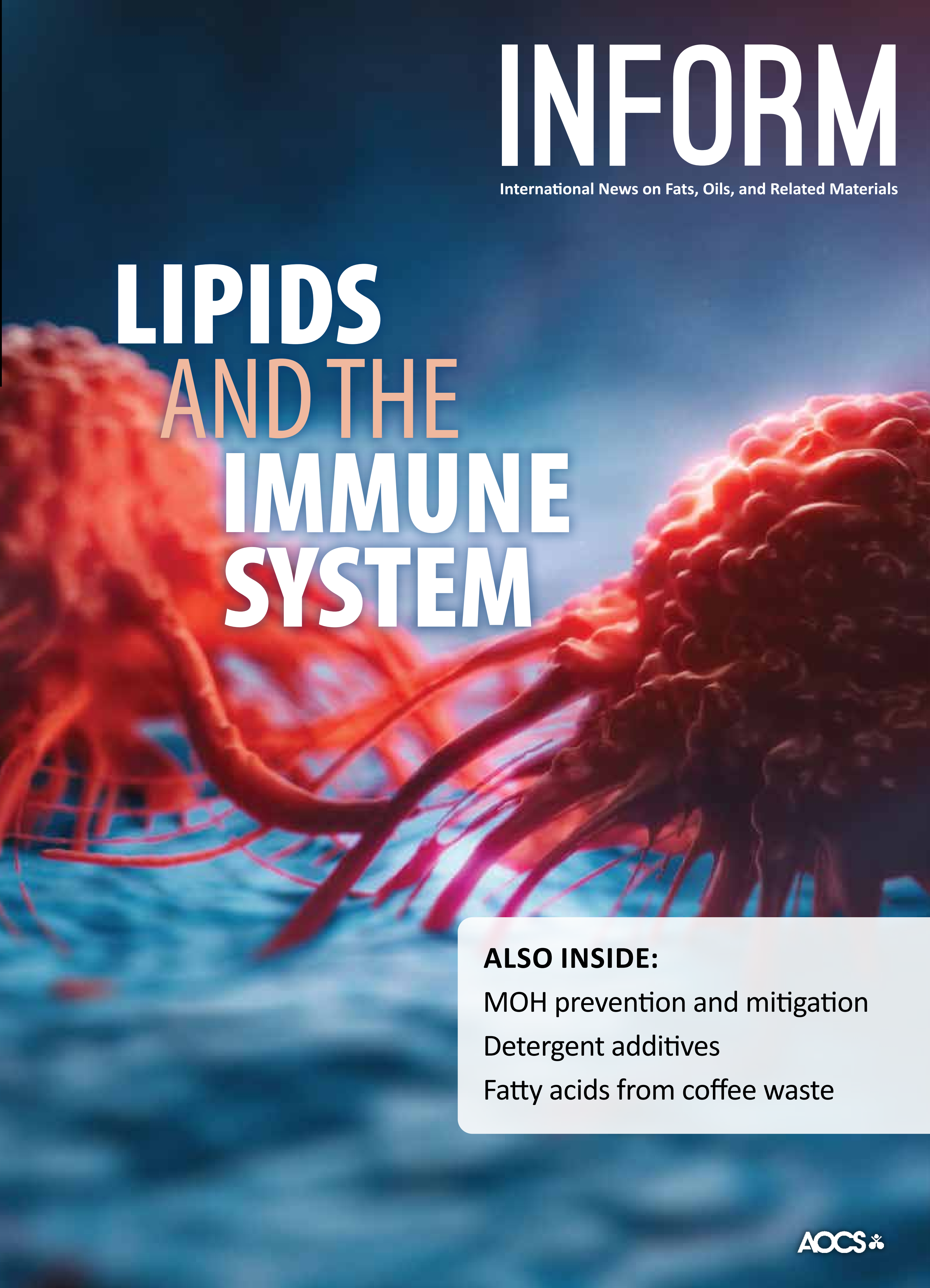
INFORM November/December 2021 Volume 32 (10)

INFORM

International News on Fats, Oils, and Related Materials



LIPIDS AND THE IMMUNE SYSTEM

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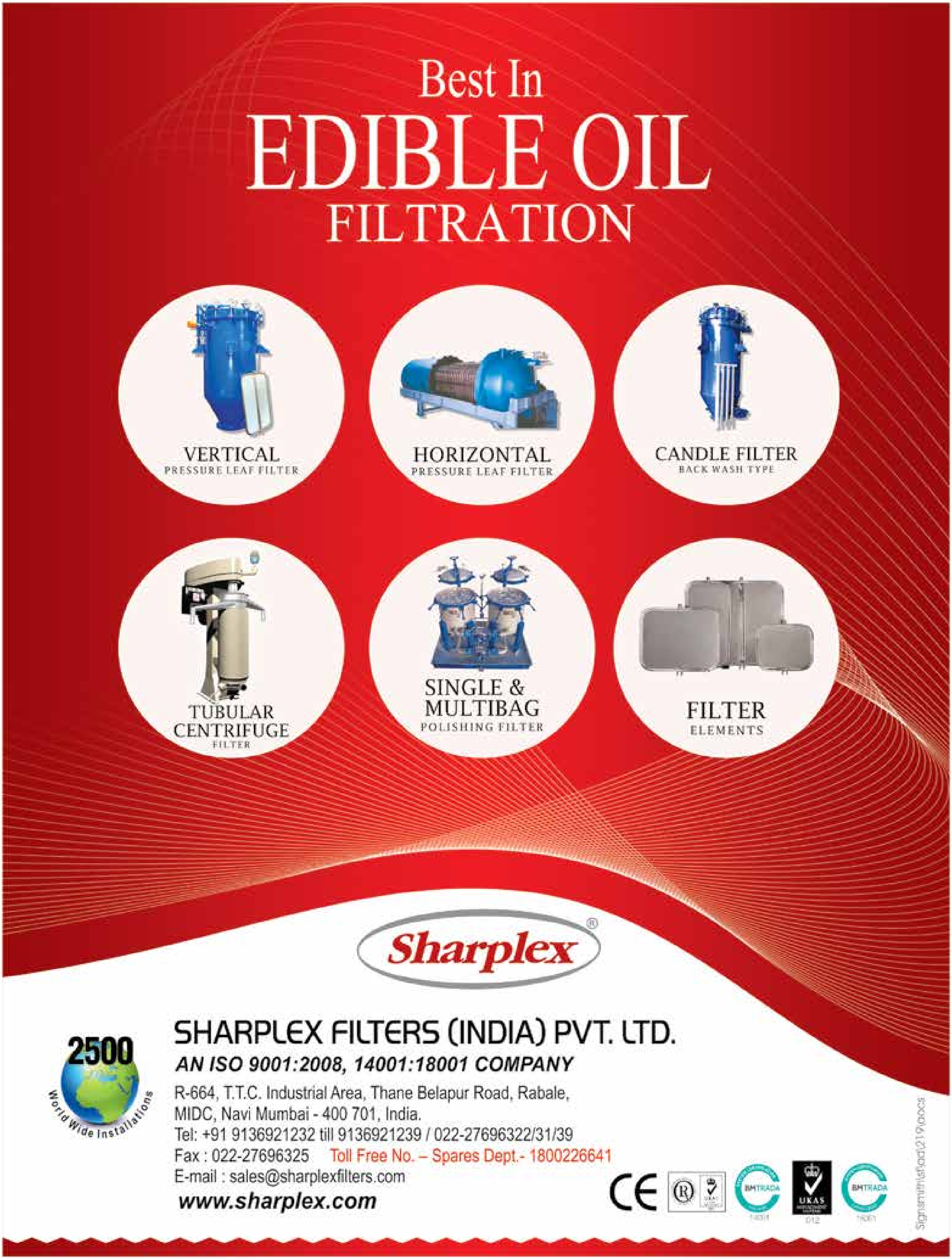


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International News on Fats, Oils, and Related Materials

ISSN: 1528-9303 IFRMEC 32 (10)

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Formerly published as *Chemists' Section*, *Cotton Oil Press*, 1917–1924; *Journal of the Oil and Fat Industries*, 1924–1931; *Oil & Soap*, 1932–1947; news portion of *JAOCS*, 1948–1989. The American Oil Chemists' Society assumes no responsibility for statements or opinions of contributors.

Inform (ISSN: 1528-9303) is published 10 times per year in January, February, March, April, May, June, July/August, September, October, November/December by AOCS Press, 2710 South Boulder Drive, Urbana, IL 61802-6996 USA. Phone: +1 217-359-2344. Periodicals Postage paid at Urbana, IL, and additional mailing offices. **POSTMASTER:** Send address changes to *Inform*, P.O. Box 17190, Urbana, IL 61803-7190 USA.

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Lipid role in the immune system

Rebecca Guenard

As the COVID-19 pandemic dragged on for almost two years, unproven, potentially dangerous treatments for the deadly respiratory disease have surfaced periodically. First there was hydroxychloroquine, then bleach, and, more recently, public health officials in Mississippi warned their citizens against taking ivermectin—a drug meant for parasitic, not viral infections (https://tinyurl.com/csr56pjd).

- **The essential fatty acids react with enzymes to produce a group of compounds known as specialized pro-resolving mediators, or SPMs.**
- **Researchers continually discover new members of the four groups of precursors that comprise the SPM family, called lipoxins, resolvins, protectins, and maresins.**
- **These stereospecific molecules shut down inflammation and restore the body to homeostasis, a mechanism researchers are targeting for treatment as an alternative to anti-inflammatory pathways.**

Meanwhile, about 2.8 billion people worldwide have been fully vaccinated against COVID-19, and vaccination remains the best way to avoid severe illness. Monoclonal antibody infusions were granted an emergency use authorization by the US Food and Drug Administration (FDA), but in most areas the treatment is reserved for patients with severe health risks due to short supply.

Patients could someday treat COVID with a pill. In October, the pharmaceutical company Merck filed with the FDA to approve its recently developed antiviral drug. However, for now, the average patient who tests positive for COVID-19 will likely get the physician's recommendation that has remained consistent throughout the pandemic: Take daily nutritional supplements that support the immune system. Vitamin D, omega-3 fatty acids, and selenium are particularly important micronutrients that modulate the body's inflammatory response to viral infections.

Viruses like SARS-CoV-2 can cause some people's immune systems to go into overdrive. If the immune system does not stop responding to the infection, symptoms may persist indefinitely, as observed in long-term COVID patients. The organ damage and eventual death experienced by millions of COVID patients is the worst-case outcome for an unabated immune response.

Researchers have puzzled over the body's mechanisms for resolving inflammation and returning tissue to homeostasis after an acute response. The process by which the immune system initiates inflammation had been identified, but researchers were not sure of the mechanism for reversing the process. Over the last decade, they have determined that essential fatty acids are crucial to putting the brakes on inflammation. The biochemical pathways that turn off inflammation after an infection center on omega-3 fatty acids which are synthesized into a family of compounds known as lipid mediators. Here is what we know about the importance of this family of fatty acid-derived compounds in the human body.

RESPONDING TO PATHOGEN INVASION

The activities of the human immune system are an exceedingly complex, invader specific set of processes that will not be detailed here. However, generally when a pathogen bursts through a cell membrane to usurp replicating power from the nucleus, it triggers alarms that rouse an immune response. Protein-signaling molecules, called chemokines, leak from the cell and create a chemical gradient which signals a call to action. The invaded tissue becomes inflamed as a team of attendants focused on killing the pathogen flood the infection site.

Scientists now appreciate that inflammation does not just subside once an invader is conquered. Resolving inflammation is an active process that occurs in concert with the inflammatory response. Shortly after the acute inflammatory response begins, the immune system has already initiated a plan for ensuring the process will eventually end. Injured tissues must be repaired. Microbes and cellular debris—the immune system's carnage—must be cleared.

Myriad compounds are in play during inflammation and its resolution. Polyunsaturated fatty acids (PUFA) nestled in the phospholipids of the cell membrane serve an important role. Along with PUFA carried to the site by blood plasma, those released locally from ruptured membranes are converted into eicosanoids that assist the chemokines in directing the molecular traffic arriving at the site to help fight the infection. No sooner have the PUFA been synthesized into inflammation-inducing compounds than they undergo what is called class-switching and start the resolution process.

Molecules that participate in the resolution of inflammation are called specialized pro-resolving mediators (SPMs). They are signaling molecules, locally biosynthesized, that bind to receptors and initiate cell-specific activity. As such, SPMs represent a group of compounds exhibiting a variety of stereospecific arrangements (Fig.1).

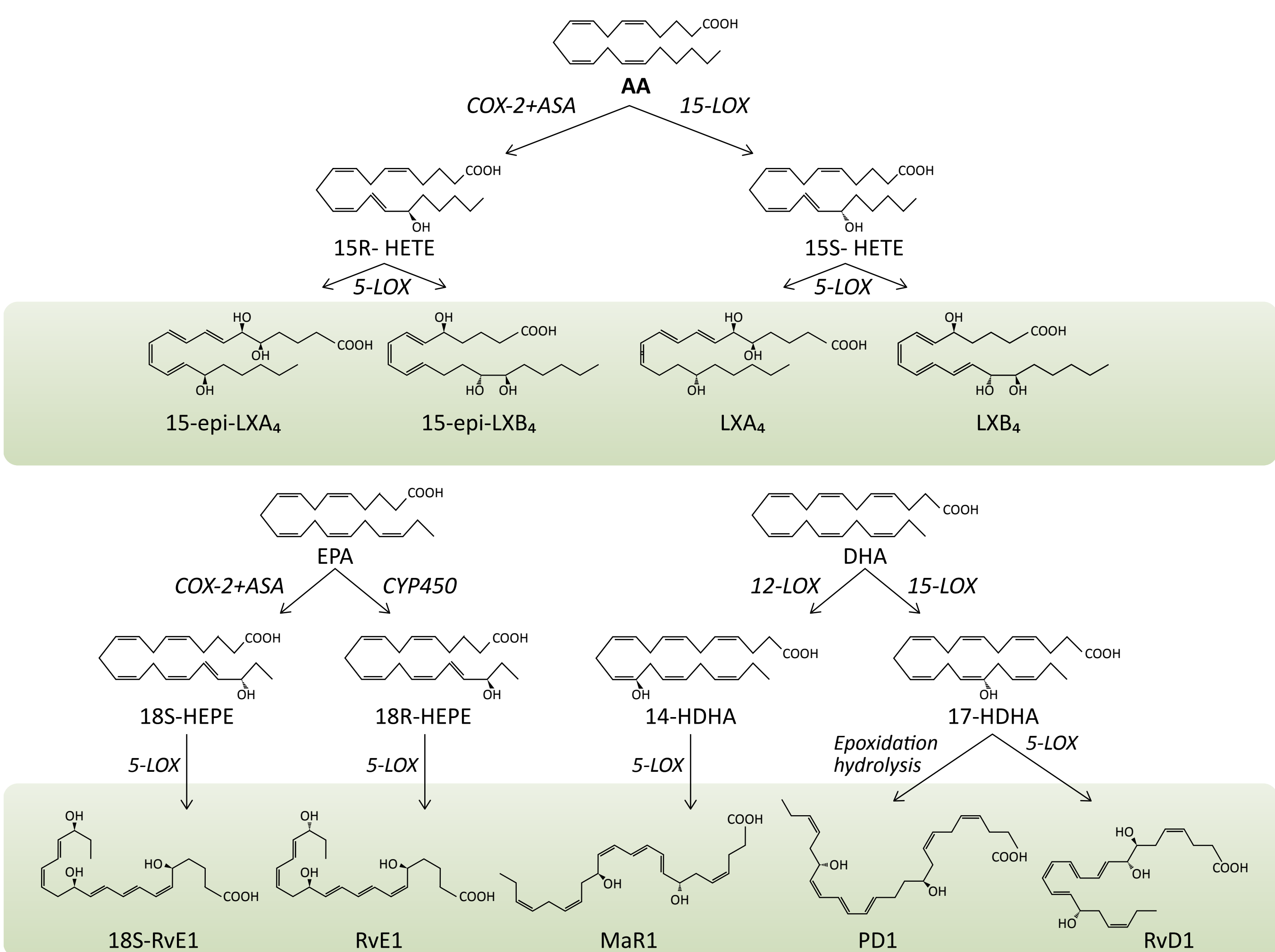

FIG. 1. A selection of pro-resolving mediators produced from polyunsaturated fatty acids. Four principal SPM families (lipoxins, resolvins, protectins, and maresins) lead to a range of stereospecific compounds that resolve inflammation and regenerate tissue. Source: Basil, M.C. and Levy, B.D., *Nat. Rev. Immunol. 16*: 51–67, 2016.

