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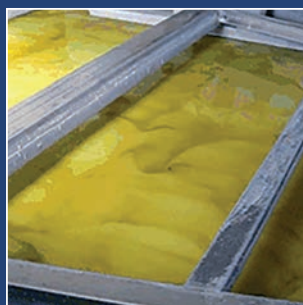
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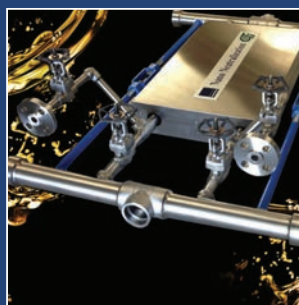
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An underwater photograph of a kelp forest. Tall, green kelp stalks with large, feathery leaves rise from the ocean floor. Several fish are swimming in the clear, blue water. The scene is illuminated by natural light filtering down from the surface.

March 2017

INFORM

CONTENTS

6 The mysterious case of the arsenolipids

Arsenolipids made by algae from inorganic arsenic in seawaters accumulate in fish and other marine animals. What are they, how are they identified and measured, and are they poisonous?



18



22



28

14 Gene combinations for production of bioactive and industrial oils

Researchers develop genetically engineered canola- and flax-producing oils enriched in punicic acid.

18 The oil of oregano (*Origanum vulgare*)

Researchers investigate the radical scavenging activities and antimicrobial properties of cold-pressed oregano oil.

22 Commercially available alternatives to palm oil

The advantages and disadvantages of several palm oil alternatives are described and compared to similar palm-based products.

28 Avocado: characteristics, health benefits, and uses

The characteristics, health benefits, current applications, and potential uses of this major tropical fruit—and its oil—are reviewed.

DEPARTMENTS

5 Index to Advertisers
20 Classified Advertising
37 AOCS Meeting Watch

Analysis/commentary
34 Olio
36 Regulatory Review
38 Latin America Update

Publications and more
42 AOCS Journals
40 Patents
44 Extracts & Distillates

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INDEX TO ADVERTISERS

*Crown Iron Works Company	C3
*Desmet Ballestra Engineering NA	C2
DVC Process Technologists	43
Editora Stilo	13
*French Oil Mill Machinery Co.	8
*Harburg-Freudenberger Maschinenbau GmbH	1
Kumar Metal Industries Pvt. Ltd.	17
*Oil-Dri Corporation of America	C4
Pope Scientific, Inc.	11
RBD Technologies	11

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The mysterious case of the arsenolipids

Laura Cassiday

In Victorian thrillers, murderers often dispatched their victims with a large dose of arsenic stirred into hot tea or sprinkled onto food. The perfect poison, arsenic mimicked symptoms of natural diseases such as cholera and was difficult to detect with the forensic techniques of the era. In more recent times, low levels of arsenic have been discovered in drinking water, rice, and seafood. Whereas the arsenic in water and rice is in the inorganic form, in fish and seaweed a substantial portion of the arsenic is sequestered in intriguing compounds called arsenolipids. As yet, very little is known about the biosynthesis, metabolism, function, or toxicity of arsenolipids. Although their levels in seafood and fish oils are unlikely to pose a risk to human health, some researchers believe that it is nonetheless important to better characterize arsenolipids, not only to guide toxicological risk assessments, but also to gain an improved understanding of arsenic cycling in the environment.

- Arsenolipids are lipid-soluble compounds that contain arsenic and a long aliphatic chain. Made by algae from inorganic arsenic in seawater, arsenolipids accumulate in fish and other marine animals.
- In recent years, about 50 arsenolipids have been identified, including arsenic-containing hydrocarbons, fatty acids, phospholipids, phosphatidylcholines, and fatty alcohols. Many more likely remain to be discovered.
- The physiological role of arsenolipids in algae and their effects on marine creatures and humans are largely unknown.

FAT-SOLUBLE ARSENIC

The presence of arsenic in marine creatures is not surprising given that arsenic is a ubiquitous component of seawater, occurring at fairly uniform levels of 0.5–2 µg/L worldwide (Sele, V., *et al.*, <http://dx.doi.org/10.1016/j.foodchem.2012.02.004>, 2012). Most inorganic arsenic is released into the ocean by natural processes, such as volcanoes and weathering of minerals, although local arsenic contamination from man-made sources, such as coal burning, pesticides, or ore smelting, can occur. In the late 19th century, arsenic had already been detected in fish. However, most studies since then have focused on water-soluble arsenic compounds, such as inorganic arsenic and arsenobetaine (trimethylarsonioacetate), an organic small molecule that usually comprises more than 80% of the total arsenic in marine organisms.

On the other hand, lipid-soluble arsenic compounds have proven much more difficult to isolate and analyze. In 1968, in the *Journal of the American Oil Chemists' Society (JAOCs)*, Lunde published the first report of lipid-soluble arsenic compounds, proposed to be arsenic-containing phospholipids, in cod liver and herring oils. Twenty years later, Morita and Shibata accomplished the first structural identification of an arsenolipid—a phospholipid with an arsenosugar head group, from a brown alga (*Chemosphere*, 1988).

