and choline phosphates.

Phospholipases exist in the forms A1, A2, B, C, and D according to the position they hydrolyze on the phospholipid backbone. Phospholipase A1 cleaves the acyl ester bonds at the sn-1 position to produce a free fatty acid and 2-acyl lyso-phospholipid [Bourtsala 2019.]

Using Purifine® PLA1 for enzymatic degumming has many benefits. It:

- increases total oil yield by up to 2%;
- reduces the carbon footprint and the energy consumption of refining due to the increased production efficiency;
- eliminates the addition of caustic chemicals;
- does not involve soapstock formation and, hence, consequent centrifuge fouling;
- is thermostable up to 70°C; and
- requires only low doses.

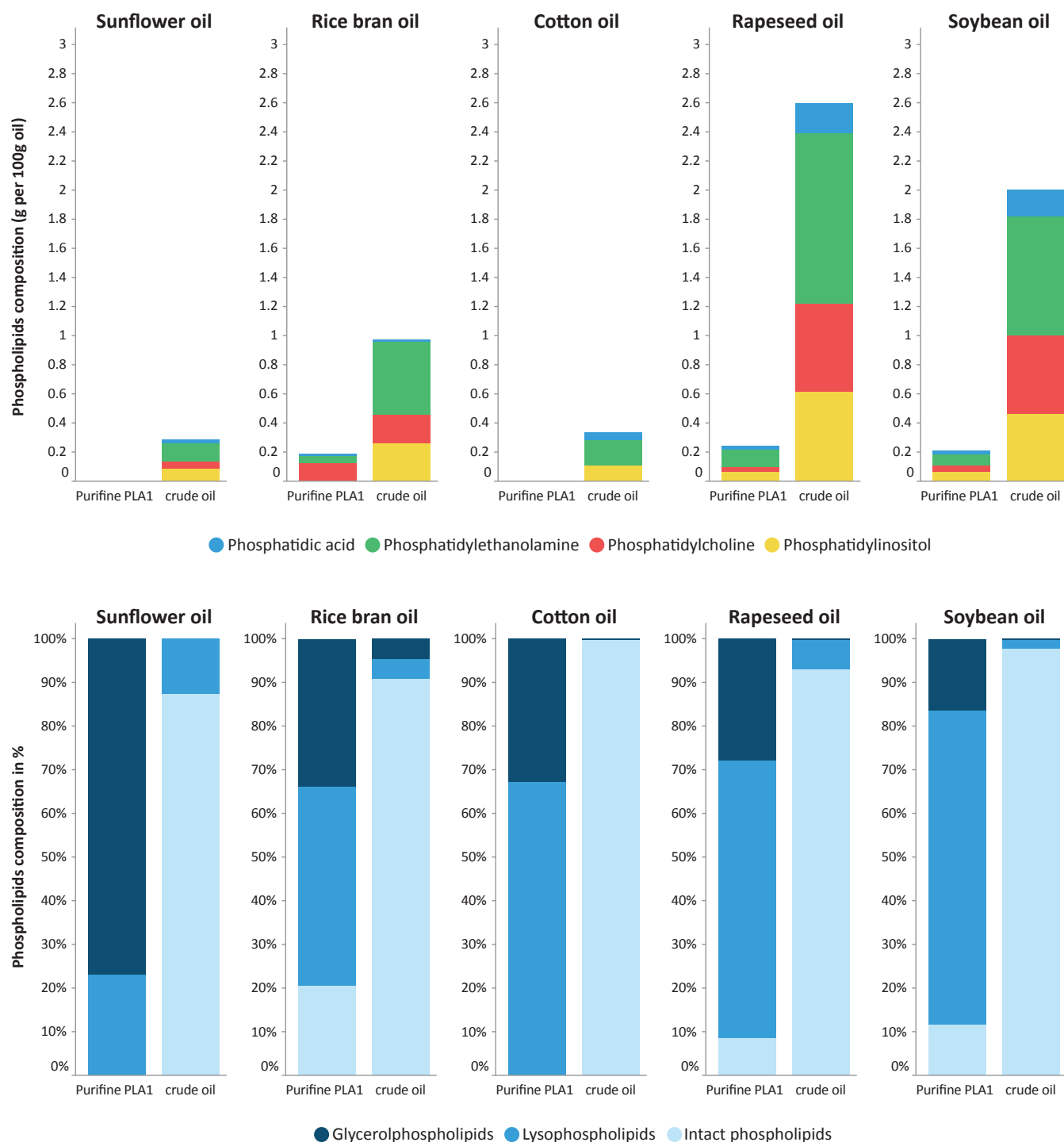


FIG. 3. Performance of Purifine® PLA1 in various vegetable oils

Purifine® PLA1 works well in most types of vegetable oil, including soybean oil, rapeseed oil, cotton oil, rice bran oil, and sunflower oil. The performance of Purifine® PLA1 in these oils is shown in Fig. 3.

In these five vegetable oil types, the conversion of intact phospholipids (PL) to their degradation form, namely lyso-phospholipids (LPL) and glycerol-phospholipids (GPL), reached 80% or more. The results show particularly impressive performance with rapeseed and soybean oil. The efficacy

of the enzyme means that phosphorous levels of less than 15 ppm can be achieved in the final refined oil, without the need for any caustic addition during the entire process.

Recent research carried out on the degumming of crude sunflower oil with phospholipase A1 showed not just the expected reduction in phospholipids, but also in the calcium and magnesium levels. Most importantly, the oxidative stability index (OSI) was reduced, which indicates that the storage life of the oil had been increased [Llamas, 2014].

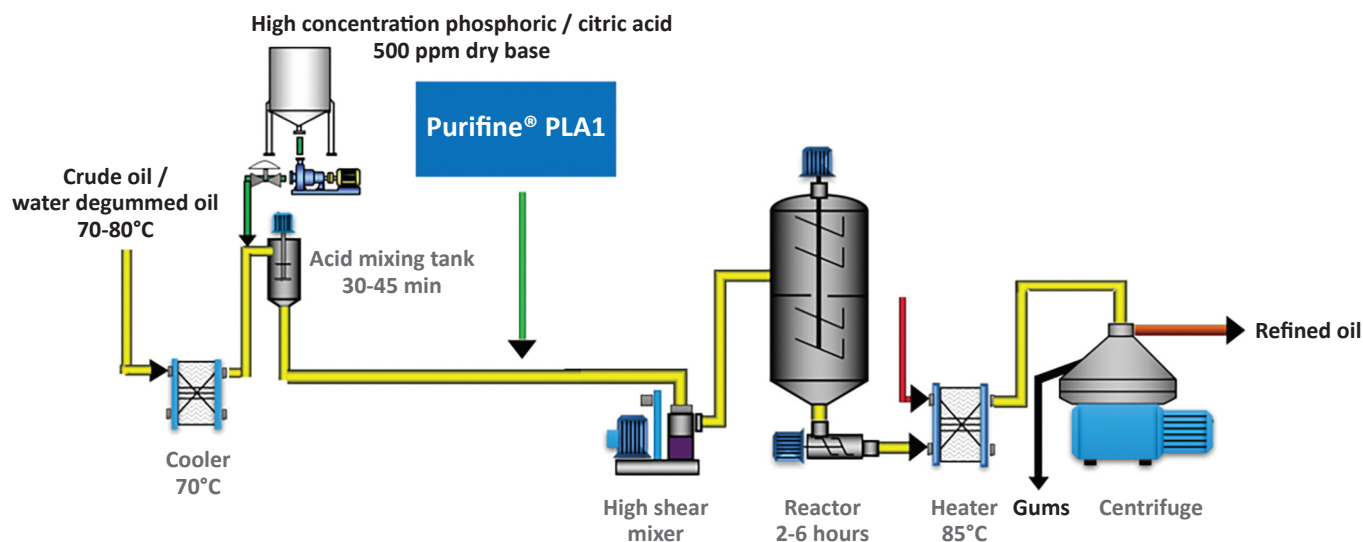


FIG. 4 Typical enzymatic degumming process design

THE EQUIPMENT REQUIRED FOR ENZYMATIC DEGUMMING WITH PURIFINE® PLA1

To use enzymatic degumming, modification to the refining plant is necessary (Fig. 4). The phospholipase enzyme should be introduced to the water in the degummed oil stream using a high-shear mixer. The oil then must be directed to a suitable batch reactor vessel that provides sufficient retention time, approximately 2–6 hours. Typically, maximum conversion of insoluble to soluble phosphatides is achieved by a dose of 30–50 ppm Purifine® PLA1 in 4–6 hours at a pH of 4–4.5

(achieved by addition of 500–1,000 ppm citric or phosphoric acid) and at a temperature of 50–65°C. Note that the Purifine® PLA1 is unusually thermostable and can operate at temperatures of up to 70°C.