Researchers distinguish four structurally distinct families of SPM; they are lipoxins, resolvins, protectins, and maresins. The essential fatty acid-derived compounds perform the functions of turning off the influx of neutrophils while clearing the infection site of microorganisms and debris. The cleanup allows inflammation to subside and initiates healing for injured tissue.

There is no one general mechanism that these compounds follow since they target different cell types. However, SPMs commonly operate by binding to inflammatory contributors, augmenting their function by changing their shape and thus limiting their ability to migrate to an infection site.

PRO-RESOLVING MEDIATOR ACTIVITY

The SPMs derived from the omega-6 PUFA arachidonic acid (AA) were the first identified by scientists and are now the most highly studied. Although several pathways are now known, the following examples should provide some understanding for the different ways SPMs work (Table 1).

In response to infection, AA is metabolized by enzymes to form eicosanoids. The two primary eicosanoids, prostaglandins and cysteinyl leukotrienes, regulate blood flow and vascular permeability to help immune cells access a pathogen easier. Shortly after producing these pro-inflammatory compounds, the lipid mediator switches to forming resolving molecules. In the resolution phase of the immune response, instead of leukotrienes AA reacts to form lipoxins. Protein receptors expressed in an array of tissues throughout the body bind with the SPM to stimulate resolution. Lipoxins are formed through multiple enzymatic pathways that can also be triggered by the presence of aspirin or statins.

Since the discovery of lipoxins, other omega-3 fatty acid-derived compounds have been identified. Researchers have uncovered pro-resolving mediators that are synthesized from eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and n-3 docosapentaenoic acid (DPA).

EPA is converted into a group of mediators referred to as E-series resolvins. The compounds serve a myriad of functions from controlling acute and chronic inflammation to helping abate neurological disorders and cancer, as well as stimulating tissue repair. In studies on inflammatory diseases, resolvins controlled vascular inflammation by modifying oxidized LDL uptake. Experiments using animal models of Alzheimer's disease indicate that resolvins reduce neuroinflammation. They have also been observed sweeping debris from tumor cells treated with chemotherapy, thus preventing the stimulation of new tumor cell growth. E-series resolvins are reliably beneficial compounds that many researchers are eyeing as a new therapeutic target.

The D-series resolvins form from DHA and represent an even broader set of compounds. Like the E-series, they protect and repair human tissue. DHA can also be metabolized into maresins and protectins. Mucosa in the respiratory tract, for example, house DHA that can react with enzymes to form a compound, called protectin D1, which clears inflammatory fluids from infected airways.

WHEN RESOLUTION FAILS

Being infected with COVID-19 opens the possibility of a persistent inflammatory response that causes tissue fibrosis, necrosis, and irreversible damage, making proper organ function impossible. A recent study showed that the blood plasma

TABLE. 1. Examples of mechanisms researchers have identified where SPMs are involved in limiting hyperinflammation and restoring homeostasis (TRPV = transient receptor potential cation channel subfamily V). Source: Basil, M.C. and Levy, B.D., *Nat. Rev. Immunol. 16*: 51–67, 2016.

Disease or procedure	Mediator	Role
Cardiac reperfusion injury	Resolvins	Cardioprotective and limit infarct size in rat cardiac ischaemia models
Atherosclerosis	Lipoxins	Protect against lipid deposition and limit plaque development in rabbit transgenic models
Ischaemic stroke	Resolvins	Neuroprotective and limit leukocyte infiltration in mouse stroke models
Atopic dermatitis	Resolvins	Improve skin lesions through reduction of interleukin-4, interferon-γ and eosinophil infiltration in murine models
Fibromyalgia	Resolvins	Inhibit allodynia and limit depressive symptoms in a mouse disease model
Inflammatory pain in murine models	Resolvins	Limit pain and revers thermal and mechanical injury in inflamed tissue
	Protectins	Inhibit capsaicin-induced TRPV1 currents and tumor necrosis factor-dependent pain hypersensitivity
	Maresins	Inhibit capsaicin-induced TRPV1 currents and reduce inflammation and chemotherapy-induced pain

of patients with severe COVID-19 contained lower levels of lipid mediators than patients with a mild form of the virus (https://doi.org/10.1161/CIRCRESAHA.121.319142). Infectious disease specialists are familiar with this outcome since pathogens commonly target SPM biosynthesis.

Resolution mediators have been widely studied in influenza infections, since different strains elicit a variety of host immune responses. Researchers found that the more aggressive the influenza strain, the more it suppressed lipoxin formation and protectin D1 production in cells. Protectin D1 interferes directly with the influenza virus' RNA, limiting its replication. Strategic, deadly influenza strains, like H5N1, debilitate this cellular defense upon invasion, leading to a cytokine storm that can be fatal for the host.

However, a foreign body is not always necessary for an overactive immune system to impose harm. The most prevalent human pathologies—cancer, arthritis, metabolic syndrome, chronic pain, periodontal, cardiovascular, and neurological diseases—are all rooted in chronic inflammation stemming from defective resolution. Now that this connection is widely accepted by researchers, more studies are focused on finding the cause in order to find solutions.

Impaired resolution may result from insufficient dietary intake of fatty acids or by genetic polymorphisms affecting SPM biosynthesis, as well as abnormal signaling upon receptor activation. Aging is also associated with increased inflammation leading to less physiological robustness overtime. Mice studies show that a decline in SPM levels contributes to delayed resolution response with age.

One interesting polymorphism study compared cardiovascular events by race and gender while documenting SPM levels. Coronary heart disease arises at similar rates in both Black and White patients, but cardiovascular events (such as myocardial infarction, rehospitalization, and mortality) are disproportionately higher in Black patients. The researchers found that an important E-series resolvin is significantly lower in Black individuals compared with White. In addition, protectin D1 is lower in White males compared with White females and Black patients. The particular factors associated with these correlations were not determined, but the results highlight a need for more research.

The roles of SPMs have been studied in a range of infections, including bacterial, viral, and parasitic. Resolving, as opposed to suppressing, an inflammatory response is now a burgeoning field in modern medicine. With continued research, clinicians may soon have treatment options beyond steroids and cytokine blocking drugs to tamp down hyperinflammation.

IMPROVING THE RESOLUTION RESPONSE

Last year, a panel of doctors from Switzerland published a set of dietary recommendations to optimize immune system health and help Swiss people have a successful recovery should they catch COVID-19. Not surprisingly, the physicians recommended increased consumption of food containing EPA and DHA.

Both human and animal experiments indicate that increasing essential fatty acids helps the body initiate resolution and heal from inflammation. These encouraging results have led many scientists to consider targeting SPMs in a pharmacological treatment.

Synthetic lipoxins and resolvins introduced into mice, successfully reduced inflammation. Researchers observed a similar effect by indirectly stimulating lipoxin synthesis. Studies on young adults taking omega-3 supplements were given an intravenous lipopolysaccharide challenge (a common method for initiating an inflammatory response). Analysis of the participants' blood plasma revealed that after a certain period of time SPM concentration in their plasma increased to resolve the inflammation.

Recently, scientist have homed in on a unique source for SPM production: the vagus nerve. The longest of the cranial nerves, the vagus nerve runs from the brainstem, down the neck, then branches in different directions towards visceral organs. The nerve transmits motor and sensory signals from the brain to every part of the body.

A number of scientific studies are currently focused on understanding less intuitive tasks that the vagus nerve performs, for example, acting as a line of communication between the gut and the brain. A nascent area of medicine involves treating chronic inflammation by stimulating the vagus nerve using electronic implants. Neurosurgeons have successfully driven serious inflammatory syndromes into remission by sending electric pulses through the nerve. Studies show that electrical stimulation resets the nerve so it produces proresolving compounds, maresins, E-series resolvins, and protectins, in the blood stream instead of inflammatory molecules, like prostaglandins.

Thankfully, we do not all need to jolt one of the body's primary nerves to keep our proresolving production processes operating smoothly. Weight loss and strenuous exercise also increase SPM production in studies on humans. Government-established dietary and fitness recommendations not only keep our bodies operating in health; they also optimize our recovery systems when we are sick.

The amount of research on SPMs has grown to a critical mass, and a database now exists to allow scientists to sort through it all. The Atlas of Inflammation Resolution (AIR) is a recently developed web resource that gathers updated data on various SPMs. The site includes information on the biological processes involved along with details on the molecular pathways and interactions of the modulators. With increased interest in this area prompted by COVID-19, the site is likely to grow at a breakneck pace.

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Mineral oil hydrocarbons: a new challenge for the oils and fats processing industry

Véronique Gibon, Wim De Greyt, Antonios Papastergiadis, and Marc Kellens

- Mineral oils are widespread food contaminants, and edible oils, like many other foodstuffs, are often contaminated.
- The lack of robust analytical methods and proper toxicological evaluation make it difficult to set a tolerance level.
- The best way to avoid mineral oil contamination is to prevent it by complying with good manufacturing practices, and the best solution to reducing mineral oil contamination in edible oils is through refining, especially during deodorization.
- This paper gives an overview of contamination sources, levels in some edible oils, regulatory aspects, analytical methods, and strategies for mitigation during refining.

Refined oil quality is primarily evaluated by traditional parameters, such as free fatty acid content, oxidative stability, color, odor, and taste. In addition, high-quality food oils will contain low amounts of trans fatty acids, high amounts of natural antioxidants (tocopherols), and very low or no "process" contaminants, such as polycyclic aromatic hydrocarbons (PAH), mycotoxins, pesticides, dioxins, polychlorinated biphenyls (PCB), phthalates, 3-monochloropropane-1,2-diol (3-MCPD), glycidyl esters (GE), trans fatty acids, di-alkyl ketones, and mineral oil hydrocarbons (Fig. 1, page 12). Contamination with mineral oil hydrocarbons (MOHs) is not new but has attracted more attention recently.

They are categorized into two main groups: mineral oil saturated hydrocarbons, or MOSH (paraffins and naphthenes), and mineral oil aromatic hydrocarbons, or MOAH (aromatics). MOSH correspond to straight and branched open-chain alkanes and largely alkylated cyclo-alkanes; MOAH are alkylated and non-alkylated polyaromatics (Fig. 2, page 12). MOHs exclude hydrocarbons naturally occurring in food and hydrocarbons from synthetic origin. They are usually classified according to their carbon number: Total MOSH refer to the sum of C10-C16, C16-C20, C20-C25, C25-C35, C35-C40 and C40-C50 fractions, and total MOAH to the sum of C10-C16, C16-C25, C25-C35, and C35-C50 fractions.

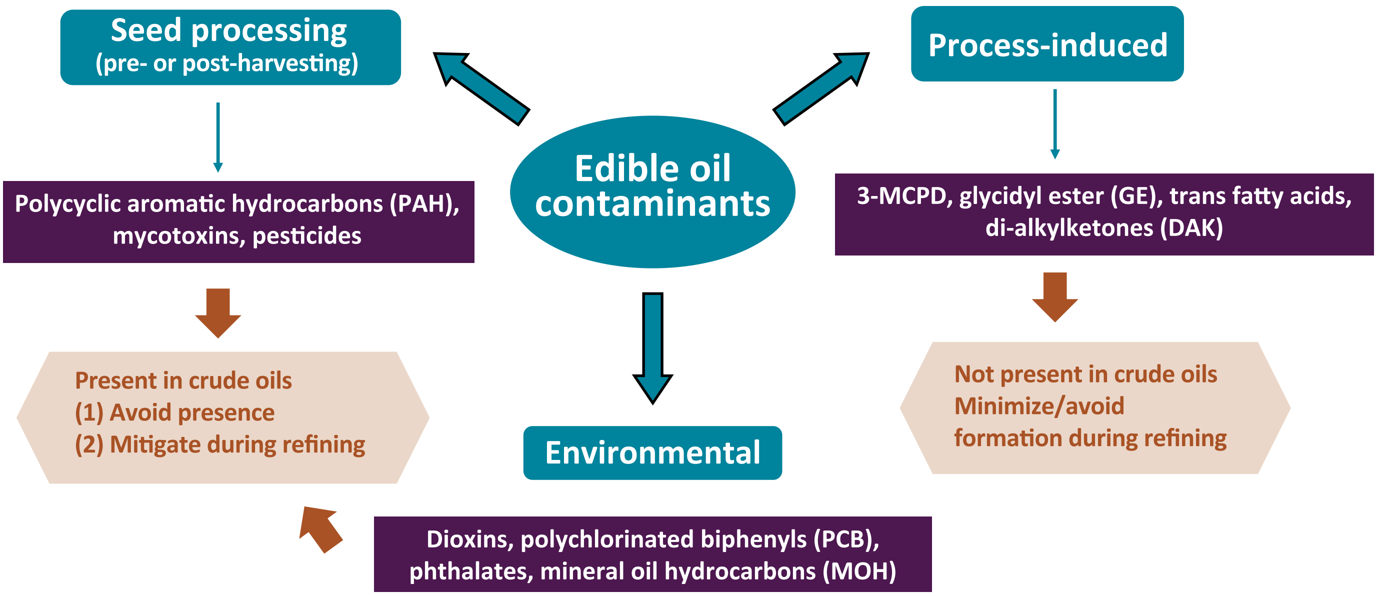

FIG. 1. Major contaminants of edible oil and fats

SOURCE AND LEVEL OF CONTAMINATION

MOHs are contaminants that can be present in food due to environmental pollution (air, soil), use of lubricants for machinery during harvesting and food production, food contact materials, processing aids, food additives, and other factors. They often come from malpractices in process operations. A contamination of walnut oil with a food-grade lubricant during refining was highlighted in 2009, followed in 2011 by a contamination of milk fat during production (2). In most cases, proper monitoring of good manufacturing practices is the best solution to avoiding such problems. The application of benchmark levels to identify unusually high loads of MOHs is informative (3). These levels are oriented to the market and allow manufacturers to respond appropriately; they are an effective tool for minimization. It is also important to monitor MOHs contents to better understand their relative presence in food commodities; variable levels of MOSH (up to 50 mg/kg) can still be detected in large food categories.