Since then, about 50 different arsenolipids have been identified, estimates Jörg Feldmann, professor of environmental analytical chemistry and director of the Trace Element

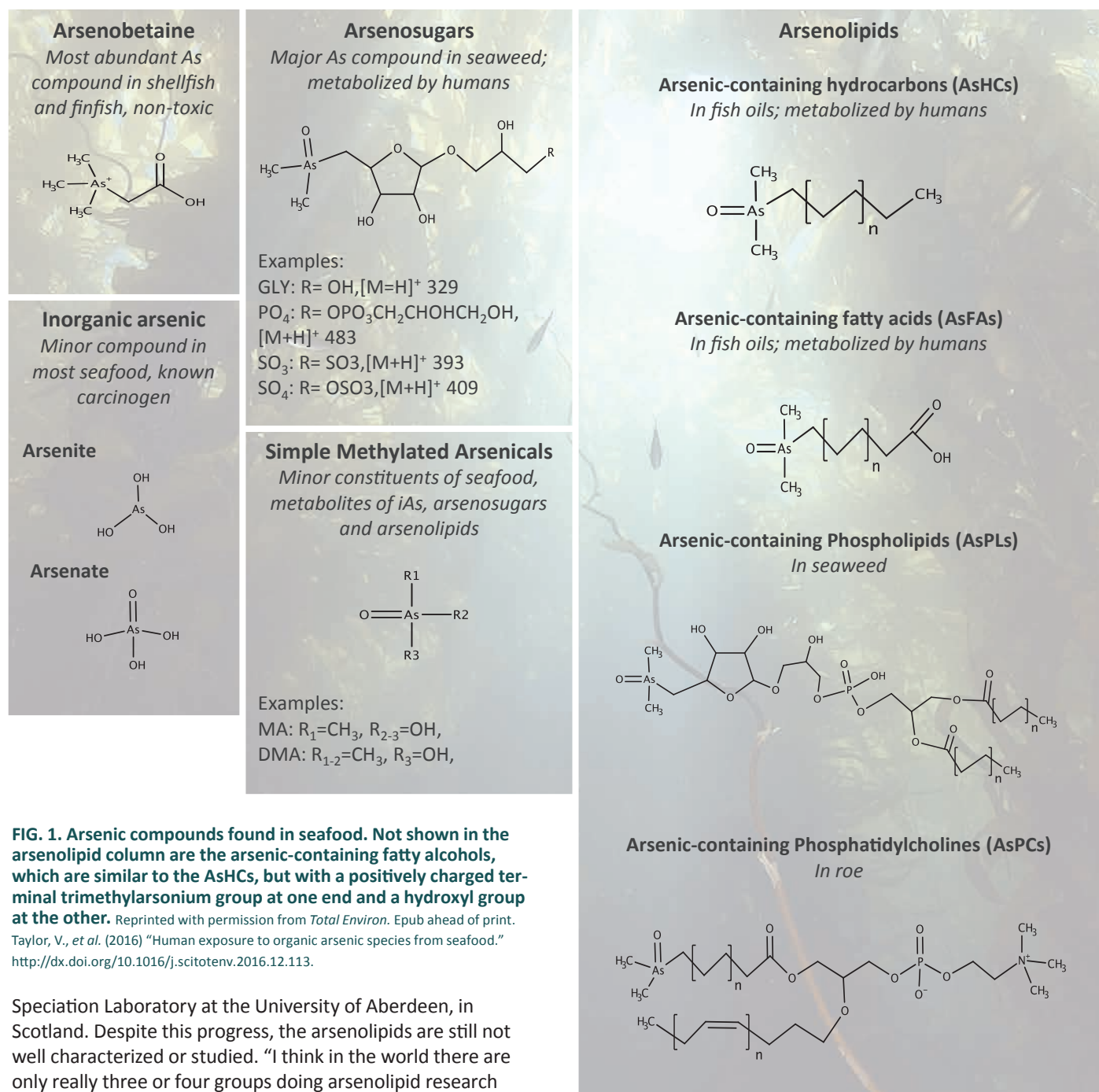


FIG. 1. Arsenic compounds found in seafood. Not shown in the arsenolipid column are the arsenic-containing fatty alcohols, which are similar to the AsHCs, but with a positively charged terminal trimethylarsonium group at one end and a hydroxyl group at the other. Reprinted with permission from *Total Environ. Epub ahead of print.*

Taylor, V., et al. (2016) "Human exposure to organic arsenic species from seafood." <http://dx.doi.org/10.1016/j.scitotenv.2016.12.113>.

Speciation Laboratory at the University of Aberdeen, in Scotland. Despite this progress, the arsenolipids are still not well characterized or studied. "I think in the world there are only really three or four groups doing arsenolipid research right now," says Feldmann. "When we started looking at arsenolipids 10 or 15 years ago, there were no real methods available to extract the arsenolipids and analyze them without changing them."