Purifine® PLA1 reduces the carbon footprint and the energy consumption of the refining process due to the increased production efficiency. Compared to a conventional oil refining process, Purifine® PLA1 typically reduces the carbon footprint of the production process by 30%–35%. For a mid-sized refinery, that means an annual reduction of 5,000 tons of carbon dioxide emissions—making processes more efficient while also reducing the impact on the planet.

Traditional method derived gum



Oil in gum ~30%

Purifine PLA1 derived gum



Oil in gum ~10%

FIG 5. Comparative appearance of gums from traditional and enzymic refining

Purifine® PLA1 enables physical refining on all types of crude and degummed oils, removing chemical usage and achieving demanding phosphorus specifications, while increasing oil yield for more efficient, sustainable processes. Typically, a crude soybean oil refining plant with 400-ton oil/day capacity can achieve a revenue of USD 250,000 a year by using enzymic refining.

Figure 5 (page 27) shows the comparative appearance of gums from traditional and enzymic refining. The gum after enzymatic degumming is less sticky and contains much less oil.

MORE PURIFINE ENZYMES

Higher oil yields in the water degumming process can be achieved using the enzyme Purifine® 3G. This blend of enzymes contains both phospholipase C and phospholipase A2, increasing oil yield by forming diglyceride. Furthermore, it reduces the total gum volume and reduces its ability to form an emulsion. Less emulsion formation means less oil yield loss in gums, due to entrapped oil, while a lower gum content means cleaner separation of the oil from the heavy phase—and lower-fat, higher-protein content in the meal. Purifine® 3G is used at an

industrial level on both crude soybean oil and rapeseed/canola oil to reach oil yield increases of up to 2.5%.

The lecithin obtained from vegetable oil gums can be modified using the lecithinase enzyme Purifine® LM. Purifine® LM can work at elevated temperatures up to 80°C, and is able to reach a phospholipid conversion in gums or lecithin of 50% within four hours reaction time. The Purifine® LM modified lecithin is an excellent oil-in-water emulsifier, and can be applied in both food and feed applications.

ENZYMES: GETTING MORE WITH LESS

In today's climate, the pressure to optimize outputs from valuable natural resources—and to do so in a sustainable way—is higher than ever for vegetable oil processors. Phospholipase enzymes offer a cleaner, safer alternative to chemicals to boost process efficiency and help producers deliver on strict yield, phosphorus specification, and performance requirements, while also delivering consistent results.

Ying is the application manager for DSM's Fats & Oils portfolio. She was previously a scientist in DSM's Biotechnology Center and holds a PhD in biotechnology from Leiden University. To find out more about how phospholipase enzymes like DSM's Purifine® PLA1 can help to boost resource efficiency and reduce energy costs, contact Ying Zha at ying.zha@dsm.com or visit https://www.dsm.com/food-specialties/en_US/products/beverage/purifine.html.

AOCS MEETING WATCH

January 24, 27, 28, 2022. Design of Lamellar Gel Network Emulsions for Personal Care and Cosmetics Applications, AOCS Continuing Education Program Certificate Course, online.

February 4–5, 2022. Canadian Lipids and Proteins Conference, online.

May 1–4, 2022. AOCS Annual Meeting & Expo, Atlanta, Georgia, USA, and online.

April 30–May 3, 2023. AOCS Annual Meeting & Expo, Denver, Colorado, USA, and online.

For in-depth details on these and other upcoming meetings, visit <http://aocs.org/meetings> or contact the AOCS Meetings Department (email: meetings@aocs.org; phone: +1 217-693-4831).

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The new research on fatty acids and migratory birds

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

Rebecca Guenard

When temperatures in the southern hemisphere start to rise in early spring, great swaths of migratory birds traverse starry skies on a journey to their northern nesting grounds. They fly in the dark to take advantage of cooler temperatures, calmer air, and fewer predators. These are among a list of measures the birds take to maximize energy efficiency across as much as 10,000 miles of flight. Along the way, migratory shorebirds also get an energy boost by stopping to feed on the biofilms that form on intertidal estuarine mudflats.

Despite taking this arduous journey to breed, shorebird populations have declined over the past three decades. North America has lost a third of its shorebirds, and globally the population of Arctic-bound migratory species is down by nearly half. In the last year, researchers have published findings that point to changes in the fatty acid content of biofilms as a possible source of the decline.

First observed under a microscope as a coating on teeth, a biofilm is a cluster of microbes confined within a polymeric matrix and attached to a surface. These films form on a variety of surfaces (from rocks in a stream to medical devices implanted in the body), and their genetics differ from the freely suspended organisms that may surround them. A surface exposed to seawater, for example, will form a film of organic material within minutes of contact.

The nutrient concentration of a film depends on its composition, which changes according to environmental conditions. Water temperature, pH, salinity, and other seasonal effects alter bacterial attachment and biofilm formation in different aqueous systems.

On intertidal estuarine mudflats, biofilms spread across the sediment in mats only 0.5 to 3 millimeters thick. They con-

sist mostly of photosynthetic diatoms (also called phytoplankton) that require sun exposure, requiring the films stay very thin. The diatoms, along with heterotrophic and cyanobacteria, secrete a mucus-like extracellular polymeric substance composed of a mixture of polysaccharides and proteins that adhere the film to the sediment.

Along their journey from South America to Alaska, where they breed, the western sandpiper stops over at the Fraser River estuary in British Columbia, Canada, to graze on intertidal biofilm. Different sandpiper species, living on the other side of the globe, travel from southeast Asia to Russia, stopping to fill up on the biofilms of the Yellow River mudflats. In fact, biofilms make up as much as 60% of the sandpiper diet.

Researchers discovered that the invertebrates sandpipers typically munch on do not provide the extra lipids and fatty acids the birds need during migration. Sandpiper tongues resemble bristles, which ecologists believe is a specific adaptation designed to scrape biofilm from mudflat surfaces. Hence, just like humans, birds rely on the sea to get most of the fatty acids they need. Terrestrial species in the food chain are deficient in these essential nutrients.

Another important factor for the birds is the biofilms' fatty acid composition. Whether the films on these estuary pit-stops provide more saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA) is of utmost importance to migratory birds. However, which fatty acids diatoms produce changes with ambient conditions. Studies have shown that, in general, fatty acid abundance at the Fraser River estuary peaks just as western sandpipers arrive for a respite from their northbound spring migration.

Seasonal triggers stimulate lipid production in diatoms. Longer spring days mean higher temperatures and greater photon density from the sun, providing ideal conditions for algal cells to photosynthesize. There is a five degree temperature window occurring in spring outside of which biomass growth is limited and cell death occurs. Fresh water permeates the tidal estuary with spring snow melt, causing changes in water salinity and pH. Although more light and warmth encourages diatom growth, a Canadian research team found that it was the change in water salinity that prompts lipid formation.

According to laboratory experiments, microalgae have two distinct phases of lipid development. With all their nutritional requirements fulfilled, diatoms make predominantly PUFA-rich membrane phospholipids. When any requirement is deficient and cells are stressed, they produce more triacylglycerol composed of SFA and MUFA. The research team has proposed that a burst of fatty acids occur in biofilms when diatoms in a growth phase are suddenly triggered to accumulate lipids by changes in tidal water chemistry.

Many questions remain about the why sandpiper fill up on biofilm. Studies on muscle tissue indicate that the increased fatty acid intake improves oxygenation. The birds could be maximizing their fitness for the final leg of the journey to their

Information

A world on the wing: The global odyssey of migratory birds by Scott Weidensaul, March 30, 2021, Norton, W.W. & Company, Inc.

Assessing diatom-mediated fatty acids in intertidal biofilm: a new conservation concern, Ollinik, J.E., *et al.*, *Environ. Syst. Res.* 10: 30, 2021.