Oil seeds, fats, and oils can accumulate MOHs from all kinds of sources along the production chain. A general overview is given in (4) for vegetable oils like sunflower oil, olive oil, palm oil, sesame oil, walnut oil, rapeseed oil, argan oil, poppy seed oil, olive oil, olive pomace oil, cottonseed oil, and grapeseed oil; contamination is also reported in oils for feed production. A specific survey is described in (5, 6) for olive oils. Contents depend on the variety: 8 to 90 mg/kg for MOSH and 1 to 13 mg/kg for MOAH, mostly in the C25-C35 fractions. Highly contaminated oils contain heavier MOSH (C35-C50).

The average MOSH and MOAH contents (C10-C50) in a series of oils are presented in (7). The highest amounts are found in coconut oils (MOSH up to 40 mg/kg and MOAH above 15 mg/kg); the contents largely depend on suppliers, and some special grades do not have MOAH. In MOSH, the major fractions are C16-C40; in MOAH the major ones are C16-C35.

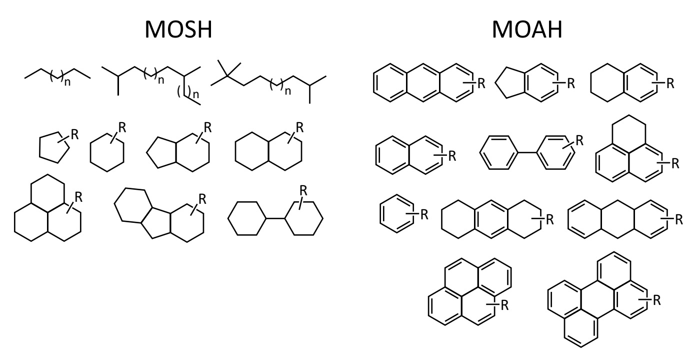

FIG. 2. Chemical structures of representative compounds found in MOSH and MOAH (1)

MOSH in soybean are also high (up to 35 mg/kg), although MOAH are usually low (< 2 mg/kg). Rapeseed, palm kernel, and sunflower oils, are also reported to have low MOAH and MOSH levels below 5–10 mg/kg.

Higher contents in MOSH (> 25 mg/kg) and in MOAH (>2.5 mg/kg) are detected in palm oil; however, palm oil from sustainable sources with low MOH contents is preferred for infant formula applications. A pilot study on MOSH status in crude palm oil was conducted by Sime Darby Plantation Palm Oil Mills and published in 2019 (8). Results revealed that MOSH in Hazard Analysis Critical Control Point (HACCP)-certified palm oil mills was at the range of 10–17 mg/kg, while non-HACCP certified-mills had an average of 44.8 mg/kg. Premium CPO showed lower MOSH, with 10.7 mg/kg compared to standard CPO at 21.9 mg/kg. The study did not establish any correlation between the free fatty acid and the MOSH contents.

A survey of MOSH and MOAH in different vegetable oils is shown in a 2020 article (9). Cocoa butter had the highest contents: up to 162 mg/kg for MOSH and 55 mg/kg for MOAH. MOSH were mostly observed in the ≤C35 faction, and

MOAH in the ≤C24 fraction; presence of benzothiophene, dibenzothiophene, and alkylated naphthalenes was detected. Palm oil contained up to 124 mg/kg of MOSH (in ≤C35 and in > C35 fractions, in lower extent in ≤C16 and ≤C24 fractions) and 39 mg/kg of MOAH (mostly in ≤C35 and > C35 fractions). Sunflower oil showed lower contents: less than 17 mg/kg of MOSH (in ≤C35 fraction) and no MOAH. In 2009, other authors (10) had already described similar levels for sunflower oil and concluded that environmental contamination and harvesting were critical factors for accumulation in the seed oil.

New data will help fill the missing gaps needed for proper risk assessment and make it possible to establish a tolerable daily intake (TDI) for MOSH in the next EFSA (European Food Safety Authority) opinion due December, 2022.

RISK ASSESSMENT

MOHs pose potential health risks in animal studies; Sprague Dawley rat seems to be a good model for humans, but it is not yet confirmed. Scientific understanding of MOHs has advanced significantly, but knowledge gaps remain, particularly regarding the risk to humans.

The risks associated with MOSH are different from those of MOAH. MOSH are neither carcinogenic nor mutagenic; there is no direct evidence of harmful effects during to humans during accumulation—even though formation of granulomas in organs of Fischer rats is reported. Critical effect was nevertheless identified by EFSA, with NOAEL (No Observed Adverse Effect Level) value of 19 mg/kg body weight/day.

MOAH are carcinogenic, mutagenic, and hormone-disruptive. Carcinogenic potential seems to correlate with increasing number of aromatic ring systems; MOAH are suspected to contain bio-accumulative genotoxic compounds, mainly related to 3- to 7-ring aromatic substances. Today, no safe thresholds are clearly defined, and presence in foods is a concern.

REGULATORY ASPECTS

There is currently no EU legislation regulating the limits of MOHs in vegetable oils—only a recommendation (2017/84) for their monitoring in food. However, large companies are setting their own standard for maximal levels in vegetable oils and fats.

In 2012, EFSA released a scientific opinion on MOHs in foods: all MOH mixtures are mutagenic unless they are treated specifically to remove MOAH, and total MOAH must be monitored with analytical methods able to separate different structural subclasses (11). In October 2015, food watch tests were organized on 120 dry products; 52 of 120 products tested were contaminated with the potentially carcinogenic MOAH. The introduction of functional barriers became mandatory, and EFSA started to consider specific limits for MOSH and MOAH in foods. In 2017, an EC (European Community) recommendation (2017/84) was released for MOHs monitoring in food. In April 2019, the JRC (Joint Research Centre of the European Commission) published a guidance on sampling, analysis, and data reporting for the monitoring of MOHs in food and food contact materials. In October 2019, a food watch test on infant formula was organized based on analytical methodology following JRC guidance; in half of the products, MOAH contamination was detected up to 3 mg/kg.

Industry publicly denied MOAH contamination, and EC immediately commissioned EFSA to rapidly assess the methodology/results published. In December 2019, the food watch test was proved to use inadequate methodology with false negative results. In February 2020, *Reuters* Malaysia published an article claiming that Nestlé was putting pressure on the palm oil industry to reduce MOHs contamination. In June 2020, the European Standing Committee on Plants, Animals, Food and Feed (SCOPAFF) defined a harmonized risk management approach with a maximum level of 1 mg/kg of MOAH per C-fraction.

Contamination with MOSH is almost avoidable, and with MOAH is nearly completely avoidable; these compounds represent an unjustifiable health risk for all consumers. An ongoing collaboration between industry and health and regulatory authorities is a promising solution with multiple benefits. There have already been many advances in analytical techniques which still need to become fully routine in the service laboratories; qualified ring trials and harmonization will drive further progress.

In anticipation of a specific EU legislation, the German Food Society has put a “benchmark level” of max. 13 ppm C10-C50 for MOSH in edible oils, while MOAH should be below the LOQ (the limit of quantification).

ANALYTICAL METHODS

Standardized analytical methods are useful in establishing internationally validated methods with precision data, and to verify product compliance with regulations. The first systematic study on the contamination of food by MOHs appeared in 1991; the LC–GC (liquid chromatography-gas chromatography) technique experienced success in the 1990s. The topic was not considered for almost 20 years, but re-emerged in 2008, with the discovery of highly contaminated Ukrainian sunflower oil (12); this led to the need for proper MOAH quantification. A method to separate the MOSH and MOAH was developed.

In 2015, the ISO 17780: 2015 method (13) was used as reference for MOSH, with a LOQ of 50 mg/kg. In 2017, the EN 16995: 2017 method (14) was released for MOSH and MOAH, with a LOQ of 10 mg/kg. From 2019 to 2021, the focus was on introducing additional clean-up steps in the EN 16995 method to lower the LOQ, for both MOSH and MOAH, from 10 mg/kg to 1–2 mg/kg. The DGF-C-VI 22 (20) method (15) was proposed in 2020, with an improved precision and comparability with a LOQ of 1 mg/kg. The general approach of the method consists in: (1) extraction of the mineral oil and enrichment, (2) separation of the MOSH and MOAH by liquid chromatography, and (3) quantification by gas chromatography.

Chromatograms are generally complex with many interferences, since food products contain varieties of liposoluble components, such as waxes, squalene, steradienes, terpinoids, and carotenoids. Sample preparation is essential, and clean-up with aluminum oxide makes it possible to retain long-chain natural n-alkanes which interfere with the quantification of

MOSH. Epoxidation is used to eliminate natural compounds like squalene, terpinoids, and carotenoids, which interfere with the quantification of MOAH. Special epoxidation treatments or additional purification steps after epoxidation may be necessary in the case of important disturbances due to matrix components.

The need to understand the source of the Ukrainian sunflower oil contamination brought into play another key hyphenated technique, GC × GC, which allows a detailed characterization of the MOSH and MOAH fractions to be obtained. GC × GC provides a sensitivity gain and a burst in separation power, along with a well-ordered 2D plot. This permits a group type separation (16). GC × GC-FID (flame ionization detection) and enables a rough separation and quantification of paraffins and naphthenes in the MOSH fractions; it also appears to be the most effective method to distinguish the different MOAH subclasses.

In May 2020, a co-authored paper by two recognized institutions, the Joint Research Centre (JRC) of the European Commission and the Official Food Cantonal Laboratory of Zürich, clearly stated that GC × GC is the best technique for verification purposes (17), confirming the previous suggestion reported by the EFSA in 2012. The combination of GC × GC with TOF-MS (time-of-flight–mass spectroscopy) permits the separation of condensed aromatics and consequently helps to identify with more confidence the occurrence of carcinogenic or mutagenic constituents, namely the 3–7 ring MOAH. The use of multidimensional GC × GC with parallel dual detection (FID and TOF-MS) was recently proposed as a confirmatory method to better distinguish MOHs fractions from interferences. The method was optimized and published (18); it shows completely overlapped 2D plots from the two detectors and helps to correct any quantification issue due to coelution with naturally present components.

LABORATORY DISCREPANCIES

The JRC guidance does not properly clarify how to report the LOQ, resulting in different approaches taken by laboratories (19). Some laboratories provide LOQs that may be different for each individual C fraction; others consider that the same LOQ applies whatever the C fraction. Some laboratories calculate the result for “total C10-50” by summing the values for the individual C fractions which are greater than LOQ. This does not take into consideration the complete signal as indicated in the JRC guidelines. In some commercial laboratories, LOQs are depicted only when a value of the corresponding C fraction is below the LOQ; in cases where the value exceeds the LOQ, the LOQ is not shown. The LOQs are therefore not always known by the company requesting the analysis. The need for confidence in laboratory performance is essential; such confidence can be demonstrated through laboratory participation in proficiency testing schemes (20).

MINERAL OIL MITIGATION DURING REFINING

There is little information available in the literature on mineral oil mitigation during edible oil refining, and data are sometimes contradictory.

In 2001, Wagner, *et al*. (21) reported the paraffin content in some refinery by-products and edible oils. By-products (fatty acids from the deodorizer condensate) showed an average concentration of 650 mg/kg. Crude, semi-, and fully refined vegetable oils from diverse origins were investigated;

with few exceptions, most of the levels detected were below 50–100 mg/kg. The authors reported the influence of press versus solvent extraction; differences observed were not related to paraffins present in the hexane migrating into the crude oil, but rather to better extractability of the paraffins from the seeds by the solvent. Removal of mineral paraffins during chemical refining of peanut oil was monitored. The crude oil contained two types of paraffins: one centered at C22 and another reaching up to C28 to beyond C40. Deodorization completely removed the paraffins up to C23 and about 50% of C25; harder conditions from physical refining were necessary to remove the longer-chain fractions. However, neutralization and bleaching did not modify the paraffin content (50 mg/kg), which only decreased after deodorization (14 mg/kg).

On the other hand, in a 2006 article (22), van Duijn claimed that much of the mineral oil that does find its way into edible oils is removed during the refining process. Gasoline and diesel can be removed during neutralizing and bleaching, and mid-fraction mineral oil (C20–C35) is stripped during deodorization. However, heavy-fraction mineral oil, such as grease and hydraulic oil, are not removed during refining.

In a 2020 article (9), crude and deodorized cocoa butters from different refineries were analyzed; the MOSH and MOAH in ≤C24 fraction were reduced by 10 to 75%, depending on the process conditions applied. In coconut oils, the MOSH ≤C24 fraction was also diminished by the refining process to levels below LOQ, while ranges of higher carbon number were reduced to less extent. The entire MOAH fraction from C10–C50 was removed, resulting in MOAH levels below LOQ. For MOSH, only single iso-alkanes were present after refining; for MOAH, naphthalenes, pyrenes, and fluoranthenes, predominantly present as crude oils, were eliminated by the refining process. The influence of deodorization temperatures (140°C to 240°C) on MOSH/MOAH reductions was investigated on laboratory scale using spiked crude cocoa butter. Significant reduction of ≤C24 in MOSH and MOAH fractions was observed at 210°C; both desired effects of free fatty acids and MOSH/MOAH reduction could be achieved.

In a 2021 survey (5), crude olive pomace oils were shown to contain MOSH ranging from 30 to 300 mg/kg and MOAH from 2 and 30 mg/kg. To better understand these important deviations, MOSH and MOAH were analyzed in both solvent and press-extracted oils. MOSH and MOAH were much higher in the solvent-extracted oils (210 mg/kg versus 65 mg/kg for MOSH, and 65 mg/kg versus 3 mg/kg for MOAH). It was shown that the hexane used in solvent-extracted olive pomace oil had a neutral effect, and that the higher content observed was only related to better oil extractability of the solvent.

The MOSH and MOAH contents were analyzed at different stages of the chemical refining. Neutralization, water washing, and bleaching did not significantly decrease the contents, but some C10-C35 MOAH were slightly reduced after bleaching. Most of MOSH and MOAH still remained in the deodorized oil apart from the lighter C10-C16 MOSH fraction which was fully removed; the C17-C24 MOSH fraction and, to a lesser extent, the C25-C35 fraction were slightly decreased. The heavy fractions of both MOSH (C36-C40 and C41-C50) and MOAH (C36-C50) were almost untouched.