"Many arsenolipids have been identified, but there are many more that haven't been identified," says Kevin Francesconi, professor in analytical chemistry at the University of Graz, in Austria. "Most of them are present at really trace amounts. There are probably 10 compounds that make up 80–90% of the total amount of arsenolipids, I would estimate."

TYPES OF ARSENLIPIDS

The definition of an arsenolipid depends, to some extent, on whom you ask. According to William Christie, consultant and

professor emeritus at the James Hutton Institute in Dundee, Scotland, some researchers define an arsenolipid as any arsenic-containing compound that is soluble in organic solvents. "Some people look at things like trimethylarsine, a very simple molecule, and call that a lipid," he says. "If you do that, there are literally hundreds of different arsenolipids, so I restrict myself to those that contain a long aliphatic chain." Using this definition, five main groups of arsenolipids have been identified: arsenic-containing hydrocarbons (AsHCs), fatty acids (AsFAs), phospholipids, phosphatidylcholines, and fatty alcohols (Fig. 1).

The AsHCs are long-chain aliphatic compounds with a terminal dimethylarsinoyl group. Odd- and even-numbered species of AsHCs have been identified, with up to 19 carbons and up to

7 double bonds (AOCS Lipid Library, <http://tinyurl.com/arsenolipids>). AsHCs have been detected in brown algae, fish, and fish oils. In 2013, Feldmann and colleagues identified seven different AsHCs in oil from the capelin (*Mallotus villosus*) fish (Amayo, K.O., *et al.*, <http://dx.doi.org/10.1021/ac4020935>). Three of these comprised the major arsenolipids in the capelin oil, together accounting for more than 80% of the total arsenic content.

AsFAs are long-chain aliphatic compounds that, like AsHCs, contain a dimethylarsinoyl group at one end. At the other end, they have a carboxylic group. AsFAs may be either saturated or unsaturated, and arsenic-containing analogs of common fatty acids such as palmitic acid, stearic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have been identified in fish and algae. "There is some speculation that there are actually arsenic-containing triglycerides, but our methods are not gentle enough to see them," says Feldmann. "The triglycerides are hydrolyzed, and we see only the arsenic-containing fatty acids." Indeed, Francesconi and colleagues isolated complex arsenic-containing compounds from blue whiting (*Micromesistius poutassou*) oil that hydrolyzed to known AsFAs, prompting the researchers to suggest that the AsFAs arose from fatty acid esters, possibly triglycerides (Taleshi, M. S., *et al.*, <http://dx.doi.org/10.1038/srep07492>, 2014).

Arsenic-containing phospholipids are analogous to regular phospholipids, but with arsenic in the head group. For example, phosphatidylarsenocholine, identified in algae, contains arsenic in place of the nitrogen atom in the choline head group. Many arsenic-containing phospholipids contain an arse-

nosugar in place of the choline group. Related compounds, the arsenic-containing phosphatidylcholines, were recently identified by Francesconi's group in herring caviar (Viczek, S. A., *et al.*, <http://dx.doi.org/10.1002/anie.201512031>, 2016). These phospholipids have a normal choline head group, but an AsFA as one of the fatty acids in their tail.

In 2013, Feldmann's group identified two arsenic-containing fatty alcohols in capelin oil (Amayo, K. O., *et al.*, <http://dx.doi.org/10.1021/ac4020935>). The compounds, present at low concentrations, were alcohols with long hydrocarbon chains and a positively charged terminal trimethylarsonium group. "So far we're the only ones who have found arsenic-containing fatty alcohols, and it was quite tricky to do that," says Feldmann. "You need some derivatization methods, and that's maybe why they are so elusive."

ARSENOLIPID ANALYSIS

The characterization of arsenolipids has proven challenging for several reasons. In comparison to regular cellular lipids, arsenolipids are present at low concentrations, making identification and quantification difficult. Care must be taken so that labile arsenolipids are not hydrolyzed during sample preparation and analysis. Analytical techniques for water-soluble arsenic species are not appropriate for the analysis of lipid-soluble species. Also, there are currently no commercially available arsenolipid standards or certified reference materials, making inter-laboratory comparisons difficult (Taylor, V., *et al.*, <http://dx.doi.org/10.1016/j.scitotenv.2016.12.113>, 2016).



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In the past, arsenolipids could only be analyzed indirectly. HPLC/ICP-MS (high-performance liquid chromatography/inductively coupled plasma-mass spectrometry) is a powerful technique that can be used to detect trace elements, such as arsenic, in samples. Initially, however, the technique could be used only for water-soluble arsenic species. So researchers were forced to chemically or enzymatically hydrolyze arsenolipid samples into water-soluble fragments, analyze the fragments by HPLC/ICP-MS, and then deduce the structures of the original compounds (Amayo, K. O., *et al.*, <http://dx.doi.org/10.1021/ac4020935>).