Natural variation in yolk fatty acids, but not androgens, predicts offspring fitness in a wild bird, Montesana, L., *et al.*, *Front. Zool.* February 18, 2021.

Peak abundance of fatty acids from intertidal biofilm in relation to the breeding migration of shorebirds, Schnurr, P.J., *et al.*, *Front. Mar. Sci.*, February 18, 2020, <https://doi.org/10.3389/fmars.2020.00063>.

nesting grounds. A German research team recently found that egg yolks with higher fatty acid concentrations lead to more robust off-spring. They found that mothers allocate resources, like fatty acids, to the yolk, influencing their offspring's phenotype. Sandpipers may not be guzzling biofilms for themselves; they could be attempting to increase the viability of the next generation. Or maybe both explanations are possible.

Scientists continue to investigate the fatty acid consumption of migratory birds in more detail to determine what effect the natural changes in diatom composition has on shorebird populations. Sampling biofilms poses a challenge. Compositions change spatially across the mudflats, so samples must be gathered in a range of locations. In addition, fatty acids degrade if not treated with care, and their source (whether diatom or invertebrate) must be distinguished.

The challenge is worth pursuing. Proving the importance of intertidal estuarine mudflats will help with their conservation. The significant loss of shorebirds coincides with increased coastal development around the world. Mudflats are bulldozed to make way for industrial sites and shrimp farms. They have been backfilled to build shoreline homes. In addition, effects of climate change threaten their existence. Rising waters shrink mudflat land mass and higher temperatures mean biofilms will not grow. Without an influx of freshwater from melting snow, diatoms will not swell with fatty acids to feed migratory birds.

The Chinese government banned the destruction of mudflats around the Yellow River in 2018, hoping to turn around dwindling shorebird numbers. Ecology researchers want to provide clear evidence of the mudflat's relevance to encourage more countries to adopt similar conservation measures.

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

Highlights from the US FDA's final guidance on sodium

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

Last October, the US Food and Drug Administration (FDA) issued a final guidance on sodium, “Voluntary Sodium Reduction Goals: Target Mean and Upper Bound Concentrations for Sodium in Commercially Processed, Packaged, and Prepared Foods” (<https://tinyurl.com/3c6hpwr9>) which provides food manufacturers, chain restaurants, and food service operators with voluntary short-term (2.5-year) targets for reducing sodium in 163 categories of processed, packaged, and prepared foods.

Sodium is widely present in the US diet, and most of it comes from eating or drinking foods to which sodium chloride, commonly referred to as “salt,” has been added. More than 70% of total sodium intake is from sodium added during food manufacturing and commercial food preparation. Average



The FDA's approach to salt reduction

The reduction guidance is based on the following principles:

- Reduction in sodium levels should progress gradually to allow time for product reformulation;
- Population-level sodium intake reduction should progress at a pace such that consumer preferences and expectations for saltiness in foods adjust;
- Reduction in sodium levels should not lead to reformulation that negatively affects the nutritional quality of the foods by modifying other nutrient levels (e.g., by increasing added sugars or saturated fat content) and should take into account all Dietary Guidelines recommendations and FDA policies;
- Population-level sodium intake reduction will involve ongoing voluntary efforts led by the food industry, in collaboration with FDA, its federal partners, and other stakeholders;
- Goals should be expressed in ways that support ongoing efforts to track modifications to the sodium content of the food supply over time;
- Successful sodium reduction is contingent upon broad participation by and distribution of impacts across the food industry; and
- Population-level sodium intake reduction can be advanced through both the categorization of the food supply based on relevant data and information (e.g., ingredient similarity, technical effects in the food, role in food safety, and range of sodium concentrations in marketed products) and the use of voluntary objectives.

sodium intake in the United States is approximately 3,400 milligrams/day (mg/day), while the recommendations of scientific groups charged with examining the totality of the evidence, including scientific bodies, qualified experts, and governments around the world, support limiting sodium intake to about 2,300 mg/day.

Ninety-six countries have implemented a national strategy for sodium reduction, and the Dietary Guidelines for Americans, 2020–2025 advises individuals 14 years and older to limit their consumption to 2,300 mg/day; this aligns with recommendations from the National Academies of Sciences, Engineering and Medicine, which set the Chronic Disease Risk Reduction Intake for sodium at 2,300 mg/day for those

14 years and older. Although a 3,000 mg/day reduction falls short of these Dietary Guidelines for Americans, the 2.5-year goals are intended to balance the need for broad and gradual reductions in sodium and what is publicly known about technical and market constraints on sodium reduction and reformulation.

The short-term goals include both a target mean concentration and an upper bound concentration of sodium for various specified categories of food. The target mean sodium concentration is the goal for the food category as a whole, rather than for every product in that category. The upper bound concentration is the goal for the highest sodium concentration for any product in that food category (a table list-

Why reduce salt?

Research shows that excess sodium consumption is a contributory factor in the development of hypertension—a leading cause of heart disease and stroke, and the first and fifth leading causes of death in the United States, respectively. Decreasing population sodium intake is therefore expected to reduce the rate of hypertension.

Research also shows that the increase in blood pressure seen with aging, common to most Western countries, is not observed in populations that consume low-sodium diets and that the US population consumes far more sodium than recommended. Moreover, dietary reduction of sodium can lower blood pressure as has been demonstrated in the Dietary Approaches to Stop Hypertension (DASH)-Sodium trial and other experimental studies.

Multiple studies have estimated the public health and economic benefits associated with broad reduction in sodium intakes in the United States. Those studies have shown that reductions in average intake (modeled at a variety of intake levels below current intake, down to an average level of roughly 2,200 mg/day) have been estimated to result in tens of thousands fewer cases of heart disease and stroke each year, as well as billions of dollars in health care savings over time. One study used three epidemiological datasets to estimate the separate public health benefits of reducing the population's average sodium intake to 2,200 mg/day over 10 years. The researchers estimated that this pattern of reduction would prevent between 280,000 and 500,000 premature deaths over 10 years and that sustained sodium reduction would prevent additional premature deaths.

In the United States, the New York City Department of Health and Mental Hygiene initiated the National Salt Reduction Initiative (NSRI), a partnership of 70 local and state health departments and health organizations, to set voluntary targets to reduce sodium in restaurant and processed foods. The goal of NSRI was to decrease average sodium intake by 20% over five years (2009 through 2014)

by developing stepwise reductions from 2009 base levels. More than 25 companies, including packaged food corporations and restaurants, responded to NSRI by committing to reductions in the sodium content of some of their products. According to the most recent report, some participating food companies achieved the 2012 NSRI targets for various categories. By 2014, NSRI reported that 26% of packaged food categories met 2012 targets and 3% met 2014 targets.

Internationally, of the 96 countries with sodium reduction strategies, more than 50 countries have developed initiatives to support the reduction of sodium in the food supply. These initiatives have included both voluntary and mandatory efforts. In an approach developed by the United Kingdom's (UK) Food Standards Agency, many companies voluntarily pledged to reduce sodium in their foods. The UK initiative resulted in a decline in average sodium intake from 3,800 to 3,240 mg/day between 2003 and 2011, and researchers concluded that decreases in blood pressure in the UK during this time were largely attributable to the reduction in sodium intake. In 2020, the UK government issued revised voluntary targets to be achieved by 2024.

Health Canada, the department within the Canadian government responsible for helping Canadians maintain and improve their health, also developed a voluntary approach to sodium reduction. Health Canada collated information from the food industry and other stakeholders to inform their “guiding benchmark” sodium reduction levels for processed foods, which were issued in 2012 to be achieved by 2016. In 2017, Health Canada evaluated the food industry's efforts to meet sodium reduction targets in processed foods. Results showed that 52% of food categories met the Canadian Phase I, II, or III targets, indicating that voluntary efforts can reduce sodium in packaged foods. In 2020, Health Canada also issued updated voluntary targets to be achieved by 2025.

ing the goals for all 163 categories of processed, packaged, and prepared foods is available at <https://www.fda.gov/media/98277/download>).

In developing sodium reduction goals, FDA reviewed various food categorization systems, identified significant contributors to the intake of sodium in the United States, and organized foods into various identified food categories. Foods were identified and categorized based on their contribution to sodium intake, the total amount of added sodium in the food (not just naturally occurring), similar functional roles for sodium-containing ingredients, similar sodium concentrations, similar technical potential for reduction in sodium content, compatibility with existing industry and regulatory categories, and comments received to the draft guidance docket.