MOSH and MOAH contents were analyzed in crude sunflower oil obtained from spiked and un-spiked seeds as well as spiked crude sunflower oil in the framework of a recent German FEI research project (23). The contents were determined at different stages of physical refining: after filtration, water washing, degumming, bleaching, and deodorization. MOSH and MOAH significantly decreased after deodorization only.

For rapeseed oil, the influence of deodorization time (60 → 150 min.) and temperature (220°C → 280°C) on MOSH removal were evaluated. Removal was time and temperature dependent, with the best removal at 260°C and 280°C; however, excessive trans fatty acids were formed. The optimal conditions for low-trans content (< 1%), with a residual MOSH content of 25–30% was 60 minutes at 240°C. Several absorption materials were tested but did not achieve significant MOSH and MOAH removal, except for active carbon (to remove PAHs). Further, cold treatment (winterization) did not show any significant removal of long-chain MOHs.

MOSH and MOAH contents in crude and fully refined soybean oil were reported in (7). In this example, MOSH in the crude oil (95 mg/kg) were principally C16-C20, C20-C25, and C25-C35. Very low MOAH contents (1.2 mg/kg) were observed (C35-C50). The C16-C20 and C20-C25 MOSH were almost completely removed in the refined oil which contained residual amounts of C25-C35; the high molecular weight fractions of both MOSH and MOAH were untouched.

Pilot trials were recently conducted using a tray-type deodorizer (24). Bleached high-oleic sunflower oil was spiked with relatively high amounts of commercial lubricants to reach 232.9 mg/kg of MOSH and 32.5 mg/kg of MOAH. Spiked oil was deodorized at 220°C, 245°C, and 260°C under varying amounts of stripping steam, and samples were analyzed (25) using the DGF-C-VI 22 (20) method (LOQ 1 ppm) (15). Almost 65% of MOSH were stripped at 245°C. The short-chain fractions (C10-C16 and C16-C20) were fully removed; ~90 and ~77% of the C20-C25 and C25- C35 fractions were also removed, but the long-chain fractions C35-C40 and especially C40-C50 remained mostly untouched. Almost 50% of the MOAH were removed at 260°; the C16-C25 fraction was fully stripped regardless of deodorization temperature.

TO SUM IT ALL UP

The quantity and relative contribution of different mineral oil fractions depends on the source of contamination, generally differ from one oil to another, and may vary over time for each oil. Removing MOSH/MOAH during the refining process is mainly possible during deodorization and is temperature dependent. The relative removal and final content depend on the initial contamination. Under process conditions, the short chains of MOSH (C10-C16 and C16-C20) are generally stripped easily. The medium ones (C20-C25 and C25-C35) can be efficiently stripped, but the heavy ones (C35-C40 and C40-C50)

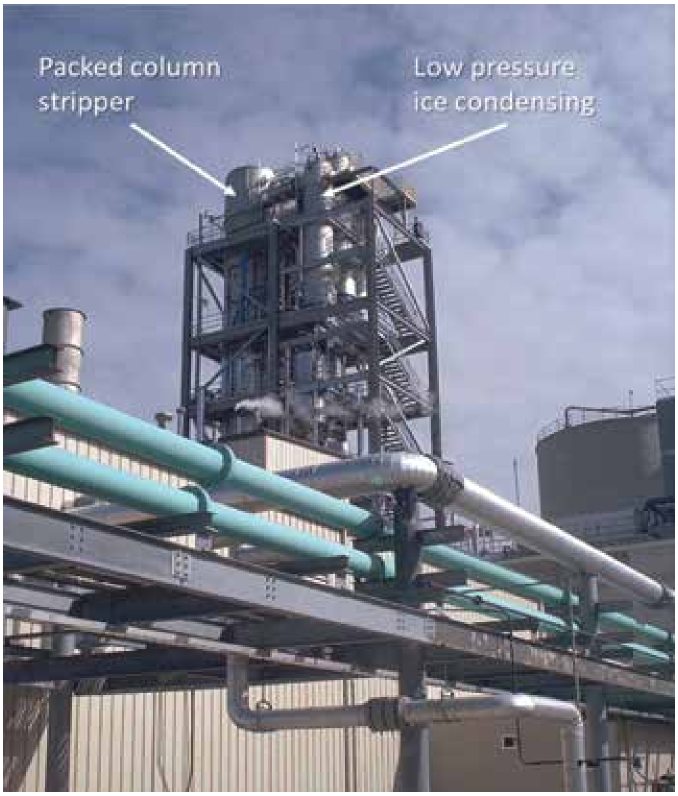

FIG. 3. General overview of a packed column stripper and low pressure ice condensing system (24)

usually remain in the oil. Similarly, the C16-C25 fraction of MOAH is easily distilled, removal of the C25-C35 is possible, but the C36-C50 fraction is more difficult to remove.

In industrial practices, mineral oil can best be stripped over a packed column stripper operating at higher temperature and at low pressure with an ice condensing system (Fig. 3). The efficient countercurrent stripping and short residence time in the packed column will provide the most efficient mineral oil stripping, with minimal formation of trans fatty acids and glycidyl esters. Mineral oil can also be stripped via molecular or short-path distillation, but use of these technologies will be difficult to justify due to high costs and higher oil losses. Higher stripping efficiency to remove MOH will also lead to more removal of essential components like tocopherols and sterols, which is disadvantageous for oil stability and nutritional quality. The best solution to mitigate MOH in edible oils is therefore to avoid contamination at the source.

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Performance enhancements in select industrial applications using surfactants, surfactant additives, and thermostable enzymes

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- **Household detergents have undergone many formulation updates to improve the cleaning power of surfactants and to adapt formulations to specific applications.**
- **This paper focuses on more recent developments in the use of surfactants for household as well as industrial use.**
- **Specific examples include the use of enzymes derived from moderately thermophilic bacteria as laundry detergent additives, the use of surfactants and surfactant additives in the manufacture of warm mix asphalt, and the use of foaming agents in chemical enhanced oil recovery.**

From a domestic perspective, the term "detergent" typically refers to a cleaning agent that is most associated with laundering and dishwashing processes. Synthetic detergents were first developed in Germany during WWI as a means of offsetting the shortage of fats and oils necessary to produce soaps. At that time, Otto Rohm (the founder of Rohm and Haas in 1907) discovered that the use of enzymes greatly enhanced the cleaning potential of textile materials, which revolutionized the use of washing detergents. In the United States, research into the production of household detergents was initiated at Procter & Gamble in the 1930s. At that time, surfactants (short for "surface-active agents") were introduced into laundry detergent formulations. Surfactants lower the surface tension of water thus providing a mechanism to release oil and grease from the textiles and suspend them in water until they can be rinsed away. Then, in 1933, Procter & Gamble's first detergent, Dreft®, hit the market.

Household detergents have since undergone many formulation updates to improve cleaning power and to adapt formulations to specific applications. The detergents of today are primarily composed of builders (chelating or sequestering agents) whose function is to remove dissolved minerals (primarily calcium and magnesium) from water to reduce the amount of soap scum that can accumulate on fabric and/or other washed materials. Unfortunately, these builders have historically been associated with different phosphate analogs that have been demonstrated to be environmental pollutants.

Other important components in detergent formulations are surfactants (*i.e.,* linear alkylbenzenesulfonates; LAS, or more recently alkyl sulfates such as sodium dodecyl sulfate; SDS) which are most responsible for the cleaning performance of any detergent, and enzymes that can replace harmful chemicals in detergents, making them more environmentally friendly and cost-effective [2]. Many different types of enzymes have been added to detergent formulations, including proteases to break down proteins, lipases to break down lipids, and amylases to break down carbohydrates. It is expected that the enzyme market will reach $13–14 billion in 2025, with the detergent industry occupying as much as 25–30% of that market. Various other ingredients have also been added to detergent formulations, such as buffering agents to control the pH balance of the washing solution, stabilizers for consistent cleaning, lather enhancers, perfumes, and brighteners for improved results [1].

There are four types of surfactants: anionic, nonionic, cationic, and amphoteric/zwitterionic [4]. Anionic surfactants dissociate in water, while nonionic surfactants do not ionize. These two surfactants are the most-used, with anionic surfactants composing about 50% and nonionic surfactants composing about 45% of global production. Cationic surfactants dissociate like anionic surfactants, with the main difference being that cationic surfactants dissolve into halogen-type amphiphilic cations and anions, while anionic surfactants dissolve into alkaline metal or quaternary ammonium amphiphilic cations and anions. Amphoteric/zwitterionic have both cationic and anionic dissociations.

The most favorable applications for individual surfactants are determined based on their hydrophilic/lipophilic balance (HLB) and their functional properties (surface-tension-lowering properties in water and critical micelle concentrations). The HLB index is a value that is correlated to the relative distribution of water-soluble (*i.e.,* hydrophilic) and oil-soluble (*i.e.,* lipophilic) segments of a surfactant molecule. In other words, the HLB value of a surfactant can be regarded as the relative water solubility of any individual surfactant; the higher the HLB value, the more hydrophilic and hence the more soluble it is in water. Conversely, the lower the HLB value, the more hydrophobic the molecule and the more soluble it is in oil.

Originally the HLB index was applied solely to nonionic surfactants; however, its use has now been extended to ionic surfactants. The range of HLB values for non-ionic surfactants is 0–20 but could go much higher in ionic surfactants (to as high as 50) based on their ionization behavior. Based on this classification system, a nonionic surfactant of certain HLB value could potentially be used as an antifoaming agent (HLB = 1–3), a water-in-oil (W/O) emulsifying agent (HLB = 3-6), a wetting agent (HLB = 7–9), an oil-in-water (O/W) emulsifying agent (HLB = 8–16), a detergent (HLB = 13–16), or a solubilization agent (*i.e.,* to facilitate the solubilization of water-insoluble substances; HLB = 16–18). The use of the HLB index has been widely adopted by many industries to ease the formulation of commercial products that require the addition of surfactants as functional ingredients.

Other popular parameters for predicting the suitability of a surfactant molecule for washing and cleaning applications are the critical micelle concentration (CMC) and the effects of the surfactant on the minimum surface tension (γ_{min}) of water in its presence. As with HLB values, the CMC and γ_{min} of a surfactant are governed by its structures and compositions,

TABLE 1. Industrial applications of surfactants [3]

Industrial Application	Consumer Products
• Foods • Agriculture • Plastic and chemical industry • Adhesives • Paints • Leather and furs • Road construction • Metal processing	• Detergents • Cleaning and dishwashing agents • Personal products

TABLE 2. Experimentally obtained crude lipase activity when mixed with chemical and commercial detergents [6]

Detergent type	Detergent	Activity
Chemical	SDS	48.3
	Tween 20	83.6
	Tween 80	107.6
Commercial	Ghandhi	84.3
	Surf Excel	95.6
	Ariel	103
	Ezee	104.3

which in turn are influenced by its production and purification processes. Unlike the HLB values, however, the CMC and γ_{min} values of any surfactant can be determined by experimental procedures using tensiometry.

Table 1 depicts a more extensive list of the several applications of surfactants. Understanding the vast potential of surfactants in industrial applications, this paper focuses on more recent developments in surfactant use, specifically on the use of enzymes derived from the moderately thermophilic *Anoxybacillus* bacteria as detergent additives, and on the use of surfactants for warm mix asphalt, and enhanced oil recovery.

LAUNDRY DETERGENT ADDITIVES

A relatively new advancement in surfactant additives is the use of extremophilic bacteria to produce new thermostable enzymes that improve stability in laundry detergent formulations, especially in warm-hot water washes. Extremophilic bacteria have adapted to live in extreme conditions, such as in environments with high temperatures, high pressures, high salinity, and/or high acidity or alkalinity (pH) levels. To withstand the environmental conditions placed upon these bacteria, they have altered their enzymatic makeup such that the vital processes within the cell can be performed under extreme conditions. Proteases are enzymes used in pharmaceuticals, detergent formulations, and other commercial products. They hydrolyze peptide bonds and account for approximately 65% of global enzyme sales. SAPA is an alkaline protease from *Anoxybacillus kamchatkensis* M_1V and is a member of the serine protease family. It is a monomer with a demonstrated optimal activity at 70°C and a pH of 11 which makes it a potentially valuable candidate for addition to laundry detergent formulations. The isolation of the SAPA protease was accomplished from water samples that were collected from the Hammam Righa hot spring in Algeria, where the bacteria *Anoxybacillus*, meaning "*Bacillus* without oxygen," was isolated. The rSAPA enzyme was found to be a good detergent additive because of its high thermal stability and high hydrolytic effect on chocolate and blood [5].

The effects of lipase activity from *Anoxybacillus* have also been studied in laundry detergent preparations. Lipase is used as a laundry detergent ingredient because it is typically eco-friendly and can facilitate the removal of oil stains [6]. Furthermore, the ability of lipase to hydrolyze carboxylic ester bonds and catalyze processes, such as esterification and transesterification, makes it a useful component to detergent formulas [**7**]. Recently, a thermostable lipase was isolated from *Anoxybacillus* sp. ARS-1 which was sampled from Taptapani Hotspring in India by solid substrate fermentation (SSF) using mustard cake as substrate. Based on statistical optimization using central composite design (CCD), the maximum lipase production of 29.4 IU/g occurred at 57.5°C, a pH of 8.31, and in the presence of 50% moisture and 1.2 mg of biosurfactants. Crude lipase (the cell-free lipase after 1 mL of sterile water and 0.1 g of biomass were centrifuged together) was obtained and was mixed with different detergent types at 1% *w/v* with the activity results shown in Table 2.

Lipase must be stable in the presence of surfactants to be used in detergent formulations because of the severe conditions they must undergo. As seen in Table 2, the crude lipase activity was maximized in nonionic detergents (Tween 20 and Tween 80). Tween 80 demonstrated higher activity in the presence of the crude lipase compared to Tween 20 due to its longer acyl ester chains. The crude lipase activity in commercial detergents varied according to the specific detergent but was more than 100% in Ariel, an anionic detergent developed in Europe by Procter & Gamble, and Ezee, a cationic detergent [8, 9] made by the *Godrej* Group, a multinational conglomerate based in Mumbai, India, that is formulated for woolens and other delicate items. The *Anoxybacillus* lipase was found to be a beneficial additive because of its activity, relative resistance, and compatibility with all the studied detergents and surfactants [10]. This indicates that these enzymes would be valuable laundry detergent additives, provided they can be obtained economically.