In the early 2000s, HPLC/ICP-MS was modified to allow for the direct analysis of arsenolipids. To separate arsenolipids by HPLC, an organic mobile phase is needed, which can destabilize the plasma ionization source of the original ICP-MS. To accommodate organic mobile phases, a modified ICP-MS set-up using low solvent flows and adding oxygen to the nebulizer gas is necessary (Sele, V., *et al.*, <http://dx.doi.org/10.1016/j.foodchem.2012.02.004>, 2012). This improvement has greatly accelerated the rate of arsenolipid discovery in the past few years.

There are four main steps to arsenolipid analysis: extraction, separation, detection, and characterization (Taylor, V., *et al.*, <http://dx.doi.org/10.1016/j.scitotenv.2016.12.113>, 2016). The extraction generally involves stepwise solvent partitioning to separate arsenic-containing compounds from the overall lipid matrix. For example, Francesconi and coworkers partitioned the lipid-soluble fraction from sashimi-grade tuna fish between hexane and aqueous methanol (Taleshi, M. S., *et al.*, <http://dx.doi.org/10.1021/es9030358>, 2010). Because many arsenolipids contain a polar (e.g., dimethylarsinoyl) group, they tend to accumulate in the aqueous methanol layer. The non-polar hexane extract contained more than 90% of the total lipids. In contrast, the polar methanol extract contained only 5% of the total lipid, but 50% of the arsenic. Therefore, the researchers analyzed the methanol extract, which was enriched in arsenolipids, by HPLC/ICP-MS and HPLC/electrospray ionization (ESI)-MS/MS. They identified two AsHCs that together accounted for about 40% of the total fat-soluble arsenic in the tuna.

In a more recent study, Francesconi and colleagues extracted the hexane layer from blue whiting oil with aqueous isopropanol to yield a “less-polar” lipid fraction that had a polarity in between the polar (hexane) and nonpolar (methanol) extracts (Taleshi, *et al.*, <http://dx.doi.org/10.1021/es9030358>, 2014). Increasing chain length reduces the polarity of arsenolipids, whereas functional groups or unsaturation can increase polarity. This strategy allowed the researchers to identify 4 novel AsHCs from the fish oil.

Reversed-phase HPLC is the most common separation step used for arsenolipid analysis. Polar compounds elute from the chromatography column more rapidly than less-polar compounds. In this way, the arsenolipids from a fraction can be further separated so that they can be detected sequentially by ICP-MS. ICP-MS has excellent detection limits and a linear range capable of quantitating compounds at concentrations ranging from low ng/L to 1 mg/L within the same run (Taylor, V., *et al.*, <http://dx.doi.org/10.1016/j.scitotenv.2016.12.113>, 2016), provided the compounds are well separated by HPLC.



FIG. 2. Seaweeds, or brown algae, produce arsenolipids, which accumulate in fish and other marine creatures that ingest the seaweed. Credit: Stef Maruch, via Wikimedia Commons

The characterization step typically involves comparing the ICP-MS data with molecular mass and fragmentation data from ESI-MS/MS to assign structures to arsenolipids. “We split the flow from the HPLC column, and we use both elemental mass spectrometry [ICP-MS], which enables us to see all the compounds that contain arsenic, and molecular mass spectrometry with a high-resolution instrument [ESI-MS/MS],” says Francesconi. “If we see a peak corresponding to an arsenic compound, we are also very often able to get the molecular mass of that compound by using the two forms of detection simultaneously. Once we have the mass and fragmentation pattern, we can start putting a structure forward.” Often, the researchers will then chemically synthesize the proposed molecule and analyze it to prove that the structure is correct.

MYSTERIOUS MOLECULES

Although much progress has been made in identifying arsenolipids, their origins and interactions are still enigmatic. Arsenate $[(H_2AsO_4)^-]$, the predominant form of arsenic in seawater, is readily taken up by algae (Fig. 2). Because arsenate is structurally similar to phosphate $[(H_2PO_4)^-]$, the algae’s phos-

phate transporters may not be able to differentiate between the two anions. Once inside the algae, arsenate undergoes a series of biotransformations that result in a variety of organoarsenic compounds, including arsenosugars and arsenolipids. “We know that algae make arsenolipids,” says Francesconi. “Whether fish make them *de novo* from inorganic arsenic, I think that’s less likely. We think that the algae are the starting points, and the fish accumulate, but also modify, the arsenolipids that they ingest.”

In marine animals and algae, total arsenic concentrations range from 1–100 mg/kg, with considerable variations between and within species (Sele, V., *et al.*, <http://dx.doi.org/10.1016/j.foodchem.2012.02.004>, 2012). Reports of total arsenic levels in commercial fish oils have ranged from 0.2–16 mg/kg, depending on the fish species. For comparison, terrestrial samples usually contain much lower levels of arsenic (below 0.02 mg/kg), with the exception of rice, which accumulates 0.02–1 mg/kg total arsenic. Although much of the arsenic in rice is in the inorganic form, marine animals typically contain low levels of inorganic arsenic. About 10–30% of the arsenic in marine animals is in the form of lipid-soluble arsenic compounds. In brown algae, however, arsenolipids can account for more than 50% of the total arsenic.