The goals account for concentrations necessary to achieve important food safety functions (e.g., antimicrobial) and functionality. The short-term targets are intended to be feasible using existing technology and are within the range of currently available top-selling commercial products. The upper bound sodium concentrations (upper bounds) are goals for the highest level of sodium for products in each food category (in milligrams sodium per 100 grams of food).

The guidance aims to inform general industry thinking about sodium content in the foods they produce and is not intended to limit industry's use of any appropriate methods or technologies to achieve sodium reduction. The goal is to

encourage gradual, efficient reduction of overall sodium content using effective and sustainable strategies that maintain other measures of nutritional quality. To ensure that no broad trends emerge that negatively affect the nutritional quality of foods (e.g., added sugars and saturated fat), the FDA in cooperation with other agencies plans to monitor the levels of other nutrients.

As most of the food consumption in the United States comes from a relatively small number of products and menu items in the marketplace that are produced by a limited number of food manufacturers, it is possible that reformulation by these food manufacturers could lead to increased demand for lower-sodium versions of ingredients used to produce packaged and prepared foods. As a result, such actions could help all members of the food industry be more readily able to provide lower-sodium products. Also, because many higher-selling products currently marketed are at or below the category means presented in this guidance, it should be possible for manufacturers to avoid disruptive changes to individual products that might result in noticeably altered taste, greatly reduced shelf life, or other undesirable product outcomes.

This article was excerpted from "Voluntary Sodium Reduction Goals: Target Mean and Upper Bound Concentrations for Sodium in Commercially Processed, Packaged, and Prepared Foods" (<https://tinyurl.com/3c6hpwr9>).

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Development, Properties, and Uses

First Edition

Edited by Frank Flider

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High Oleic Oils: Development, Properties and Uses is the first complete reference to address practical applications for this new and dynamic category of fats and oils that are essentially replacing partially hydrogenated oils in various food and nonfood uses. As a category, high oleic oils are highly stable, but like other fats and oils, there are differences in the composition and applications of the various types of high oleic oils. Their compositions allow for the production of a range of frying oils, increased shelf-life foods, functional shortenings and hard fats, and even industrial products not easily produced with nonhigh oleic oils. Information and know-how on these applications and advantages has been in high demand and short supply until now.

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Meet Selina Wang

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

PROFESSIONAL

What's a typical day like for you?

Every day is different, but they almost always consist of lots of meetings with collaborators, industry stakeholders, commodity group representatives, university colleagues, and students. Otherwise, I work on answering questions about food quality and authenticity from industry professionals and consumers, preparing educational/extension materials, developing research plans and analyzing results, and helping the students and researchers in my lab.



Selina Wang with her children (left to right) Linden, 1, and Tilden, 5, in Berkeley, California, USA.

My favorite part of my job is...

Doing applied research to solve timely problems. Being an extension specialist allows me to work closely with industry professionals and share knowledge.

Why did you decide to do the work you are doing now?

I was a postdoctoral researcher at the UC Davis Olive Center, where I discovered the joy of doing research and extension to help an industry have sustainable growth. My current work allows me to do what I was doing with olives and olive oil while expanding to cover all the crops that are important in California. It's challenging and rewarding.

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

I moved by myself to the United States from Taiwan on the day I graduated from high school. I worked multiple jobs while completing my college education as a full-time student. There were many times I wondered how I was going to pay the rent, how I was going to find time to study, how I was going to figure everything out in a foreign country, but I stayed the course. I'm proud of my grittiness.

Fast facts

Name	Selina Wang
Joined AOCS	2010
Education	Ph.D. (2008); University of California, Davis
Job title	Cooperative Extension Specialist, Department of Food Science and Technology; Research Director, Olive Center
Employer	University of California, Agriculture and Natural Resources (Davis, California, USA)
Current AOCS role	Session chair, AOCS Annual Meeting & Expo; chair of AOCS Avocado Oil Expert Panel; member, AOCS Smouse Award, Dutton Award, and Student Award Selection Committees

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

Like many people, I've found that having children changes how I want to live my life and how I view the world. The African philosophy of *ubuntu*, loosely translated as "I am because we are," resonates with me. Through my learning as a parent, I value community, connection, and humanity in a deeper way than I did before.

PERSONAL

What skill would you like to master?

I would like to master having fun. After decades of working very hard to establish a career and a family, I seem to have forgotten how to have fun and enjoy fun for what it is without feeling the pressure of being productive.

What are some small things that make your day better?

I have had a daily practice of meditation and yoga for the past decade. I still struggle with being present, but these practices help me focus on each moment of each day, which makes the day better.

PATENTS

Rubber composition for tire tread and tire manufactured using same

Park, J.H., *et al.*, Hankook Tire Co., Ltd., US10982076, April 20, 2021

The present disclosure relates to a rubber composition for a tire tread, the rubber composition comprising 100 parts by weight of a raw material rubber, 10 to 30 parts by weight of a vegetable oil having a weight ratio of linolenic acid to oleic acid of 1:0.5 to 1:1.5, 10 to 20 parts by weight of a terpene-based resin having a softening point of 110 to 130°C, and 75 to 120 parts by weight of a reinforcing filler, and a tire manufactured using the rubber composition for the tire tread. The present disclosure provides a tread rubber composition for four seasons which improves the braking performance on the ice and snow road surface and the abrasion resistant performance and improves the braking performance on the wet road surface at the same time while minimizing a drop in rotational resistance.

Methods for oil degumming

Vetcher, L., *et al.*, Keclon SA, US10982171, April 20, 2021

The present disclosure provides compositions and methods for enzymatic oil degumming.

Low dust powdered seed treatment

Ochampaugh, J., *et al.*, Agrilead, Inc., US10986769, April 27, 2021

A powdered lubricant composition that minimizes fugitive dust and a method of treating seeds with dry lubricant that minimizes fugitive dust. Addition of oil powder to dry lubricants is found to minimize fugitive dust created during the application of the dry lubricant. Mica coated titanium dioxide is an ideal dry lubricant for combining with oil powder.

Systems and methods for fatty acid alkyl ester production with recycling

Blanchard, C.O., *et al.*, Inventure Renewables, Inc., US10988708, April 27, 2021

Provided are industrial processes for producing a fatty acid alkyl ester from a natural oil feedstock or a mixed lipid feedstock, wherein the natural oil feedstock comprises a free (un-esterified) organic acid such as a free fatty acid, comprising: producing fatty acid alkyl esters using an alcoholysis reaction such as a vapor phase alcoholysis reaction or a supercritical alcoholysis reaction, wherein the alcoholysis reaction takes place under conditions comprising: mixing the natural oil feedstock and/or mixed lipid feedstock and alcohol into an alcoholysis reactor or equivalent, and then depressurizing, and then recovering the fatty acid alkyl esters from the

alcoholysis reactor or equivalent by distillation in a distillation column, leaving a still pitch or distillation bottoms in the distillation column or equivalent, wherein the fatty acid alkyl esters can be removed from the alcoholysis reactor or equivalent with or without cooling of the reaction mixture prior to depressurization.

Defoaming composition comprising a tall-oil-derived surfactant

Chavan, S.V., *et al.*, Halliburton Energy Services, Inc., US10988672, April 27, 2021

The present disclosure may be directed to defoaming methods and compositions. A method of treating a subterranean formation may comprise providing a defoaming composition, wherein the defoaming composition comprises a tall-oil-derived surfactant. The method may further comprise mixing the defoaming composition with an aqueous fluid to form a treatment fluid such that foaming in the treatment fluid is reduced, wherein the treatment fluid is a water-based fluid. The method may further comprise placing the treatment fluid into the subterranean formation.

Non-meat food products having appearance and texture of cooked meat

Cully, K.J., *et al.*, Societe des Produits Nestle S.A., US10993445, May 4, 2021

Non-meat food products are provided that have the appearance and the texture of cooked meat. Methods for making such non-meat food products are also provided. In an embodiment, a method includes mixing dry ingredients comprising vegetable proteins with wet ingredients including water and/or oil to form a non-meat dough; heating the non-meat dough under pressure; maintaining the pressure while transferring the heated non-meat dough to a cooling device; and gradually cooling the heated non-meat dough while gradually decreasing pressure on the heated non-meat dough, to form a non-meat food product. The mixing can be performed by a batch or continuous mixer; the heating can be performed by a device selected from the group consisting of a high shear emulsifier, a heat exchanger, and a dielectric heater; and the gradual cooling can be performed by a heat exchanger.