WARM MIX ASPHALT

Asphalt is used in many construction projects, such as highways, driveways, and sidewalks. Hot mix asphalt (HMA) is typically manufactured at about 160°C, while warm mix asphalt (WMA) is manufactured at temperatures that are approximately 20–40°C lower than HMA. As such, WMA may allow for important economic and environmental benefits as it requires lower energy consumption for construction projects com-

TABLE 3. CO_2 emissions and energy consumption values of road materials [13]

Material	CO_2 emissions (kg/1,000 kg)	Energy consumption (MJ/1,000 kg)
Base asphalt	288	5,000
Aggregate	9	110
Cement	375	4,000
Steel	1,900	20,000

pared to HMA [11]. WMA can also be used during cold weather for paving and construction, while this weather condition is not ideal for HMA utilization [12]. However, road construction materials can be hazardous to the environment, as working with these materials releases CO_2 and requires high energy consumption (Table 3).

These large numbers indicate that more eco-friendly approaches to construction are necessary to reduce these environmental threats. Studies have shown that WMA manufacturing maintains a 30–40% reduction in CO_2 emissions, a 35% reduction in SO_2 emissions, and a 10–30% reduction in CO emissions when compared to HMA manufacturing [14] thus demonstrating a lower environmental footprint than HMA and making WMA a beneficial alternative for large-scale use.

In a 2017 study, three surfactant additives were used to lower the WMA manufacturing temperature. Additive 1 was a traditional surfactant possessing an amine composition, additive 2 was a nano-additive, and additive 3 was a vegetable additive. Results of that study showed that the nano-additive changed the alkalinity of the asphalt surface, and the vegetable additive possessed surface-active properties. Each of these additives was included with WMA at a 0.5% dosage by weight, and the mixtures were observed at temperatures of 145 and 120°C (Table 4).

Results of the 145°C mixtures showed a decrease in water sensitivity, while the 120°C mixtures did not show as promising results. However, additives 1 and 2 were more promising compared to additive 3 at 120°C because the two showed around 98% density values, which was the same density value for HMA. Overall, the nano-additive (additive 2) was found to be the most promising of the three because of its high resistance to water and plastic deformations. After further testing of additive 2, a dosage of 0.05% showed even higher resistance

TABLE 4. Experimental plan for a 2017 study involving three distinct additives mixed with WMA with HMA as a standard [11]

Temperature (˚C)	Mix
165	HMA
145	WMA + Additive 1
	WMA + Additive 2
	WMA + Additive 3
120	WMA + Additive 1
	WMA + Additive 2
	WMA + Additive 3

to plastic deformations and less dependence on manufacturing temperature.

ENHANCED OIL RECOVERY

Enhanced oil recovery (EOR) involves methods to improve the extraction of oil from matured reservoirs. CO_2 foams are helpful for EOR because they are more environmentally friendly than other extraction methods due to their propensity to reduce greenhouse gas emissions by trapping carbon [16], thus making them a more popular alternative in the gas and oil industries [15]. The structure of surfactants (a polar head and hydrocarbon tail) results in foam formation when a gas mixes with the surfactant [15]. Foam flooding is the process of placing foamed gas in an oil reservoir. As the gas dissolves in the oil, the oil's mobility increases for oil recovery. "Thief zones" are defined as areas of high permeability in oil reservoirs that are reaching residual oil saturation [17]. By adding surfactants, the CO_2 flow through these thief zones within the rock sections can be minimized, thereby resulting in increased oil recovery [16].

A principal purpose of using surfactants in EOR is to decrease the interfacial tension (IFT) between oil and water. Surfactants also can change the wettability, which is the change in oil-wetting state to water-wetting state when the surfactant is adsorbed on the rock surface and interacts with oil molecules [18, 19]. The surfactant can promote the water-wetting state, which enhances oil recovery as residual oil saturation of the reservoir decreases [20].

Table 5 shows how each type of surfactant works to reduce IFT in chemical EOR, with cationic and zwitterionic

TABLE 5. The mechanisms and advantages of the four types of surfactants in enhanced oil recovery [20]

Surfactant	Mechanism	Advantages	Surfactant	Mechanism	Advantages
Cationic	Reduces IFT and effects wettability	Best EOR surfactant in carbonate reservoirs	**Nonionic**	Reduces IFT	Useful for surfactant flooding
Anionic	Reduces IFT	Stable foam formation in sandstone reservoirs	**Zwitterionic**	Reduces IFT and effects wettability	High foaming performance; high thermo stability

surfactants also affecting wettability. It has previously been determined that cationic surfactants are not efficient for sandstone reservoirs and anionic surfactants are not efficient for carbonate reservoirs, which is consistent with Negin's conclusions.

FomaxII, FomaxVII, and UTP-Foam are foaming agents for surfactant alternating gas flooding (SAG) that have been studied previously. These foaming agents have CO_2-philic groups with branched tail groups that stabilize foams and the CO_2/brine interface during SAG flooding [21]. In a 2015 study where different alkalis functioned as additives for water alternating gas flooding (WAG), the studied anionic surfactants produced more stable foams compared to nonionic surfactants. Sodium hydroxide decreased foam stability while sodium carbonate and sodium borate increased foam stability [22]. A critical finding of studies like these was that anionic surfactants were

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more efficient in CO_2 EOR than nonionic surfactants. However, an ecological drawback to this discovery was that several anionic surfactants that can potentially be used in CO_2 EOR are potentially toxic to aquatic life [16].

These are just a few examples of the industries that have benefitted from the expanded use of surfactants, surfactant additives (mixing of different surfactants), and new enzymes. Suffice it to say that improvements and new applications continue to be made in numerous industries.

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Fatty acid production from coffee waste

Danyelle Andrade Mota, Laiza Canielas Krause, and Cleide Mara Faria Soares

Environmental problems are a global concern. A circular bioeconomy, following the biorefinery concept, is a promising approach toward resolving these global issues by converting renewable biological resources and waste streams into value-added products. How can this be applied to the coffee industry?

- **The valorization of coffee processing waste by producing products with high added value is a promising approach toward a circular bioeconomy.**
- **Crude coffee silverskin oil can be directly used in enzymatic hydrolysis catalyzed by non-specific lipases or *sn*-1,3 regioselective lipases.**
- **Converting coffee silverskin oil into free fatty acids or enriched polyunsaturated fatty acids in the form of acylglycerols with promoting properties can reduce the environmental impact and increase the profitability of the coffee industry.**

COFFEE SILVERSKIN AS A SOURCE OF CRUDE OIL

Coffee is of the most traded commodities in the world. According to the International Coffee Organization (ICO), the total exports over the first eight months of the coffee year between 2020 and 2021 amounted to 87.3 million 60-kg bulk bags, compared with 85.4 million 60-kg bulk bags during the same period in the coffee year between 2019 and 2020 [1]. Brazil is the largest producer and exporter of coffee in the world. However, the industrial production of coffee causes serious environmental concerns that are primarily related to the huge amount of residues generated as husks, pulp, low-quality or defective beans, spent coffee grounds, and coffee silverskin (CS) [2].

The development of sustainable and integrated bioprocesses, under the concept of the circular bioeconomy, is important in the use of waste. Coffee silverskin is the by-product of the coffee roasting process. It is rich in dietary fiber, protein, minerals, and antioxidants, offering potential applications in the cosmetic, food, and health industries, among others. About 1 ton of silverskin is produced from every 120 tons of roasted coffee. Despite its potential application in several fields, coffee silverskin has recently gained attention as a source of residual oil for the production of free fatty acids (FFA) [3].

Its total lipid content is usually between 2.4–3.8% with bioactive molecules, such as phytosterols, that can reduce the low-density lipoprotein (LDL) cholesterol concentration. The variation found in the lipid profile of CS can be attributed to the different blends of coffee varieties, their geographical origin, and the processing method. The triacylglycerols (TAG) are the major components of CS oil (≅48%), followed by FFA (≅21%), esterified sterols (≅15%), free sterols (≅13%), and diacylglycerols (≅4%).

CS oil is highest in saturated fatty acids (SFA: 55.5–86%), followed by polyunsaturated (PUFA: 10–36.1%), and monounsaturated (MUFA: 5–10%) fatty acids. The main fatty acid is linoleic acid (C18:2: 24.4–36.1%), followed by palmitic acid (C16:0: 22–34.4%), behenic acid (C22:0: 15.4–11.5%), and arachidic acid (C20:0: 13.5–10.8%) [2,3].

The free fatty acids produced by hydrolysis are promising due to their various applications in the cosmetic and food industries. It is worth mentioning that lipase-catalysed selective hydrolysis is a promising

method to enrich PUFA in the form of acylglycerols, but the main method of producing them is through the hydrolysis of vegetable oils and fat, such as coffee silverskin oil.

ENZYMATIC HYDROLYSIS

The hydrolysis of oils and fats aims to produce free fatty acids, partial acylglycerols, and glycerol. Acids or alkalis are used as catalysts to hydrolyse oils, but they present environmental and disposal concerns. To solve these problems, enzyme-catalyzed oil hydrolysis is a green alternative, as enzymes can be produced from renewable resources and are biodegradable. Lipases can be used free or immobilized on solid supports to be reused in the reactions [4].

Enzymes can be highly selective catalysts that interact with a single substrate. Lipase reacts with TAG through a hydrolysis reaction, leading to numerous products: diacylglycerols, monoacylglycerols, FFA, and glycerol (G) [4]. Enzymatic hydrolysis can be affected by the presence of water and oil as a reactant, forming an emulsion (oil-in-water). Lipase-mediated hydrolysis reactions of oils and fats from different sources have been carried out in the presence of emulsifiers to increase the oil/water interfacial area which, in turn, increases the catalytic activity of the enzyme. The nature of the oil (long or medium fatty acid chains) and the quality of the emulsification have an influence on lipolysis activity, with triacylglycerol breaking down into fatty acid and glycerol molecules.

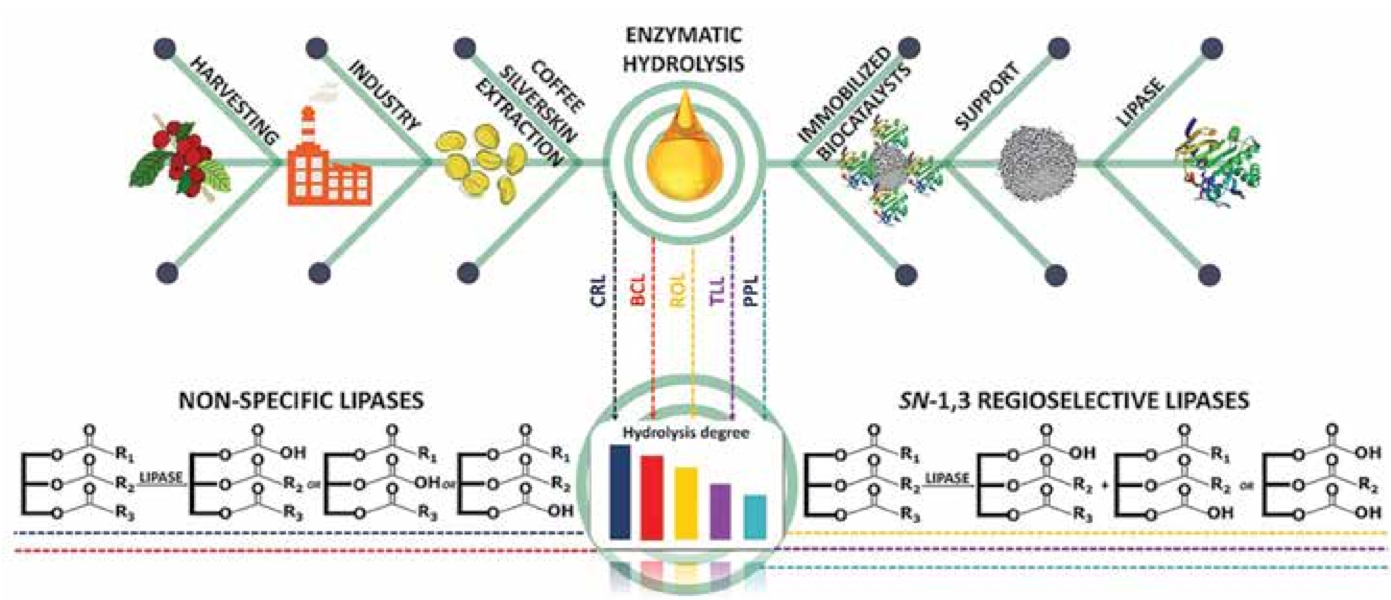

FIG. 1. Schematic integrated process for the valorization of coffee silverskin oil by enzymatic hydrolysis with non-specific lipases and sn-1,3 regioselective lipases

With respect to selectivity, lipase can be divided into three main groups. Non-specific lipases catalyze the complete hydrolysis of triacylglycerols into fatty acids and glycerol in a random way, producing mono- and diacylglycerols as intermediate products. The second group includes enzymes with a specific or selective type of fatty acid where TAG hydrolysis depends on the nature of the alkyl chains (mainly chain length and number of double bonds). The last group includes regiospecific enzymes (*sn*-1,3 regioselective) that hydrolyse triacylglycerols at the C1 and C3 glycerol bonds, producing fatty acids, 2-monoacylglycerols, and 1.2- or 2.3-diacylglycerols [4]. Figure 1 (page 25) shows an integrated process for enzymatic hydrolysis of coffee silverskin oil using non-specific and *sn*-1, 3 regioselective lipases.

Coffee silverskin oil was used as the feedstock for fatty acid production by enzymatic hydrolysis using five commercial lipases immobilized on silica as biocatalysts: *Burkholderia cepacia* lipase (BCL), *Candida rugosa* lipase (CRL), *Thermomyces lanuginosus* lipase (TLL), *Rhizopus oryzae* lipase (ROL), and porcine pancreas lipase (PPL). Figure 2 shows that CRL had the highest hydrolytic activity (≅1144 $U.g^{-1}$) in the hydrolysis reaction of CS oil, followed by BCL (≅787 $U.g^{-1}$), ROL (≅741 $U.g^{-1}$), TLL (≅499 $U.g^{-1}$), and PPL (≅443 $U.g^{-1}$). Lipases showed the same performance in evaluating the degree of hydrolysis, since the biocatalysts CRL, BCL, ROL, TLL and PPL presented approximately 52%, 38%, 29%, 24%, and 23%, respectively [3].

CRL and BCL are non-specific lipases with the ability to hydrolyze fatty acids at any position in the triacylglycerol and were therefore more efficient. While the ROL, TLL, and PPL are *sn*-1,3 regiospecific, these lipases showed a lower degree of hydrolysis. To ensure the specificity in catalytic reactions with oils, we have used an enzymatic approach using lipases. The use of *sn*-1,3 regioselective lipases is important for the synthesis of FFA since the original position *sn*-2 is preserved.

APPLICATION OF ENZYMATIC HYDROLYSIS PRODUCTS

Enzymatic hydrolysis of lipids makes it possible to obtain interesting products with improved functionality. The conversion of fats and oils into products with high added value, such as monoacylglycerols, free fatty acids, and derivatives has been of great commercial interest.