When algae take up arsenate, which is highly toxic, they rapidly detoxify it by adding two methyl groups to produce dimethylarsinate [DMA; $\text{Me}_2\text{As}(\text{O})\text{O}^-$]. Many organisms, including humans, can likewise convert arsenate into DMA. However, unlike humans, marine algae use DMA as an intermediate to produce a wide range of arsenolipids (Francesconi, K. A., and Schwerdtle, T., <http://dx.doi.org/10.1002/lite.201600024>, 2016).

An unanswered question is why algae produce arsenolipids. One possibility is that, like the phosphate transporters, biosynthetic enzymes mistake arsenic-containing compounds for other molecules, and then use them as building blocks for cellular lipids. If this were the case, the arsenolipids in marine organisms would likely reflect the natural abundance of non-arsenic-containing lipids. Although more research is needed, abundant fatty acids such as palmitic and stearic acids are indeed more likely to appear as arsenolipids than less abundant fatty acids (Francesconi, K. A., and Schwerdtle, T., <http://dx.doi.org/10.1002/lite.201600024>, 2016). Another idea is that arsenolipid production could help with detoxification. Adding arsenic to lipids may facilitate its diffusion through the membrane and out of the cell.

An intriguing possibility is that, instead of being mere accidents or detoxification products, arsenolipids might actually serve a useful purpose in marine organisms. The early ocean, like now, contained appreciable amounts of arsenic. “Arsenic was always available, and usually life forms use all of the elements available at higher concentrations in their chemistry,” says Feldmann. “Arsenic chemistry is distinctively different from any other chemistry. Therefore, there’s a gut feeling that arsenic is somehow an essential element at a low concentration.”

When Feldmann and colleagues challenged brown algae in the lab with different forms of environmental stress (low

nitrate, low phosphate, oxidative stress), the algae responded by making more arsenolipids (Pétursdóttir, Á. H., *et al.*, <http://dx.doi.org/10.1071/EN14229>, 2016). Low-phosphate conditions preferentially increased the biosynthesis of arsenic-containing phospholipids. Therefore, it is possible that arsenolipids are an adaptive mechanism to certain forms of environmental stress.

The presence of arsenic-containing phospholipids in cell membranes may alter their structure or fluidity, which in turn could affect cell growth, signaling, and ligand-receptor interactions (Sele, V., *et al.*, <http://dx.doi.org/10.1016/j.foodchem.2012.02.004>, 2012). However, arsenic-containing phospholipids would not normally be expected to have large effects on membranes because their concentration would be much lower than that of regular phospholipids. Perhaps the contribution of arsenolipids to membranes would become more important for algae living under phosphorus-deficient conditions.

“You can do various experiments to suggest that algae are quite happy with low levels of arsenic, and some grow even better when there’s a little bit more arsenic in the water,” says Francesconi. “It’s certainly a fascinating topic, but we haven’t got any firm evidence to say, yes, arsenic is useful, and it is being used by some organisms.”

CAUSE FOR CONCERN?

Chronic exposure to inorganic arsenic in drinking water can have serious health effects, including skin, bladder, and lung cancers. The International Agency for Research on Cancer (IARC) has classified inorganic arsenic as a Group 1 human carcinogen. Seafood is one of the major contributors of arsenic in the diet (more than 90% in the United States); however, most arsenic in seafood is present as organoarsenic compounds with largely unknown health effects (Taylor, V., *et al.*, <http://dx.doi.org/10.1016/j.scitotenv.2016.12.113>, 2016).

The major organoarsenic compound in seafood, arsenobetaine, is known to be nontoxic. Arsenobetaine is metabolically inert, meaning that it is excreted unchanged from the human body in the urine. In contrast, arsenolipids and arsenosugars are metabolized by humans, mainly to DMA—an IARC Group 2B (possible) carcinogen, and the same metabolite produced upon ingestion of inorganic arsenic. There is also a concern that arsenolipids could produce toxic intermediates along the pathway to DMA.

To gain a better understanding of human metabolism of arsenolipids, Francesconi and coworkers performed a small study in which two male volunteers ingested canned cod liver (Schmeisser, E., *et al.*, <http://dx.doi.org/10.1007/s00216-006-0401-x>, 2006). Arsenic metabolites in the volunteers’ urine were monitored by HPLC/ICP-MS for 66 hours afterwards. As expected, the arsenobetaine from the cod liver samples was excreted in the urine unchanged. The arsenolipids, however, were metabolized. For both volunteers, the most abundant arsenolipid metabolite was DMA, followed by four novel, short-chain AsFAs. The peak arsenic concentration in urine occurred between 7 and 15 hours, and the AsFAs reached background levels by 24 h after ingestion in both volunteers. By the end of the experiment, about 90% of the ingested arsenic had been accounted for in the urine of both volun-

teers. This preliminary study suggests that ingested arsenolipids are rapidly metabolized to water-soluble compounds and excreted. The time profile indicates that arsenolipids are first metabolized to AsFAs, and then further to DMA.