Powdered oil and/or fat, food and drink containing powdered oil and/or fat, and method for manufacturing powdered oil and/or fat

Kitatani, Y., *et al.*, The Nisshin OilliO Group, Ltd., US10993455, May 4, 2021

A powdered oil and/or fat that includes a high proportion of a medium-chain fatty-acid triglyceride, has excellent solubility and fluidity, and gives rise to little oil flotation during dissolution. The powdered oil and/or fat includes a medium-chain fatty-acid triglyceride, an octenylsuccinic acid-treated starch mixture, and a

dextrin, in which the octenylsuccinic acid-treated starch mixture includes a first octenylsuccinic acid-treated starch of which the viscosity of a 10 mass % aqueous solution at 25°C is less than 30 mPaS, and a second octenylsuccinic acid-treated starch of which the viscosity of a 10 mass % aqueous solution at 25°C is 30 mPaS or more; the content of the second octenylsuccinic acid-treated starch is 12–45 mass %, inclusive, relative to the entire octenylsuccinic acid-treated starch mixture; the dextrose equivalent weight of the dextrin is 10 or more; and the content of the medium-chain fatty-acid triglyceride is 65–85 mass %, inclusive, relative to the entire powdered oil and/or fat.

Baking lipase and methods of use

Pop, C., *et al.*, BASF SE, US10993446, May 4, 2021

Lipase enzymes and methods of using the lipases in a baking for improving the volume, stability, tolerance of a baked product and/or reducing and reducing or eliminating the use of DATEM.

Curable compositions

Konarski, M.M., *et al.*, Henkel IP & Holding GmbH, US10995210, May 4, 2021

A curable composition, includes a diluent, an epoxy functionalized resin derived from a nutshell oil, an epoxy-rubber copolymer adduct, and a curing agent. There is disclosed a method for making the curable composition, a method of imbuing improved flexibility to a curable composition and a method for improving oily metal adhesion of a curable composition. The method of imbuing improved flexibility to a curable composition includes providing a curable composition, adding an epoxy functionalized resin derived from a nutshell oil and adding a liquid modified hydrocarbon resin derived from a nutshell oil.

Biorenewable kerosene, jet fuel, jet fuel blendstock, and method of manufacturing

Abhari, R., *et al.*, REG synthetic fuels, LLC, US11001774, May 11, 2021

The present technology provides compositions that include at least about 98 weight percent (“wt %”) n-paraffins which, among other surprising features, may be suitable for use as a diesel fuel, an aviation fuel, a jet fuel blendstock, a blendstock to reduce the cloud point of a diesel fuel, a fuel for portable heaters, and/or as a charcoal lighter fluid. The composition includes at least about 98 wt % C.sub.7-C.sub.12 n-paraffins, where at least about 10 wt % of composition includes n-decane, at least about 20 wt % of the composition includes n-dodecane, and at least about 75 wt % of the composition includes even carbon number paraffins. The composition also includes less about 0.1 wt % oxygenates and less than about 0.1 wt % aromatics. The composition may be produced by a process that includes hydrotreating a biorenewable feedstock comprising at least one of palm kernel oil, coconut oil, babassu oil, microbial oil, or algal oil.

Methods for producing hydrocarbon compositions with reduced acid number and for isolating short-chain fatty acids

Bressler, D., Forge Hydrocarbons Corporation, US10995276, May 4, 2021

The methods described herein provide an efficient way to remove and isolate short chain fatty acids from hydrocarbons that are produced upon the heating of a fatty acid resource. The short chain fatty acids can be continuously isolated and fed into the pyrolysis reactor, which in turn increases the overall efficiency of the production of the hydrocarbons. Alternatively, the short chain fatty acids can be isolated and used in other applications.

Medium-chain triglyceride compositions

Cuenoud, B., *et al.*, Societe des Produits Nestle S.A., US11000496, May 11, 2021

A composition comprising medium-chain triglycerides (MCTs) wherein the composition comprises (i) a MCT comprising three fatty acid moieties each with 8 carbon atoms (MCT-C8) and (ii) a MCT comprising three fatty acid moieties each with 10 carbon atoms (MCT-C10); wherein the ratio of MCT-C8 to MCT-C10 is from 10:90 to 90:10 (mol/mol) and wherein the combined amount of MCT-C8 and MCT-C10 make up at least 50 mol % of the MCTs in the composition.

Ceramide containing capsules, ceramide compositions, and cosmetic compositions thereof

Le Claire, L., *et al.*, L’Oreal, US11007134, May 18, 2021

The ceramide composition includes capsules dispersed in the ceramide composition, the capsules comprising a shell and a core. The shell includes a polycaprolactone, a block copolymer, and a surfactant. The core includes ceramide NP, hydroxypalmitoyl sphinganine, 2-oleamido-1,3-octadecanediol, and a hydrophobic solvent. The core has a weight ratio of the ceramide NP of (i) and the hydroxypalmitoyl sphinganine of (ii) to the 2-oleamido-1,3-octadecanediol of (iii) is from 1:2.5 to 1:5. Additionally, the ceramide composition includes hydroxyacetophenone and hydrophilic solvent.

Modified lecithin, preparation thereof, and use as an antioxidant

Decker, E., The University of Massachusetts, US11001865, May 11, 2021

Disclosed are methods of making a modified lecithin by conducting an enzymatic conversion of a naturally derived lecithin to form a modified lecithin, e.g., having an enhanced level of phosphatidylethanolamine, phosphatidylserine, or a combination thereof. Compositions prepared from the modified lecithin and use to inhibit lipid oxidation are described.

Pastry premix compositions and methods for preparing same

Belitz, A.S., inventor, US11006638, May 18, 2021

A pastry product premix preparation method includes the steps of freezing a shortening material in a condition below about 40°F to make a solidified shortening material, breaking the solidified shortening material into particles that may have the shape of a sphere, a flake, an irregular shape, or a combination thereof; combining the shortening material with at least one dry ingredient that may include flour, salt, or baking powder to make a dry mix; vacuum-sealing the dry mix; and maintaining the dry mix at a temperature below the melting point of the shortening particles until further use.

Emulsion stabilization method

Vieira, J.B., *et al.*, Societe des Produits Nestle S.A., US11006651, May 18, 2021

The present invention relates to the use of cocoa particles as the emulsifier system for the stabilization of a water-in-oil or oil-in-water emulsion. In another aspect there is now provided a confectionery product having cocoa particles as the emulsifying agent that does not contain any synthetic or artificial emulsifiers, and to methods for producing such confectionery product.

Oil-containing rubber compositions and related methods

Srinivasan, P., *et al.*, Bridgestone Americas Tire Operations, LLC, US11008448, May 18, 2021

Disclosed herein are rubber compositions comprising bio-oil produced by a recombinant cell. Also disclosed are methods of controlling the variability of fatty acid content in bio-oil containing rubber compositions or tires comprising at least one component incorporating the bio-oil containing rubber composition, and a method of providing a bio-oil-containing tire with a reduced carbon footprint.

Methods of refining a grain oil composition to make one or more grain oil products, and related systems

Lamprecht, B.A., *et al.*, POET Research, Inc., US11008531, May 18, 2021

The present disclosure is related to refining one or more grain oil composition streams (e.g., distillers corn oil or syrup) in a biorefinery to provide one or more refined grain oil products, where each grain oil product has targeted amounts of a free fatty acid component and the fatty acid alkyl ester component.

Process for enzymatic production of triglycerides

Dragan, V., Stepan Company, US11008595, May 18, 2021

An enzymatic process for producing fatty acid triglycerides using a lipase catalyst system that includes a mixture of a supported lipase catalyst and an additive, such as silica gel. The additive is used without adsorbing any of the acyl group donor or acyl group acceptor reactants onto the additive. Use of the catalyst system decreases production reaction time, decreases the temperatures required for reaction, and allows for a single reaction vessel. The catalyst system can be used to efficiently produce medium chain triglycerides or conjugated linoleic acid triglycerides.