Among the hydrolysis products, free fatty acids have several applications, such as in soap, surface-active agents, lubricants, plastics, paints, coatings, pharmaceuticals, food, and personal care products, among others. They can also be used as substrates for production of biodiesel from esterification.

Enzymatic hydrolysis can also be an attractive approach to obtain PUFA concentrates directly as natural glycerides. Linoleic acid (LA) is an essential polyunsaturated fatty acid (PUFA) that cannot be synthesized by humans and must be supplied by food. Coffee silverskin oil is rich in LA and constitutes about 32% of the total fatty acid content. Food adequate levels with essential PUFA have been associated with health promotion [3].

A study with molecular docking showed that different lipases (CRL, BCL, ROL, TLL, and PPL) have a higher affinity and will preferentially hydrolyse SFA and MUFA, such as palmitic and oleic acids, over PUFA. On the other hand, ω-6 PUFA showed low binding energy and no interaction of fatty acids with amino acids. However, it is noteworthy that SFA are mainly bound to *sn*-1 and *sn*-3 positions of the glycerol structure, making them more susceptible to hydrolysis by lipases, principally *sn*-1,3 regioselective [3].

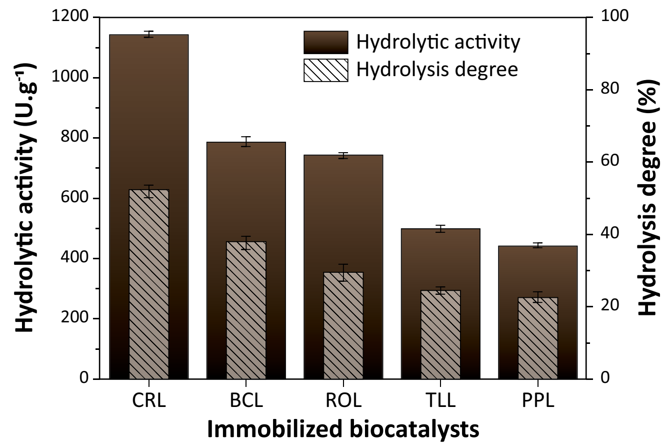

FIG. 2. Hydrolytic activity and hydrolysis: degree of different lipases immobilized on silica for hydrolysis in crude silverskin oil

Overall, these results show crude coffee silverskin oil can be successfully used to produce FFA or enrich PUFA in the form of acylglycerols. Converting coffee by-products into value-added products could play an important role in reducing the environmental impact and increase the profitability of the coffee industry.

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The authors acknowledge the financial support from Fundação de Apoio à Pesquisa e à Inovação Tecnológica do Estado de Sergipe (FAPITEC) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). D. A. M. acknowledges the PhD grant (88882.176405/2018-01) and the financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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The latest research on coconut oil and human health

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

Rebecca Guenard

After five years of trendiness, in certain circles coconut oil still enjoys the acclaimed title of "superfood." Celebrities and health food aficionados encourage consuming the oil at each meal to improve everything from weight-loss to mental cognition. But could their enthusiasm for the product be based on poorly designed or misinterpreted research showing that the oil alleviates inflammation, improves glucose homeostasis, and reduces body fat?

Prior to its recent popularity, the tropical oil composed of nearly 90% saturated fat was more often labeled a guilty pleasure than a superfood. Coconut oil has a variety of uses: baking, frying, as a spread on toast, or in coffee as creamer. It is undeniably delicious for popping popcorn (at least according to this author). However, the latest meta-analysis has researchers, once again, pleading with consumers to stop considering coconut oil a healthy food.

Last year, researchers from the National University of Singapore published a meta-analysis of clinical trials comparing the effects of coconut oil consumption with other fats. They evaluated the findings from 17 articles on clinical trials that measured LDL cholesterol, HDL cholesterol, total cholesterol, and triglycerides. In addition, the trials assessed biological markers for glycemia, inflammation, and adiposity (https://doi.org/10.1161/CIRCULATIONAHA.119.043052).

The paper's authors defined specific criteria for including studies in their analysis. They focused only on controlled clinical trials performed on human adults with a duration exceeding two weeks (long enough to let blood lipid concentrations stabilize). They eliminated animal and cell studies, as well as studies involving fresh coconut, coconut milk, lauric acid, or mixed oils. The trials that met the researchers' criteria represent only about 2% of papers published on the health benefits of coconut oil. The resulting analysis compared the health outcomes of 730 individuals.

As part of the discussion of their findings, the author's write: "In our meta-analysis of clinical trials, coconut oil consumption significantly increased total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations compared with non-tropical vegetable oils. Coconut oil also significantly increased total cholesterol and LDL-cholesterol concentrations compared with palm oil (another tropical oil with ≈50% saturated fat vs ≈90% saturated fat in coconut oil). Coconut oil consumption did not significantly change C-reactive protein, fasting glucose concentrations, or measures of body fatness compared with non-tropical vegetable oils."

The primary argument for categorizing coconut oil as healthy is the presence of lauric acid. Based on analysis by different research groups, the fatty acid composition of coconut oil is about half lauric acid, mostly in the form of triglycerides. Some researchers suggest that since it is composed of only 12 carbons, lauric acid should be categorized as a medium-chain fatty acid.

Since they are more water soluble, medium-chain fatty acids (MCFA) bypass the heart and are transported directly to the liver via the portal venous system. In the liver, they are metabolized for energy, resulting in the formation of ketones that are dispersed to vital organs like the brain and the heart. These organs contain enzymes that turn the ketones into a beneficial energy source.

Some researchers contend there are no consistent clinical trial results indicating that lauric acid deserves an MCFA label. Frank M. Sacks, a professor and specialist in clinical hyperlipidemia at Harvard T.H. Chan School of Public Health in Boston, Massachusetts, USA, wrote an editorial in the journal *Circulation* to accompany the meta-analysis publication (https://doi.org/10.1161/CIRCULATIONAHA.119.044687). Sacks argues that lauric acid "acts biologically like a long-chain fatty acid absorbed by packaging into chylomicrons."

In other words, lauric acid is not transported directly to the liver like MCFA, according to Sacks. Instead, its behavior in the body indicates it is too big to be water-soluble enough to transfer into the portal venous system after digestion. Coconut oil significantly increased LDL-cholesterol, according to the meta-analysis, because it must be transferred through the bloodstream with the help of chylomicrons, just like long-chain fatty acids.

We all know the consequences of increased LDL-cholesterol in the bloodstream. These bigger, fatty acid-transport particles are more likely to get stuck in the arteries. A build-up of these deposits restricts blood flow and leads to cardiovascular disease.

The Singapore research team estimates that if consumers replaced their non-tropical vegetable oil with coconut oil, the associated increase in LDL-cholesterol could "translate to a 6% increase in risk of major vascular events and a 5.4% increase in the risk of coronary heart disease mortality." In addition, they support other trials and epidemiological studies that encourage consumers to replace any saturated fats with polyunsaturated fats, a switch that has shown to reduce cardiovascular disease risk by as much as a 13%.

As noted, the researchers did conclude that eating coconut oil raises HDL-cholesterol. However, they argue that the association between higher HDL levels and lower risk of cardiovascular disease should be questioned given recent findings by other research groups on signal nucleotide dependent outcomes. Then there are pharmacological studies showing that treatments to increase HDL-cholesterol have no effect on heart health. "It is unknown which, if any, foods or nutrients that raise HDL-cholesterol do so in a way that reduces atherosclerosis and coronary events," writes Sacks. Therefore, the team does not see this positive result as indicating that coconut oil is a beneficial food.

Finally, the team dismisses any claim that indigenous cultures that consume more coconuts have lower incidents of heart disease. Such correlations fail to consider that fish provide the main source of protein for these populations, and their diet includes large amounts of fruits and vegetables.

Sacks says that comparisons like those used in the Singapore team's meta-analysis could eventually be used to form a hierarchy of healthy cooking oils that can inform consumers' dietary choices. Until then, if you like the taste of food cooked in coconut oil, enjoy it sparingly. Despite what the influencers say, there is no clinical evidence to suggest that eating coconut oil will make you thinner or reduce inflammation.

Information

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Excerpts from an overview on most common and emerging fraud in the olive oil sector

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

Enrico Casadei, Enrico Valli, Alessandra Bendini, and Tullia Gallina Toschi

Within the H2020 OLEUM project, a review of most common infringements (fraud or non-compliance) was conducted with the support of two questionnaires specifically addressed to all the stakeholders and regulatory bodies in the olive oil sector. This review was undertaken with great urgency after the European Commission's 2019 annual report on food fraud identified fats and oils as the most notorious category in the system, and olive oil was found to be the most reported product [1].

Europe is the world's largest olive oil producer, exporter, and consumer, having produced about 64% of the olive oil produced worldwide during the 2019/20 crop year. [2]. Nonetheless, growing competitiveness, expanding markets, and different levels of regulation are encouraging fraud. Furthermore, the high price of extra virgin olive oils provides a great temptation to adulterate them. The EU framework for conformity checks has effectively contributed to and is currently improving the quality of the products on the market. Although these checks reduce the prevalence of fraudulent practices, many studies have identified disparities and problems with the current conformity check system. Among these, the work conducted by Areté Research (2020) [3] emphasizes that the most common infringements are the marketing of virgin olive oil as extra virgin olive oil, or marketing blends of olive oil with other vegetable oils (sunflower, corn, palm, rapeseed, etc.) as pure olive oil.

These types of fraud are also highlighted by the results of the quality controls and anti-fraud inspections carried out between 2011 and 2014 by the Government of Catalonia (in Spain), discussed in the article by Cugat and Biel (2016) [4]. This work reported cases in which oils labelled with a protected designation of origin (PDO) were produced from olives harvested in areas outside the PDO, as well as cases in which oils had a geographical origin on the label that did not correspond to the real one. Mislabeling, dilutions, and unauthorized enhancements specifically related to the composition of the oils detectable through quality and purity parameters—as well as false declarations on the labels (or labels made in a way that does not comply with the legislation)—are among the other types of fraud pointed out by Cugat and Biel (2016).

To ensure the health and protection of consumers, the Joint Research Center of the European Commission (JRC), as the Commission's internal scientific service, publishes a monthly summary with press and media articles on food fraud, with the aim of informing all the stakeholders and giving them the opportunity to act on these irregularities [5]. In particular, the foods which are most often subjected to fraudulent activities are those specified by the European Parliament resolution of January 14, 2014, which addresses the food crisis, fraud in the food chain, and the control thereof. Specifically, of the 32 reports registered from September 2016 to December 2019 with JRC in a monthly summary of articles on food fraud and adulteration concerning olive oils, 20 occurred in Europe. Sixteen concerned substitution; 11, mislabeling; 6, dilution; 5, intentional distribution of contaminated products/counterfeiting; 4, untrue origin; and 1, theft. The sum of the incidents of fraud appears to be higher than the number of reports, since a single case often involves two different types of issues. Moreover, almost all types of fraud in the olive oil sector (e.g. dilution, substitution, untrue origin) can also be considered as cases of mislabeling if the mislabeling is intentional.

Currently there are numerous databases that collect data and monitor problems related to the safety and authenticity of food products. The Rapid Alert System for Food and Feed (RASFF) [6] with its annual reports is a key tool to ensure the rapid flow of information to enable swift action when risks to public health are detected in the food chain. RASFF allows information to be shared efficiently between its members and provides a round-the-clock service to ensure that urgent notifications are sent, received, and responded to collectively and efficiently. Other examples are HorizonScan [7], a proprietary tool owned by Fera, and The Food Authenticity Research Network Hub (FARNHub [8]), which is a web-based platform developed within the EU H2020 AUTHENT-NET Project.

To gain a general and up-to-date picture of the emerging trends in olive oil fraud and possible countermeasures coming from all the key players in the olive oil sector, an online survey for stakeholders (e.g., exporters, importers, control laboratories) was carried out within the H2020 OLEUM project. This effort included an additional questionnaire specifically addressed to the EU Food Fraud Network (FNN) National Contact Points. The purpose of this latter survey, developed under the guidance of the European Commission DG AGRI (Unit G.4 – Arable crops and OO) and DG SANTE (Unit G.5 - Alerts, Traceability and Committees), was to gain a clear understanding of how the authorities are trying to combat and face this problem. From the results of the first online survey, which was completed by 111 respondents, it was possible to notice that of seemingly high priority to industry were emerging issues with regards to fraud arising from the addition of deodorized oil and from mixing with oil obtained by a second centrifugation of the olive paste (remolido). Regarding the second questionnaire, an analysis of the 17 responses received (out of 31 questionnaires sent), showed that the most frequent fraudulent practice is mixing with lower quality olive oil, and that misrepresentation of EU and non-EU oils, as well as mixing non-EU with EU oils are cases which need more control activities related to false designations of origin.

In conclusion, OLEUM underscored how the EU regulation of olive oil is both extensive and concrete, and that the level of attention and high requests for conformity checks have improved the quality of the olive oil on the market in the last 30 years. Nevertheless, to better guarantee olive oil quality and authenticity, there is still the need to ameliorate conformity checks, reduce cases of disagreement about classifications, include robust and validated methods into the regulation, and make market screening tools widely available that guarantee quality during production, in a concerted effort to stay one step ahead of fraudsters.

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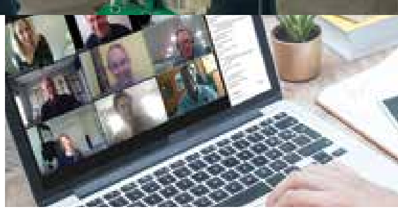
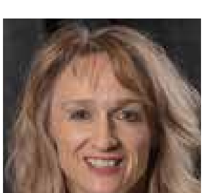
[8] FARNHub http://farnhub.authent.cra.wallonie.be/.





Meet Michael Eskin

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

Michael Eskin poses in the lab kitchen at the University of Manitoba.

Fast facts

Name	Michael N. A. Eskin
Joined AOCS	1980
Education	Ph.D. from University of Birmingham, UK, in physiological chemistry
Job title	Professor, Department of Food and Human Nutritional Sciences, Faculty of Agricultural and Food Sciences
Employer	University of Manitoba, Winnipeg, Manitoba, Canada
Current AOCS involvement	Associate editor (education), AOCS Lipid Library; several award-selection committees

PROFESSIONAL

What's a typical day like for you?
My morning starts around 4:30 a.m. when I have a coffee, read the paper, and do the crossword puzzle. I am always working on chapters or a book or organizing the next one. I am also usually working on papers, as I collaborate with several colleagues in my department.

My favorite part of my job is…
…interacting with students and colleagues. Teaching online to 250 students does not allow for any real interactions, and that is something I miss.

Flash back to when you were 10 years old. What did you want to be when you grew up?
I don't think I ever dreamed of becoming a professor. My three sisters and I were the first generation in my family to attend university, which opened new and unknown opportunities for us.