In an *in vitro* study, Schwerdtle, Francesconi, and colleagues investigated the cellular toxicity of AsHCs in cultured human bladder and liver cells (Meyer, S., *et al.*, <http://dx.doi.org/10.1039/c4mt00061g>, 2014). Surprisingly, the cytotoxicity of the three tested AsHCs was comparable to that of inorganic arsenic, in the low μM range. However, the mechanism of toxicity appeared to differ between the two arsenic forms: AsHCs reduced the cellular energy (ATP) level, rather than causing DNA damage as inorganic arsenic did. The AsHCs readily accumulated in the cultured cells, where they were localized in the membranous structures. The authors concluded that the study “cannot exclude a risk to human health related to the presence of arsenolipids in seafood, and indicates the urgent need for further toxicity studies in experimental animals to fully assess this possible risk.”

In a similar study with AsFAs, the same researchers found that AsFAs and three of their proposed water-soluble metabolites (DMA, DMAr, and thio-DMAr) were less cytotoxic than inorganic arsenic or AsHCs (Meyer, S., *et al.*, <http://dx.doi.org/10.1039/c5tx00122f>, 2015). The AsFAs and DMA had cytotoxic effects at concentrations of about 50 μM and higher, whereas the other two metabolites were not cytotoxic. The researchers concluded that the tested AsFAs were 10–20-fold

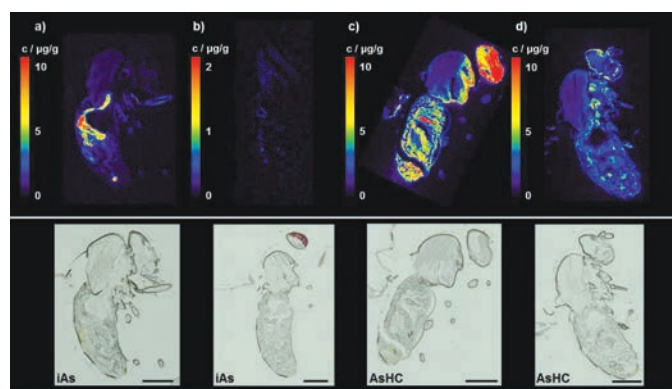


FIG. 3. Arsenic distribution in *D. melanogaster* adult flies after feeding of inorganic arsenic (iAs) or an AsHC for 3 days, followed by 2 days without arsenic-containing feed. LA-ICP-MS images of the ^{75}As distribution in adult flies fed for 3 days with 50 μM (a) inorganic arsenic (arsenite), (c) AsHC-spiked feed (3.2 μg As/g), and (b, d) subsequent feeding without arsenic for 2 days, respectively. Microscopic images of the analyzed sections are shown for comparison. Scale bars, 500 μm . Reprinted with permission from Niehoff, A. C., *et al.* (2016) “Imaging by elemental and molecular mass spectrometry reveals the uptake of an arsenolipid in the brain of *Drosophila melanogaster*.” *Anal. Chem.* 88, 5258–5263. <http://dx.doi.org/10.1021/acs.analchem.6b00333>. Copyright 2016 American Chemical Society.

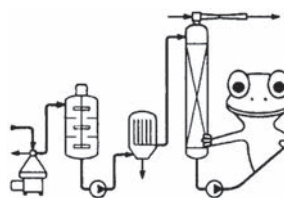
less toxic than AsHCs in the same cell lines. Because AsHCs are amphiphilic, they may be able to interact with cell membranes better than AsFAs, which have two polar end groups.



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To study the fate of an ingested AsHC in a model organism, Karst, Francesconi, and coworkers used elemental laser ablation (LA)-ICP-MS and matrix-assisted laser desorption/ionization (MALDI)-MS to image and quantify the uptake of an AsHC in fruit flies (*Drosophila melanogaster*) (Niehoff, A.-C., *et al.*, <http://dx.doi.org/10.1021/acs.analchem.6b00333>, 2016). The researchers fed larvae or adult flies either inorganic arsenic or an AsHC (1-dimethylarsinoylpentadecane) and then examined arsenic accumulation at different stages of development. The distribution patterns of inorganic arsenic and the AsHC varied substantially, and both larvae and adult flies accumulated higher concentrations of the AsHC than the inorganic arsenic. Disturbingly, perhaps, the AsHC was able to cross the blood-brain barrier and enter the brain of adult flies (in contrast to inorganic arsenic, which was not detected in the brain) (Fig. 3, page 11).

In 2009, the European Food Safety Authority (EFSA) concluded that a risk assessment of arsenolipids in seafood is urgently needed. Currently, regulations exist only for inorganic arsenic in drinking water and food. “We finally have legislation now for

inorganic arsenic in rice, and I think the next big topic is really the arsenolipids,” says Feldmann. “But we still need to convince the analytical community to use all their power to do the identification. We need more than a handful of groups working on this topic. Since it’s in our food, we should be concerned about it.”