Methods of reducing foam during ethanol fermentation

Shen, X., *et al.*, Novozymes A/S, US11015212, May 25, 2021

The invention relates to methods of reducing foam during ethanol fermentation by adding a phospholipase A and/or a phospholipase C during fermentation.

Composition containing vegetable oil, caramel, and phenolic compounds

Acharya, P., *et al.*, Conopco, Inc., US11019830, June 1, 2021

The present invention relates to a composition comprising vegetable oil, caramel and one or more phenolic compounds. The caramel and one or more phenolic compounds together prevent oxidation of the vegetable oil, such that the amount of EDTA in the composition can be reduced. The caramel has been heated such that it does not impart a dark color to the composition. The invention also relates to a method to prepare the composition. Finally, the invention relates to use of caramel and one or more phenolic compounds to decrease the oxidation of vegetable oil.

Mixture of fatty acids for use in the treatment of inflammatory pathologies

Burattin, L., Again Life Italia SRL, US11020365, June 1, 2021

This invention relates to a mixture of at least three fatty acids selected from palmitic acid, oleic acid, stearic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), azelaic acid, and myristic acid. This invention also relates to the use of the aforesaid mixture in the treatment of inflammatory pathologies.

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



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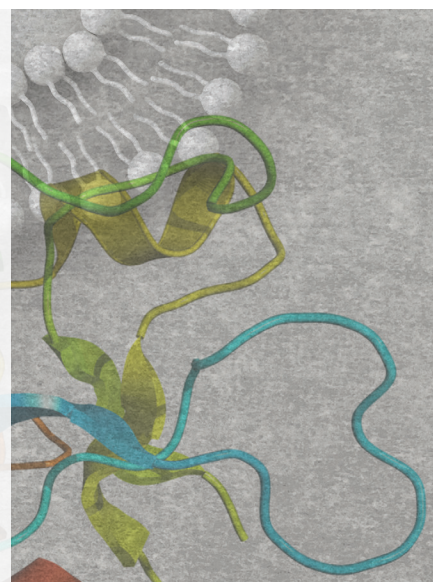
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Review Articles

H&N How should we judge edible oils and fats? An umbrella review of the health effects of nutrient and bioactive components found in edible oils and fats

Teasdale, S.B., *et al.*, *Crit. Rev. Food Sci. Nutr.* online March 11, 2021, <https://doi.org/10.1080/10408398.2021.1882382>.

Dietary guidelines for many Western countries base their edible oil and fat recommendations solely on saturated fatty acid content. This study aims to demonstrate which nutritional and bioactive components make up commonly consumed edible oils and fats, and explore the health effects and strength of evidence for key nutritional and bioactive components of edible oils. An umbrella review was conducted in several stages. Food composition databases of Australia and the United States, and studies were examined to profile nutrient and bioactive content of edible oils and fats. PUBMED and Cochrane databases were searched for umbrella reviews, systematic literature reviews of randomized controlled trials or cohort studies, individual randomized controlled trials, and individual cohort studies to examine the effect of the nutrient or bioactive on high-burden chronic diseases (cardiovascular disease, type 2 diabetes mellitus, obesity, cancer, mental illness, cognitive impairment). Substantial systematic literature review evidence was identified for fatty acid categories, tocopherols, biophenols, and phytosterols. Insufficient evidence was identified for squalene. The evidence supports high mono- and polyunsaturated fatty acid compositions, total biophenol content, phytosterols, and possibly high alpha-tocopherol content as having beneficial effects on high-burden health comes. Future dietary guidelines should use a more

sophisticated approach to judge edible oils beyond saturated fatty acid content.

ANA PRO EAT Newly marketed seed oils. What we can learn from the current status of authentication of edible oils?

Przykaza, K., *et al.*, *Food Control* 130: 108349, 2021, <https://doi.org/10.1016/j.foodcont.2021.108349>.

Concerns regarding the quality and authenticity of newly marketed seed oils are based on a high risk of identity theft, tampering with purity, and adulteration. Seed oils are a rich source of dietary fat and various phytochemicals, and as such are an object of health-related and commercial interest. However, the lipid composition of these oils is poorly monitored, and there is a lack of information on what methods are appropriate for detecting adulteration. In this review, current knowledge about detecting adulteration of well-known edible oils was collected and systematized. Most methods of identification and determination of lipidomic profiles of oils are based on infrared and Raman spectroscopy as well as chromatographic separation combined with mass spectrometry. Quantitative and qualitative determination of fatty acids, tri- and di-glycerols, phospholipids, glycolipids, sterols, and tocopherols can contribute to overcoming the problem of food adulteration.

IOP PRO Mono- and diglyceride production from microalgae: challenges and prospects of high-value emulsifiers

Ferreira, G.F., *et al.*, *Trends Food Sci. Technol.* 118: 589–600, December 2021, <https://doi.org/10.1016/j.tifs.2021.10.027>.

Monoglycerides (MAG) and diglycerides (DAG) belong to the category of naturally occurring glycerolipids. They have wide applications in the food, pharmaceutical, and cosmetic industries, with commercial demand supplied by the consolidated industrial catalytic route of vegetable oil glycerolysis. Despite the economic competitiveness of producing these types of emulsifiers from vegetable oils, the increasing demand for products with high nutrition value makes some kinds of microalgae oil potential feedstock of high-quality fatty acids to serve this growing market. An alternative to the use of vegetable oils is the production of triacylglycerols from microalgae. Usually, microalgal oils have a high content of polyunsaturated fatty acids (PUFA) and the cultivation of microalgae may present fewer environmental impacts, considering reduced use of arable land, efficient CO₂ biofixation, and high productivity. Microalgae lipids are mostly studied for biodiesel production, but this work shows the potential to explore more valuable applications due to their composition, discussing the possibility of producing MAGs and DAGs from microalgal lipids. While biodiesel B99-B100 costs US\$56/gallon, according to the US Energy Department, (April 2021), a food emulsifier (soybean lecithin) is sold for US\$147/kg (Alfa Aesar, August 2021). Hence, it is imperative to consider high-value bioproducts from an economic point of

view. Microalgal oil can be rich in omega-3 and omega-6 fatty acids, being a promising source of MAGs and DAGs with higher nutritional value. Glycerolysis studies of this feedstock are restricted to the enzymatic route, but different alternatives are shown in this work.

IOP PRO BIO Microbial hosts for metabolic engineering of lignin bioconversion to renewable chemicals

Bugg, T.D.H., *et al.*, *Renew. Sust. Energ. Rev.* 152: 111674, 2021, <https://doi.org/10.1016/j.rser.2021.111674>.

This review discusses the use of engineered microbes for bioconversion of lignin from plant biomass to produce renewable chemicals. Existing bacterial hosts for lignin bioconversion are discussed, such as *Pseudomonas putida* KT2440 and *Rhodococcus jostii* RHA1, and case studies where they have been engineered to generate aromatic and non-aromatic products are described, and the different types of lignin substrates used for these studies. Other bacteria identified as lignin degraders are described, and the prospects for using other bacteria as hosts for metabolic engineering of lignin degradation are discussed. Recent advances in genetic modification of fungi are also covered, which could lead to the metabolic engineering of lignin-degrading fungi for bioproduct formation from lignin. Prospects and challenges in this field are considered, as the field moves from the laboratory to industrial

application, including: choice of chassis organism, choice of lignin feedstock, the complexity of polymeric lignin breakdown, and considerations for scale-up and choice of bioproduct.

Original Articles

BIO PRO IOP Increased lipid production in *Yarrowia lipolytica* from acetate through metabolic engineering and cosubstrate fermentation

Chen, L., *et al.*, *ACS Synthetic Biology*, October 29, 2021, <https://doi.org/10.1021/acssynbio.1c00405>.