Why did you decide to do the work you are doing now?
I am curious and never short of ideas. I am interested in a few areas, including bioactive phenols in canola and mustard as well as fetal alcohol spectrum disorder, and I was the guest editor for a special issue of *Antibiotics* on botanicals and antibiotic-resistant organisms.

Is there an achievement or contribution you are most proud of? Why?
I was truly fortunate to work on the development of canola oil when I first arrived in Canada in 1968. For this and related work, my colleagues nominated me for the Order of Canada, which I received in 2016, and the Order of Manitoba, which was awarded this year. Receiving them—as well as the Supelco AOCS Research and Stephen S. Chang Awards—ranks among the proudest moments in my life.

What event, person, or life experience has had the most influence on the direction of your life?
I have had wonderful mentors and collaborators, mostly remarkable women. The first was my late mother, Ethel Eskin, who made sure I could sew and iron before I left home. The second was my late Ph.D. supervisor, Dr. Sybil James. The third was my late colleague, Professor Marion Vaisey-Genser, with whom I worked on canola oil for more than 25 years. Others include Rajannah Bird, Miyoung Suh, and the late Usha Thiyam-Hollander. The most remarkable woman in my life, however, is my wife Nella, whose support and patience were key to whatever successes I have enjoyed over the past half-century.

PERSONAL

How do you relax after a hard day of work?
I enjoy playing Super Scrabble, but beating my wife is a real challenge. In the summer we are dedicated gardeners. I also enjoy writing raps and other ditties that make learning science fun. This year I composed a song to celebrate the discovery of insulin in Canada 100 years ago, which was presented at the Virtual 2021 AOCS Annual Meeting & Expo.

What skill would you like to master?
I would like to play the banjo. My sons bought me one, but I have yet to really work on it.

PATENTS

Compositions and methods for lowering levels of high-sensitivity C-reactive protein (hs-CRP) in a subject

Manku, M., *et al.*, Amarin Pharmaceuticals Ireland Limited, US10973796, April 13, 2021

The present disclosure provides methods for treating and/or preventing cardiovascular-related disease and, in particular, a method of blood lipid therapy comprising administering to a subject in need thereof a pharmaceutical composition comprising eicosapentaenoic acid or a derivative thereof. In some embodiments, the method comprises lowering high sensitivity CRP (hs-CRP) levels in a subject including, for example, a subject with a HbA.sub.1c value of about 5.00%–8.50% or at least about 6.8%.

Powder lubricant based on fatty acids and fatty acid glycerides and use thereof

Odink; G. J., *et al.*, Henkel AG & Co. KGaA, US10975324, April 13, 2021

A dry lubricant composition in powder form is provided based on a mixture of alkali metal salts of fatty acids and fatty acid glycerides which is useful in the production of aluminium cans in a deep drawing process, wherein the formed aluminium cans are immediately further processed to yield thin inorganic and/or organic protective coatings. The invention also encompasses the use of the lubricating powder for cold forming of aluminium as well as a process for the deep drawing of aluminium cans.

Methods for making free fatty acids and fatty acid derivatives from mixed lipid feedstocks or soapstocks

Long, R.A., *et al.*, Inventure Renewables, Inc., US10975328, April 13, 2021

Provided are methods and systems for treating a soapstock. Provided are systems and methods for treating a soapstock to generate free fatty acids and/or fatty acid derivatives, and for realizing the full fatty acid yield of a soapstock by first converting substantially all of the saponifiable material in a soapstock to fatty acids and acidulating the soaps to generate free fatty acids and/or fatty acid derivatives, wherein the soapstock comprises soaps and saponifiable lipids, and the generating of free fatty acids is achieved. Provided are systems and methods for realizing the full fatty acid yield of a soapstock by first converting substantially all of the saponifiable material in a soapstock to salts of fatty acids and acidulating the soaps to generate free fatty acids and/or fatty acid derivatives, wherein the soapstock comprises soaps and saponifiable lipids, and the generating of free fatty acids is achieved.

Lipid compositions containing bioactive fatty acids

Remmereit, J., *et al.*, Sciadonics, Inc., US10980763, April 20, 2021

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

Exogenous ketone supplements for reducing anxiety-related behavior

D'Agostino, C.A., *et al.*, University of South Florida, US10980764, April 20, 2021

Methods of treating anxiety disorders or reducing anxiety-related behaviors. The methods include administering a therapeutically effective amount of ketone supplementation, such as butanediol, ketone esters (e.g., 1,3-butanediol-acetoacetate diester) and/or ketone salts (e.g., beta-hydroxybutyrate-mineral salt), chronically, sub-chronically, or acutely, with or without admixture with a medium-chain triglyceride or in combination. It was determined herein that ketone supplementation reduced anxiety in rats on elevated plus maze as measured by less entries to closed arms, more time spent in open arms, more distance travelled in open arms, and delayed latency to entrance to closed arms, when compared to control. Along with reducing anxiety-related behavior, the chronic, sub-chronic, and acute ketone supplements also caused significant elevation of blood beta-HB levels and changed blood glucose levels.

Method of preparing combustible oil

Miyata, K., *et al.*, Fusion Group Holdings Co., Ltd., US10982160, April 20, 2021

Provided is a method of preparing a combustible oil, the method comprising adding and mixing: a petroleum-based combustible oil; a water having an oxidation-reduction potential of -300 mV or lower, a pH of 9.0 or higher, and a dissolved hydrogen concentration of 0.8 ppm or higher; a fatty oil; and an activated carbon to obtain a mixture.

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

Stay informed. Gain insights. Get answers.

November 4, 11 a.m. CDT (Chicago, USA; UTC-5)

Accelerated Procedure for the Determination of Lipid Oxidation Stability

Corey Letizio, Manager, VELP Scientifica, USA, and Dr. Antonella Cavazza, Lead Researcher, University of Parma, Italy

For food and feed companies and laboratories specializing in the food industry, the prediction of fat stability and oxidation of raw materials and finished products is a matter of urgency. Learn about accelerated testing, using internationally recognized procedures, that can quickly reveal oxidation processes that normally take weeks or months and provide fast, accurate, and reliable results.

November 5, 2 p.m. CDT (Chicago, USA; UTC-5)

Enzyme Selection and Use for Optimized Sweetness and Sugar Levels in Oat Beverages

James D. Chapa, Scientist, Novozymes North America, Inc., USA

Oat beverage production typically includes liquefaction to increase the solubility of the oat starch and saccharification to release glucose and maltose and provide sweetness. Learn about a study that compares protocols highlighting carbohydrate profile, viscosity, dissolved solids and the results of sensory profiling.

November 10, from 12:30 p.m. CDT (Chicago, USA; UTC-5)

Special Symposium: Impact and Response to Food Fraud in Canada

Public attention has been captured by recent reports on food fraud concerning food oil, honey, juice, syrup, and meat products. Economically motivated adulteration of food is of increasing concern. A growing catalog of detection methods is used to monitor and enforce regulations and quality standards to protect consumers and producers from health and economic impacts. An expert panel will highlight current issues and practices of food fraud defense.

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Tips on using academic translation services effectively

Anselmo Martyres

Are you interested in submitting an article to one of AOCS's three journals but don't have time to wrestle with the fine points of written English? Non-native English-speaking researchers who do not have the luxury of spending time writing in English and grappling with the grammatical rules and nuances of the language can benefit considerably from English language translation services. The researchers draft their manuscripts in a language they are familiar with and use a translation service to convert their draft into an English-language document. English second language (ESL) researchers looking to get published have the option of using package services that offer both translation and editing. With this option, authors can choose to get the translated paper checked for language and formatted according to the requirements of the target journal, and in the process ensure that the final draft meets the standard of publishing.

While a lot depends on the skill and experience of the translator, input from the author of the paper plays a significant role in ensuring that the product is closer to the desired output. In this article, I will discuss what steps the author can take to ensure that the final draft is publication ready.

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PROVIDE A GLOSSARY/TERM LIST

While translating some technical terms is straightforward, some translations may vary with the translator and the clarity offered by the source text. These differences may be nuanced ("expenditure incurred on drugs" vs. "drug expenditure") or drastic ("measurement of corneal refractive power" vs. "keratometry"). To ensure that technical terms are translated as per your liking or are consistent with the parlance used in your industry, authors are advised to provide the translator with a glossary that contains technical terms in the source language along with the preferred translation. The translator will then make sure that these terms are used as instructed. However, if the translator or editor feels that the author-recommended translation is unsuitable or inaccurate, he/she may provide a more suitable alternative. It is in the author's best interest to review these suggestions.

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PROVIDE CLEAR INSTRUCTIONS/ REFERENCE FILES

It helps to know if certain parts of the source text need to be excluded from the translation or approached differently. For example, PowerPoint documents may contain presenter notes that may have to be translated; or you may require translation of a few stray sentences in an otherwise English paper. In this case, let the translator know if he needs to refer to the English text to maintain consistency. While this may be a reasonable expectation from a translator, remember that it puts a premium on the translator's time and the timeline for completing the project may need to be extended.

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Anselmo Martyres manages training operations of the editorial team and oversees quality and training in the translation team at Editage. As the quality manager of the translation team, Anselmo has been working with experienced translators to acquire an in-depth understanding of the academic translation industry.

EXTRACTS & DISTILLATES

The full version of all AOCS journal articles are available online to members at www.aocs.org/journal. This column builds on that member benefit by primarily highlighting articles from other journals.

ANA Analytical
BIO Biotechnology
EAT Edible Applications
LOQ Lipid Oxidation and Quality
H&N Health and Nutrition
IOP Industrial Oil Products
PRO Processing
PCP Protein and Co-Products
S&D Surfactants and Detergents

Review Articles

ANA IOP The employment of analytical techniques and chemometrics for authentication of fish oils: a review

Rohman, A., *et al.*, *Food Control 124*: 107864, 2021, https://doi.org/10.1016/j.foodcont.2021.107864.

The objective of this review was to provide a scientific view on some analytical methods based on molecular spectroscopy and chromatography combined with chemometrics which are widely used for authentication of fish oils. It highlights various analytical methods, including molecular spectroscopy and chromatography techniques, which have been deeply investigated with respect to the quality of information provided for fish oil authentication. Combined with multivariate analysis or chemometrics, Fourier transform infrared (FTIR) spectroscopy and other molecular spectroscopies offered suitable methods for rapid screening and detection of adulteration. Further, chromatographic methods hyphenated with mass detector (GC x GC-MS and LCMS/MS) are reliable methods for confirming the specific adulteration practice by identifying specific components present in authentic or in adulterated fish oils. This review assisted regulators and producers to choose the most suitable methods for their responsibilities.

EAT H&N PRO Microencapsulation of healthier oils: an efficient strategy to improve the lipid profile of meat products

TeresinhaHeck, R., *et al.*, *Curr. Opin. Food Sci. 40*: 6–12, 2021, https://doi.org/10.1016/j.cofs.2020.04.010.

Meat products have high SFAs levels and a high n-6/n-3 ratio, with negative effects on human health. The replacement of animal fat by oils rich in n-3 PUFAs is an efficient strategy to improve the lipid profile of meat products. However, the technological properties, oxidative stability, and sensory quality of meat products can be impaired by this lipid reformulation. Recently, microencapsulation has stood out as a promising tool to improve the oxidative stability of oils, allowing their application in meat products. Considering the growing interest in this topic among the population, this short review aims to highlight the recent studies on the microencapsulation of healthier oils as a strategy to improve the lipid profile of meat products.

H&N LOQ A critical review on the health benefits of fish consumption and its bioactive constituents

Chen, J., *et al.*, *Food Chem. 369*: 130874, 2022, https://doi.org/10.1016/j.foodchem.2021.130874.

Diverse nutrients in fish make it an important nutrient source proven to possess anti-oxidative, anti-inflammatory, wound-healing, neuroprotective, cardioprotective, hepatoprotective, and other health-promoting properties. Fish proteins, such as immunoglobins, act as defense agents against viral and bacterial infections and prevent protein-calorie malnutrition. Fish oil constituents, such as polyunsaturated fatty acids (PUFAs), regulate signaling pathways, such as the nuclear factor kappa B pathway, toll-like receptor pathways, transforming growth factor-β (TGF-β) pathway, and peroxisome proliferators activated receptor (PPAR) pathways. Literature about the health benefits of fish consumption accumulated from PubMed, Google Scholar, Scopus—and the mechanistic action of health benefits—are summarized in this review.

IOP PRO BIO Biodiesel production with enzymatic technology: progress and perspectives

Pasha, M.K., *et al.*, *Biofpr 15*: 1526–1548, 2021, https://doi.org/10.1002/bbb.2236.

Biodiesel, as a renewable fuel, has long been recognized as one of the solutions to meet energy demand in a climate-constrained world. Although several routes have been devised for biodiesel production, the enzymatic route has attracted substantial research interest. Here we discuss enzymatic esterification/transesterification technology and its contribution to the green synthesis of biodiesel. This review provides a comprehensive assessment of the fundamentals of enzymatic reaction, such as the impact of different reaction components (lipase, acyl acceptor, substrate, and reaction media) on the process parameters, along with recent developments in improving enzymatic biodiesel production, such as different lipases, combinations of lipases, and two-step methods. Kinetics and the mechanism of enzymatic reaction when lipase is used as a catalyst are also explained in greater detail. Finally, the opportunities and challenges for the development of potentially sustainable and eco-friendly enzymatic biodiesel technology are discussed.

PRO BIO PCP Sustainable production of polyhydroxybutyrate from autotrophs using CO_2 as feedstock: challenges and opportunities

Sirohi, R., *et al.*, *Bioresour. Technol. 341*: 125751, 2021, https://doi.org/10.1016/j.biortech.2021.125751.

Due to industrialization and rapid increase in world population, global energy consumption has increased dramatically. Consequently, there is increased consumption of fossil fuels, leading to a rapid increase in CO_2 concentration in the atmosphere. This accumulated CO_2 can be efficiently used by autotrophs as a carbon source to produce chemicals and biopolymers. There has been increasing attention on the production of the biopolymer polyhydroxybutyrate (PHB), with focus on reducing production cost. Cheaper renewable feedstocks and molecular tools, including metabolic and genetic engineering, have been explored to improve microbial strains along with process engineering aspects for scale-up of PHB production. This review discusses the recent progress on utilization of CO_2 as feedstock, especially by engineered autotrophs, for sustainable production of PHB. Innovations in cultivation technology and process monitoring are also discussed, along with the underlying mechanisms for CO_2 to biopolymer conversion.

Original Articles

ANA IOP Differential scanning calorimetry as a method for the control of vegetable oils

Rudakova, O.B., *et al.*, *J. Anal. Chem. 76*: 258–266, 2021, https://doi.org/10.1134/S1061934821020118.