However, at the moment there is no cause for panic, or even for reducing fish consumption, in response to the emerging research on arsenolipids. Indeed, fish consumption in general is linked to positive health outcomes, such as reduced mortality from coronary heart disease, and neurological benefits. “Anything that has arsenic is potentially troublesome, but it’s too easy to exaggerate the risk,” says Christie. “People say, ‘Oh, arsenic, it’s a deadly poison,’ but as poisons go, it’s not that bad. It very much depends on the form of arsenic that is presented. So while people are right to keep an eye open, it doesn’t do to overstate the risk.”

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Due to the ever-increasing demands for edible oils and energy resources, oilseed researchers are preparing themselves for emerging challenges. Over the past several decades, considerable interest has been focused on understanding the molecular biology and enzymology of seed oil biosynthesis and modification. Metabolic engineering is useful in generating oilseed crops that produce oils with desired fatty acid compositions for specific high-value nutritional, nutraceutical, and industrial applications. This article describes metabolic engineering approaches for producing punicic acid (18:3 $\Delta^{9cis,11trans,13cis}$) and α -linolenic acid (18:3 $\Delta^{9cis,12cis,15cis}$; ALA)-enriched seed oils.

- A**



Sliced pomegranate fruit revealing the arils

Seeds isolated from the arils

Punicic acid is a conjugated fatty acid and a main component of pomegranate (*Punica granatum*) seed oil (Fig. 1), whereas ALA is highly abundant in flax (*Linum usitatissimum*) seed oil (Fig. 2).

Punicic acid has been shown to exhibit strong anti-cancer and anti-inflammatory properties. It also prevents diet-induced obesity. In addition, oils with conjugated double bonds, such as punicic acid, have the potential to be used as industrial drying agents, such as tung tree (*Vernicia fordii*) oil. Pomegranate only grows in sub-tropical and tropical climates, and the oil is considered a by-product of pomegranate fruit production. In an effort to make punicic acid more widely available for possible nutraceutical and industrial applications, our research group is developing a temperate source of seed oil enriched in punicic acid.

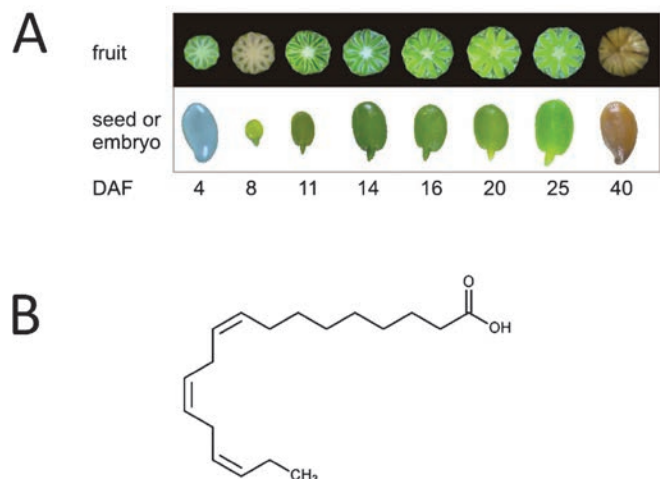


FIG. 2. Flax seed at different stages of development (A). Flax seed oil is enriched in α -linolenic acid (B). Seed development occurs in the fruit known as the boll or capsule. DAF, days after flowering.

Figure 2A was originally published in *J. Biol. Chem.*, Pan et al., 2013, 288: 24173–24188. © the American Society for Biochemistry and Molecular Biology.

Our metabolic engineering approach is based on the introduction of genes that encode enzymes involved in the production of punicic acid-enriched oil in pomegranate. The model plant *Arabidopsis thaliana* (thale cress) and two temperate oil crops were used in our investigations. In developing pomegranate seed, punicic acid is formed on phosphatidylcholine (PC) from linoleic acid (18:2 $\Delta^{9cis,12cis}$) at the *sn*-2 position through the catalytic action of a fatty acid conjugase, which is a divergent form of fatty acid desaturase (FAD) 2 known as FADX (Fig. 3). Regular FAD2 catalyzes the formation of linoleic acid from oleic acid (18:1 Δ^{9cis}).