Bioconversion of acetate, a byproduct generated in industrial processes, into microbial lipids using oleaginous yeasts offers a promising alternative for the economic utilization of acetate-containing waste streams. However, high acetate concentrations will inhibit microbial growth and metabolism. In this study, the acetate utilization capability of *Yarrowia lipolytica* PO1f was successively improved by overexpressing the key enzyme of acetyl-CoA synthetase (ACS), which resulted in an accumulation of 9.2% microbial lipids from acetate in shake flask fermentation. By further overexpressing the second key enzymes of acetyl-CoA carboxylase (ACC1) and fatty acid synthase (FAS) in *Y. lipolytica*, the lipid

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content was increased to 25.7% from acetate. Finally, the maximum OD₆₀₀ of 29.2 and a lipid content of 41.7% were obtained with the engineered strain by the adoption of cosubstrate (glycerol and acetate) fed-batch fermentation, which corresponded to an increase of 68 and 95%, respectively. These results presented a promising strategy for economic and efficient microbial lipid production from the waste acetate

EAT ANA Use of confocal Raman imaging to understand the microstructure of anhydrous milk fat-based oleogels

Gómez-Mascaraque, L.G., *et al.*, *Food Struct.*, online October 30, 2021, <https://doi.org/10.1016/j.foostr.2021.100228>.

This work explores confocal Raman microscopy as a novel approach to study the microstructure of oleogels, using milk fat-based oleogels as a case study. Anhydrous milk fat (AMF) was sequentially fractionated to obtain two olein and two stearin fractions with different thermal properties. These and the unfractionated AMF were structured into oleogels using ethyl cellulose as gelling agent. Although the fatty acid profile of the different fractions showed only minor changes (in most cases < 10% change), the microstructure of the various oleogels was markedly different, especially the unfractionated AMF-based oleogels, which exhibited distinct pseudo-spherical features suggesting segregation of components. Different univariate and multivariate data analysis approaches were compared to map the distribution of components in the oleogels using confocal Raman microscopy. The univariate approach only provided accurate microstructural information for oleogels prepared with olein (liquid at the temperature of analysis). The multivariate approaches, although more complex, provided reliable results for all oleogels. Mapping lipids with different degrees of mobility (liquid vs solid) within the oleogels was possible with this technique. Solid lipids were located preferentially in close proximity to ethyl cellulose, and were confirmed to be the main component of the microspheres found in the unfractionated AMF oleogels.

EAT ANA PRO Qualitative identification of the edible oil storage period using a homemade portable electronic nose combined with multivariate analysis

Jiang, H., *et al.*, *J. Sci. Food Agric.* 101: 3448–3456, 2021, <https://doi.org/10.1002/jsfa.10975>.

The edible oil storage period is one of the important indicators for evaluating the intrinsic quality of edible oil. The present study aimed to develop a portable electronic nose device for the qualita-

tive identification of the edible oil storage period. First, four metal oxide semiconductor gas sensors, comprising TGS2600, TGS2611, TGS2620 and MQ138, were selected to prepare a sensor array to assemble a portable electronic nose device. Second, the homemade portable electronic nose device was used to obtain the odor change information of edible oil samples during different storage periods, and the sensor features were extracted. Finally, three pattern recognition methods, comprising linear discriminant analysis (LDA), K-nearest neighbors (KNN) and support vector machines (SVM), were compared to establish a qualitative identification model of the edible oil storage period. The input features and related parameters of the model were optimized by a five-fold cross-validation during the process of model establishment. The recognition performance of the non-linear SVM model was significantly better than that of the linear LDA and KNN models, especially in terms of generalization performance, which had a correct recognition rate of 100% when predicting independent samples in the prediction set. Overall, the results demonstrated that it is feasible to apply the homemade portable electronic nose device with the help of the appropriate pattern recognition methods to achieve the fast and efficient identification of the edible oil storage period, which provides an effective analysis tool for the quality detection of the edible oil storage.

EAT LOQ Changes of phenolic profile and antioxidant activity during cold storage of functional flavored yogurt supplemented with mulberry pomace

Du, H., *et al.*, *Food Control* 132: 108554, 2022, <https://doi.org/10.1016/j.foodcont.2021.108554>.

Changes in the main phenolic compounds and *in vitro* antioxidant activity of functional flavored yogurt supplemented with mulberry pomace (MP) during cold storage were investigated. Three anthocyanins (cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, and pelargonidin-3-O-glucoside) and six non-anthocyanin monomeric phenolics (resveratrol, catechol, catechin, rutin, quercitrin, and quercetin) were identified in the MP. The MP powder addition significantly and dose-dependently increased the contents of total phenol (TPC), total anthocyanin (TAC), and individual phenolics and DPPH and ABTS free radical scavenging activity of the products. During the cold storage, TPC, TAC, and contents of individual phenolics and antioxidant activity of MP-fortified yogurts gradually and significantly increased. Antioxidant activity of MP-fortified yogurts is highly related to TPC/TAC. MP-fortified yogurts exhibited the highest antioxidant activity on the 21st day of storage. The results showed that MP can be used as a natural antioxidant, providing a promising natural ingredient for the production of functional dairy products with improved nutritional value and biological activity.



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EAT LOQ Açai extract powder as natural antioxidant on pork patties during the refrigerated storage

Bellucci, E.R.B., *et al.*, *Meat Sci.* 184: 108667, 2022, <https://doi.org/10.1016/j.meatsci.2021.108667>.

The current trends among consumers are pushing for the use of natural antioxidants options. Açai fruit is rich on polyphenolic components, but no studies have been carried out to evaluate their effect in meat products. The objective was to investigate the effect of açai extract on refrigerated pork patty quality. Five treatments were done: without antioxidant (CON), sodium erythorbate 500 mg.kg⁻¹ (ERY), açai extract: 250 (AEL), 500 (AEM), and 750 mg.kg⁻¹ (AEH). Açai extract did not affect the proximate composition, pH, and cooking parameters. The concentrations of açai extract studied increased antioxidant activity and reduced lipid oxidation (0.379, 0.293, and 0.217 vs. 0.889 mg MDA.kg⁻¹ for AEL, AEM, AEH vs. CON, respectively). However, only the AEL treatment did not affect the color parameters, showing the best option for the application in pork patties. Thus, açai extract at 250 mg.kg⁻¹ can be used as a natural antioxidant, replacing sodium erythorbate to preserve the quality of refrigerated pork patties.

EAT LOQ Viability of probiotics and antioxidant activity of cashew milk-based yogurt fermented with selected strains of probiotic *Lactobacillus* spp.

Shori, A.B., *et al.*, *LWT* 153: 112482, 2022, <https://doi.org/10.1016/j.lwt.2021.112482>.

This study investigated the effects of three strains of probiotic *Lactobacillus* spp.,—*Lactobacillus rhamnosus* (Lr), *Lactobacillus casei* (Lc), or *Lactobacillus plantarum* (Lp)—in co-cultures with *Streptococcus thermophilus* (St) and *Lactobacillus delbrueckii* subsp. *Lactis* (Ll) with respect to post-acidification changes, viability of lactic acid bacteria (LAB), total phenolic and flavonoid contents, and antioxidant activity of cashew milk (W)-based yogurt (Y) during 0, 7, 14, and 21 days of storage. In addition, sensory evaluation of all yogurt samples was run during the first day of storage. The addition of Lr significantly decreased the pH in cashew milk-based yogurt compared to control-yogurt (C-Y; containing only St and Ll) throughout 21 days of storage. Cashew milk-based yogurt showed an increment in LAB counts, total phenolic and flavonoid contents, and antioxidant capacity content, which is relatively dependent on the probiotic *Lactobacillus* spp. strains. WLP-Y showed lower sensory attributes scores. In conclusion, *L. rhamnosus*, *L. casei*, and *L. plantarum* could be used as a starter culture in cashew milk-based yogurt production with a potential source of natural antioxidants.

EAT LOQ Response surface methodology for the optimization of biophenols recovery from “alperujo” using supercritical fluid extraction. Comparison between Arbequina and Coratina cultivars

Dauber, C., *et al.*, *J. Supercrit. Fluids* 180: 105460, 2022, <https://doi.org/10.1016/j.supflu.2021.105460>.

Alperujo is a semi-solid residue from the olive oil industry that accounts for about 80–85% of the total processed olives; it contains phenolic compounds that could be used as natural preservatives in the food industry. The objective of this study was to optimize supercritical fluid extraction (SFE) of antioxidant compounds from alperujo using a Box-Behnken Design and response surface methodology. Antioxidant (ABTS and ORAC assays) and antimicrobial (MIC) potential of SFE extracts from Arbequina and Coratina were compared for extracts obtained in the optimal conditions to maximize the antioxidant activity (200 bar, 60°C and 10% ethanol as modifier). Extracts from Coratina presented a significantly higher ($p < 0.05$) TPC (1487–2073 mg GAE/kg) and tocopherol content (345–454 ppm), although, in general, a correlation between these values and antioxidant activity of extracts was not observed. Moreover, Arbequina and Coratina SFE extracts showed moderate bacterial inhibitory potential against most of the bacteria studied.