Differential scanning calorimetry (DSC) was used to study the thermophysical properties of amaranth, corn, flax, sunflower, rapeseed, milk thistle, camelina, and pumpkin seed oils, liquid at room temperature. The characteristic thermal effects of these oils (temperatures of the maxima of endothermic peaks and their areas in the DSC thermograms) were determined. Endothermic peaks of different intensities on the melting curves of liquid vegetable oils

in the ranges from −40 to −15°C, from −25 to −8°C, from −19 to +6°C, and from −10 to +4°C as identification factors are discussed. The coordinates of the maxima of these peaks on the abscissa axis (Ti) and their areas (Si) significantly correlate with the concentrations of basic fatty acids and triacylglycerols (Wi, %), determined by reversed-phase HPLC. We demonstrated that the authenticity of vegetable oils could be effectively controlled by DSC.

ANA H&N A comparative study of supercritical fluid and ethanol extracts of cannabis inflorescences: chemical profile and biological activity

Fernández, S., *et al.*, *J. Supercrit. Fluids 179*: 105385, 2021, https://doi.org/10.1016/j.supflu.2021.105385.

We compared chemical profiles and *in vitro* biological activity of extracts obtained through maceration with ethanol or supercritical fluid extraction with CO_2 ($scCO_2$), from two different cannabis inflorescences chemotypes, one with high total tetrahydrocannabinol (THC) and the other with high total cannabidiol (CBD) content. $scCO_2$ was performed to obtain extracts with highest total THC and CBD recovery using ethanol as a co-solvent. In addition, a short maceration with ethanol was performed for both chemotypes. Cannabinoid and terpene composition of the extracts were determined using chromatographic and spectroscopic methods, and their cytotoxicity and cell proliferation effects were evaluated *in vitro* using HT-29 colon adenocarcinoma cell line. *In vitro* biological activity confirmed that cannabis extracts from both chemotypes have anti-proliferative activity, but the $scCO_2$ extract from high total THC chemotype was more cytotoxic. These results underscore the importance of chemotype and extraction method selection in the preparation of cannabis extracts for different therapeutic applications.

EAT Foams of vegetable oils containing long-chain triglycerides

Liu, Y. and B.P. Binks, *J. Colloid Interface Sci. 583*: 522–534, 2021, https://doi.org/10.1016/j.jcis.2020.09.043.

The cooling of vegetable oil results in the formation of crystals of certain triglyceride chain lengths and composition dispersed in liquid oil of other chain lengths and composition. Do such oleogels allow the formation of oil foams stabilized by adsorbed crystals? Using two vegetable oils, the temperatures for crystal formation were determined. Crystal dispersions were characterized using rheology and optical microscopy. Oleogels were aerated using a double beater and the effects of temperature and aeration time were investigated. The stability and microstructure of the oil foams were studied visually and using microscopy. A stable oil foam was progressively destabilized on heating. Upon cooling/warming vegetable oils, crystals of high melting triglyceride formed in a low-melting liquid oil—an oleogel. Such oleogels can be whipped to fabricate oil foams stabilized by fat crystals. Optimum foaming yielded an over-run of ~ 40% for peanut oil and ~ 110% for olive oil. The result is oil foams which do not exhibit drainage, coarsening, or coalescence. We showed that high-melting triglyceride crystals possess a higher fraction of saturated fatty acids than the original oil. Ultra-stable oil foams can be rendered unstable by heating upon approaching the melting point of the crystals.

EAT LOQ Effect of triacylglycerol structure on the antioxidant activity of γ-oryzanol

Toorani, M.R. and G. Mohammad-Taghi, *Food Chem. 370*: 130974, 2022, https://doi.org/10.1016/j.foodchem.2021.130974.

In this study, the triacylglycerol structure of vegetable oils was removed and the effects on the antioxidant efficiency of γ-oryzanol were observed. A sigmoidal model was used to calculate kinetic parameters relevant to the initiation and propagation phases during the peroxidation of soybean, corn, sesame, and olive oils, as well as their fatty acid methyl esters (FAME). Removing the triacylglycerol structure caused an increase in the antioxidant activity of γ-oryzanol (26.49%) by affecting both inhibitory mechanisms, i.e., hydrogen-donating (7.80%) and electron-transfer (14.72%). Unexpectedly, the antioxidant performance of γ-oryzanol continued even when the induction period had ended. During the propagation phase, the highest antioxidant activity was observed in the FAME of soybean oil (3.86) based on hydroperoxides decomposition. An evaluation of how the endergonic-activated complexes formed could indicate that the removal of the triacylglycerol structure increased the effective collisions between the γ-oryzanol molecules and free radicals.

EAT Stabilization mechanism of water-in-oil emulsions by medium- and long-chain diacylglycerol: post-crystallization vs. pre-crystallization

Lia, G., *et al.*, *LWT 146*: 111649, 2021, https://doi.org/10.1016/j.lwt.2021.111649.

In this work, medium- and long-chain diacylglycerols (MLCD) with high nutritional features and surface activities were used to prepare emulsions. The influence of crystallization procedures (pre- or post-crystallization) on the emulsions' stability was examined in terms of the change in droplet size distribution (DSD), sedimentation, microstructure, and thermal properties. The sedimentation and coalescence of emulsions were reduced when a higher amount (8%, w/w) of MLCD was used. The post-crystallized emulsions showed narrower DSD and less sedimentation compared to the pre-crystallized emulsions. Pre-crystallized emulsions prepared using shear speed of 10,000 rpm showed improved stability due to the reduction of crystal size. MLCD was able to form typical interfacial crystal shells in post-crystallized emulsions, whereas only large crystals were formed in the continuous phase in the pre-crystallizations. Therefore, the post-crystallized emulsions were thicker and sedimentation was effectively reduced.

EAT LOQ Effect of feeding pigs with bergamot by-product on fatty acid composition and oxidative stability of meat and salami

Scerra, M., *et al.*, *Meat Sci. 183*: 108662, 2022, https://doi.org/10.1016/j.meatsci.2021.108662.

This work investigated the effects of feeding ensiled bergamot pulp to pigs on meat and salami quality. Eighteen pigs were assigned to two experimental treatments and fed a cereal-based concentrate diet (control) or the same diet in which ensiled bergamot pulp replaced 15% dry matter of the diet fed (BP). The BP treatment increased alpha-linolenic acid (+250%; $P < 0.05$), docosapentaenoic acid (+62%; $P < 0.05$), docosahexaenoic acid (+43%; $P < 0.05$) and consequently n-3 PUFA (+15%; $P < 0.01$) in meat. In salami, the content of alpha-linolenic acid, total PUFA, and n-3 PUFA increased (+320%, +25% and + 258%, respectively) by feeding the BP diet ($P < 0.001$). The inclusion of bergamot pulp in the diet did not alter the oxidative stability in raw and cooked meat and color descriptors. In salami, TBARS values were lower after 5 days of storage ($P < 0.001$) in BP group (1.54 vs 2.96). Finally, dietary supplementation with ensiled bergamot pulp to pigs improved the nutritional value of meat and meat products.

IOP PRO BIO Production cost and carbon footprint of biomass-derived dimethylcyclooctane as a high-performance jet fuel blendstock

Baral, N.R., *et al.*, *ACS Sustain. Chem. Eng. 9*: 11872–11882, 2021, https://doi.org/10.1021/acssuschemeng.1c03772.

Near-term decarbonization of aviation requires energy-dense, renewable liquid fuels. Biomass-derived 1,4-dimethylcyclooctane (DMCO), a cyclic alkane with a volumetric net heat of combustion up to 9.2% higher than Jet A, has the potential to serve as a low-carbon, high-performance jet fuel blendstock that may enable paraffinic bio-jet fuels to operate without aromatic compounds. DMCO can be produced from bio-derived isoprenol (3-methyl-3-buten-1-ol) through a multistep upgrading process. This study presents detailed process configurations for DMCO production to estimate the minimum selling price and life-cycle greenhouse gas (GHG) footprint considering three different hydrogenation catalysts and two bioconversion pathways. The platinum-based catalyst offers the lowest production cost and GHG footprint of \$9.0/L-Jet-Aeq and 61.4 gCO2e/MJ, given the current state of technology. However, when the supply chain and process are optimized, hydrogenation with a Raney nickel catalyst is preferable, resulting in a \$1.5/L-Jet-Aeq cost and 18.3 gCO2e/MJ GHG footprint if biomass sorghum is the feedstock. This price point requires dramatic improvements, including 28 metric-ton/ha sorghum yield and 95–98% of the theoretical maximum conversion of biomass-to-sugars, sugars-to-isoprenol, isoprenol-to-isoprene, and isoprene-to-DMCO. Because increased gravimetric energy density of jet fuels translates to reduced aircraft weight, DMCO also has the potential to improve aircraft efficiency, particularly on long-haul flights.

IOP PRO Biodiesel production from alternative raw materials using a heterogeneous low ordered biosilicified enzyme as biocatalyst

Ferrero, G.O., *et al.*, *Biotechnol. Biofuels 14*: 67, 2021, https://doi.org/10.1186/s13068-021-01917-x.

The combination of enzymatic and inorganic heterogeneous catalysis generates a platform for fuel and chemical production that combines the advantages of both the catalytic efficiency and selectivity of enzymes with the ordered structure, high porosity, mechanical, thermal and chemical resistance of mesoporous materials to obtain enzymatic heterogeneous catalysts. Enzymatic mineralization with an organic silicon precursor (biosilicification) is a promising and emerging approach for the generation of solid hybrid biocatalysts with exceptional stability under severe use conditions. Herein, we assessed the putative advantages of biosilicification technology for developing an improved efficient and stable biocatalyst for sustainable biofuel production. A series of solid enzymatic catalysts denominated LOBE (low ordered biosilicified enzyme) were synthesized from *Pseudomonas fluorescens* lipase and tetraethyl orthosilicate. The microscopic structure and physicochemical properties characterization revealed that the enzyme formed aggregates that were contained in the heart of silicon-covered micelles, providing active sites with the ability to process different raw materials (commercial sunflower and soybean oils, *Jatropha excisa* oil, waste frying oil, acid oil from soybean soapstock, and pork fat) to produce first- and second-generation biodiesel. Ester content ranged from 81 to 93% wt depending on the raw material used for biodiesel synthesis. A heterogeneous enzymatic biocatalyst, LOBE4, for efficient biodiesel production was successfully developed in a single-step synthesis reaction using biosilicification technology. LOBE4 showed to be highly efficient in converting refined, non-edible and residual oils (with high water and free fatty acid contents) and ethanol into biodiesel. Thus, LOBE4 emerges as a promising tool to produce second-generation biofuels, with significant implications for establishing a circular economy and reducing the carbon footprint.

LOQ H&N Effects of roasting temperature and time on aldehyde formation derived from lipid oxidation in scallop (*Patinopecten yessoensis*) and the deterrent effect by antioxidants of bamboo leaves

Hu, Y., *et al.*, *Food Chem. 369*: 130936, 2022, https://doi.org/10.1016/j.foodchem.2021.130936.

This study aimed to investigate the effects of roasting temperature and time on aldehyde formation derived from lipid

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oxidation in scallop, and the deterrent effect of natural antioxidants extracted from bamboo leaves (AOB). Results showed that roasting process significantly increased the peroxide value (PV), thiobarbituric acid-reactive substances (TBARS), p-Anisidine value (p-AV), and total oxidation (TOTOX) in scallop lipids. Additionally, 16 different aldehydes in scallop lipids were detected using an HPLC-ESI-MS/MS method. Among them, the content of hexanal, pentanal, nonanal, trans, trans-2,4-octadienal, and 4-hydroxy-2-hexenal increased in a time- and temperature-dependent manner during the roasting process. After roasting at 210°C for 40 min, their content increased by 1.23-, 0.81-, 1.44-, 0.59-, and 2.12-fold compared with the unroasted group, respectively. However, pretreatment with AOB effectively prevented aldehyde formation in roasted scallops by reducing the oxidation of polyunsaturated fatty acids and scavenging free radicals.

PRO A hybrid ultrafiltration/nanofiltration/pervaporation membrane process for intensifying the refining of crude canola oil and solvent recovery

Abdorrezaee, Z. and R. Ahmadreza, *Chem. Eng. Process.—Process Intensification* 169: 108598, December 2021, https://doi.org/10.1016/j.cep.2021.108598.

Conventional edible oil processing methods with all their drawbacks have not changed over the past decades. Therefore, the main novelty of this study is to develop a three-stage hybrid membrane process to remove phospholipids, pigments, and solvent from the crude canola oil miscella by combining ultrafiltration, nanofiltration, and pervaporation processes. For the first and second stages, ultrafiltration and nanofiltration membranes were prepared by phase inversion method using polyethersulfone as polymer, and triethylene glycol and polyethylene glycol as additives. Pervaporation for the first time was conducted for solvent recovery, using commercial polydimethylsiloxane composite membranes with different active layer thicknesses. Almost full rejection of phospholipids (>99.9%) was obtained by addition of both additives, and approximately remained constant by changing the additive concentration. A 91.49% reduction in the color of crude oil with a flux of 1.17 L/m2.h was obtained at the end of the bleaching stage. The solvent recovery by the pervaporation process with the polydimethylsiloxane composite membranes showed the 100% oil rejection with acceptable flux. The proposed hybrid ultrafiltration/nanofiltration/pervaporation membrane process showed an excellent performance in terms of permeation flux, phospholipids rejection, and color reduction for refining crude canola oil and solvent recovery processes and meets the criteria of process intensification.

PRO IOP Plasma-assisted catalytic route for transesterification reactions at room temperature

Palm, M.O., *et al.*, *Fuel* 307, 121740, January 1, 2022, https://doi.org/10.1016/j.fuel.2021.121740.

Biodiesel has been currently obtained from the transesterification reaction of vegetable oils, including residual oil as waste cooking oil (WCO), usually in the presence of a catalyst. Advanced methods, such as plasma, have been studied to produce biodiesel since it allows for milder conditions, mainly by decreasing both reaction temperature and time of production. The objective of this work is to investigate the plasma-assisted catalytic route for the monoesters transesterification reactions. H_3PMo and $NaOCH_3$ were used as acid and basic catalysts, respectively. The batch plasma reactor used in this work was composed by a borosilicate glass tube with concentric electrodes, in which ethyl acetate and methanol were fed. The effects of the plasma associated with both acid and basic catalysts were investigated in the ambient temperature and atmospheric pressure. In general, plasma-assisted catalytic routes showed higher ethyl acetate conversions compared to the routes with no plasma under the same experimental conditions. For example, the ethyl acetate conversion increased from 38% to 77% with plasma assistance over 90 min. This level of conversion is comparable with values achieved for the reaction in the presence of basic catalyst without plasma, although in a shorter time of reaction. The synergistic effect between the plasma and the catalysts provided an increase in the reaction rate constants, and high ethyl acetate conversions in shorter reaction times. These findings indicate that plasma-assisted catalyst routes are promising for production of biodiesel under room temperature, especially for production from waste oils, which requires the use of acid catalysts, usually under extreme conditions.

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