IOP PRO PCP Retrospective analysis of the US corn ethanol industry for 2005–2019: implications for greenhouse gas emissions reductions

Lee, U., *et al.*, *Biofuels, Bioprod. Biorefining* 15: 1318–1331, 2021, <https://doi.org/10.1002/bbb.2225>.

Since 2000, US corn ethanol production has increased significantly—from 1.6–15 billion gallons (6.1–57 billion liters)—due to supportive biofuel policies. In this study, we conduct a retrospective analysis of the changes in US corn ethanol greenhouse gas emission intensity, sometimes known as carbon intensity (CI), over the 15 years from 2005–2019. Our analysis shows a significant decrease in CI: from 58 to 45 gCO₂e/MJ of corn ethanol (a 23% reduction). This is due to several factors. Corn grain yield has increased continuously, reaching 168 bushels/acre (10.5 metric tons/ha, a 15% increase) while fertilizer inputs per acre have remained constant, resulting in decreased intensities of fertilizer inputs (e.g., 7% and 18% reduction in nitrogen and potash use per bushel of corn grain harvested, respectively). A 6.5% increase in ethanol yield, from 2.70–2.86 gal/bushel corn (0.402–0.427 L kg⁻¹ corn), and a 24% reduction in ethanol plant energy use,

from 32 000–25 000 Btu/gal ethanol (9.0 to 6.9 MJ L⁻¹ ethanol) also helped reduce the CI. The total GHG emission reduction benefits through the reduction in the CI and increased ethanol production volume are estimated at 140 million metric tons (MMT) from 2005–2019 in the ethanol industry. Displacement of petroleum gasoline by corn ethanol in the transportation fuel market resulted in a total GHG emission reduction benefit of 544 MMT CO₂e during the period 2005–2019.

IOP PRO BIO Elucidating the mechanisms of enhanced lignin bioconversion by an alkali sterilization strategy

Zhao, Z.-M., *et al.*, *Green Chem.* 23: 4697–4709, 2021, <https://doi.org/10.1039/D1GC00911G>

Biological lignin valorization represents an emerging green approach to upgrade lignin for sustainable and economic biorefineries. However, lignin generally exhibits poor water solubility and inhomogeneous distribution in an aqueous medium, significantly limiting its bioconversion efficiency. Herein, we develop a novel alkali sterilization strategy to effectively enhance the dispersion and fermentation performance of lignin substrates. The colloidal

particle size and molecular structure variations of lignin during the sterilization were thoroughly investigated to reveal the mechanisms of enhanced fermentation performance. Results showed that alkali sterilization achieved a completely aseptic effect when mixing lignin medium at an initial pH of 12.7 for 24 h. Dynamic light scattering (DLS) analysis demonstrated that the hydrodynamic volume of colloidal lignin particles decreased by 96.3% by alkali sterilization compared with the conventional thermal sterilization. Moreover, lignin characterizations by nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography (GPC) suggested that alkali sterilization modified the lignin molecular structure by generating 50% more hydrophilic carboxyl groups, reducing the weight-average molecular weight (M_w) by 23.0%, and narrowing the molar-mass dispersity (D_M) by 23.8%. The generation of lignin substrates with more uniform distribution and lower molecular weight improved *Rhodococcus opacus* PD630 cell growth and metabolism. Microbial cell amount, lignin degradation, and lipid production in alkali sterilized medium increased by 309%, 30.3%, and 48.3%, respectively, compared to those in thermally sterilized medium. These results clearly demonstrated that alkali sterilization dramatically improved the lignin bioconversion performance. This work presents a facile and effective sterilization strategy to overcome inhomogeneous lignin distribution in aqueous fermentation media, showing great potentials as a platform technique for promoting biological lignin valorization.

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LOQ H&N Antioxidant capacity of phenolic compounds separated from tea seed oil *in vitro* and *in vivo*

Liu, G., *et al.*, *Food Chem.* 371: 131122, 2022, <https://doi.org/10.1016/j.foodchem.2021.131122>.

Tea seed oil is rich in phenols with good antioxidant capacity. However, the antioxidant capacity of tea seed oil polyphenols is usually evaluated superficially by evaluating the chemical system. Thirty-nine phenols were tentatively identified by UPLC-ESI-MS/MS analysis, including flavonoids and phenolic acids. The antioxidant capacity of phenol extracts was investigated *in vitro* and *in vivo*. The chemical assays showed the extracts had good proton and electron transfer capabilities. The CAA assay indicated the IC₅₀ of the extracts was 77.93 ± 4.80 $\mu\text{g/mL}$ and cell antioxidant capacity of the extracts was 101.05 ± 6.70 $\mu\text{mol-QE/100 g}$ of oil. The animal experiments suggested phenol extracts could significantly improve the organ index, reduce malondialdehyde content, and increase superoxide dismutase, glutathione peroxidase, and total antioxidant capacity ($p < 0.05$).

PRO IOP Green diesel production from oleic acid deoxygenation using subcritical water under hydrogen-free condition

Hongloi, N., *et al.*, *Eng. J.* 25: 115–122, October 2021, <https://doi.org/10.4186/ej.2021.25.10.115>.

Green diesel or bio-hydrogenated diesel (BHD) is a second generation renewable liquid fuel that can be produced from several types of renewable sources such as triglyceride in vegetable oils or animal fats, free fatty acid in waste from refining palm oil industry, and their derivatives via a catalytic reaction involving hydrogenation and deoxygenation provided n-alkanes as a main product. In this work, the aim was to investigate the effect of reaction time and catalyst type on green diesel production in a batch mode without H₂ feed. The green diesel was produced from oleic acid using activated carbon and commercial catalyst at reaction temperature of 250°C and total pressure of 40 bars under DI water as a hydrogen

source. The results showed that 100% oleic acid conversion was obtained by using both type of catalysts. Pentadecane was the main product with 96% percentage at 3 h reaction time for commercial catalyst and 100% percentage at 5 h reaction time for activated carbon. In addition, the products in gas phase were CO₂ and CO for both types of catalyst and CH₄ only appeared when commercial catalysts were used.

PRO IOP Biodiesel feedstock selection strategies based on economic, technical, and sustainable aspects

Anwar, M., *Fuel*, 283: 119204, 2021, <https://doi.org/10.1016/j.fuel.2020.119204>.

The interest in sustainable energy sources such as biodiesel is growing due to unpredictable fossil fuel prices, depletion of their origins, inconsistent supply, geopolitical instability, conflicts of fuel/oil-producing countries, global politics, and sanctions. Selecting the right biodiesel feedstocks is the first step of mass biodiesel production. This paper aims to define the best possible biodiesel feedstocks using various multiple criteria decision analysis (MCDA) processes. This study concentrated on 15 criteria based on economics (cost of biodiesel production), technical aspects (physicochemical properties and fatty acid composition), and environmental aspects (sustainable land usage) for the selection process. Sixteen of the most popular biodiesel feedstocks, namely palm, soybean, sunflower, moringa, jatropha, pongamia, mustard, coconut, tallow, peanut, corn, cottonseed, rice bran, beauty leaf, rapeseed, and waste cooking oil were investigated as alternatives. Five weighting methods in percentage, namely equal, critic, entropy, analytical hierarchical process (AHP), and fuzzy analytical hierarchical process (FAHP) were used to determine the weight of each criterion. Four MCDA processes, namely PROMETHEE Graphical Analysis for Interactive Assistance (GAIA), Weighted sum method (WSM), Weighted product method (WPM), and Technique for order preference by similarity to ideal solution (TOPSIS) were implemented for this investigation. The findings indicate that coconut rated best and soybean the worst among the alternatives.

A photograph of a man with dark hair and a beard, wearing a grey sweater, leaning over a young child with blonde curly hair. The child is wearing a blue and white patterned cardigan over a white shirt. They are in a kitchen, looking down at a wooden rolling pin and a bowl of butter on a wooden surface. The background is softly blurred, showing kitchen shelves with various items.

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