Various biochemical pathways are involved in channeling unusual fatty acids, such as punicic acid, from PC into triacyl-

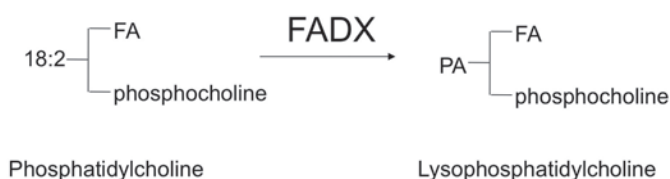


FIG. 3. Fatty acid desaturase (FAD) X (fatty acid conjugase) catalyzes the conversion of a linoleyl (18:2) moiety to a punicyl (PA) moiety at the *sn*-2 position of phosphatidylcholine.



glycerol (TAG), which is the major component of seed oil. Early attempts to produce punicic acid in the model plant *A. thaliana* (Columbia wild type background) through expression of the *PgFADX* gene led to the limited accumulation up to 4.4% of the total fatty acids. The low accumulation of punicic acid was linked to the limited pool of linoleic acid present in the *A. thaliana* background and an increased level of oleic acid.

The observed increase in oleic acid suggested that the expression of *PgFADX* may have suppressed endogenous *A. thaliana* FAD2 expression, thereby limiting the amount of linoleic acid available for conversion to punicic acid. To overcome this limitation, we developed a new approach for increasing punicic acid accumulation through a combined over-expression of pomegranate FADX and FAD2 in a high linoleic acid *A. thaliana* *fad3fae1* double mutant background. Our new approach resulted in increased accumulation of punicic acid up to 21.8% of total fatty acids in the seed oil and restored the natural level of oleic acid found in *A. thaliana* *fad3fae1* mutant seeds.

The same gene combination was then introduced into canola-type *Brassica napus*, resulting in the development of oil enriched in punicic acid up to about 10% of the total fatty acids; the punicic acid content of the oil was stable through two seed generations. Punicic acid content was further increased to 24.8% in the *A. thaliana fad3fae1* mutant background through combined over-expression of *PgFADX*, *PgFAD2*, and the gene encoding a type-2 pomegranate acyl-Coenzyme A (CoA):diacylglycerol acyltransferase (DGAT2). DGAT catalyzes the acyl-CoA-dependent formation of TAG through the transfer of a fatty acid from acyl-CoA to *sn*-1,2-diacylglycerol (see Fig. 4).

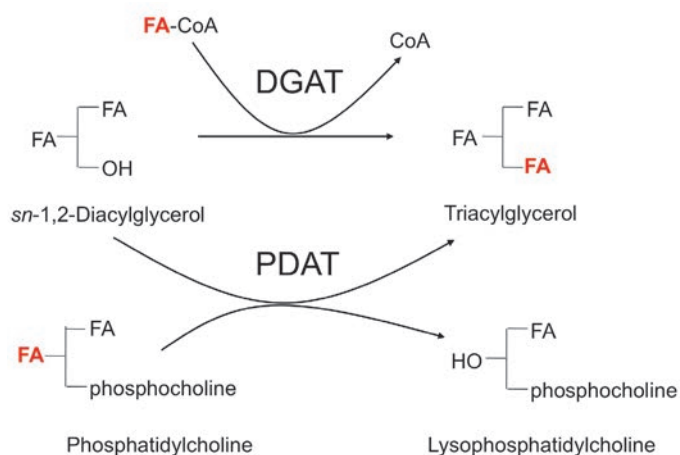


FIG. 4. Seed triacylglycerol can be formed by acyl-CoA-dependent and acyl-CoA-independent processes. Both reactions result in the incorporation of the donated fatty acyl (FA) moiety at the *sn*-3 position of triacylglycerol. CoA, Coenzyme A; DGAT, acyl-CoA:diacylglycerol acyltransferase; PDAT, phospholipid:diacylglycerol acyltransferase.

The results suggested that pomegranate DGAT2 may exhibit increased preference for substrates containing punicic acid. In addition, the pomegranate genes were also used to engineer solin type-flax (*Linum usitatissimum*) to produce oil with about 10% punicic acid. Normally, flax oil is enriched in ALA. In the past, mutagenesis breeding was used to produce solin-type flax through inactivation of the gene encoding FAD3, which catalyzes the conversion of linoleic acid to ALA.

ENHANCING α -LINOLENIC ACID CONTENT

In addition to the strategies developed for producing punicic acid-enriched oils, we also identified genes which may be useful in engineering increased levels of ALA and other polyunsaturated fatty acids (PUFA) in oil crops. As indicated previously, flax oil naturally contains a high amount of ALA and is the most well-known plant-based source of ALA. The polymer-forming properties of ALA make flax oil especially suitable for many types of domestic and industrial coatings such as varnishes, paints, and linoleum. Moreover, as an essential omega-3 fatty acid, ALA is a precursor for nutritionally beneficial very-long-chain omega-3 PUFA (≥ 20 carbons).

Considering the wide range of applications of ALA in the manufacture of food, feed, and industrial products, we investigated the molecular mechanisms behind high ALA accumulation in flax oil. By exploiting the publicly available flax genome database, we identified several genes encoding TAG-biosynthetic enzymes, including seven genes encoding DGAT and six genes encoding phospholipid:diacylglycerol acyltransferase (PDAT).

In contrast to DGAT, PDAT catalyzes the acyl-CoA-independent formation of TAG by transferring a fatty acid from the *sn*-2 position of PC to *sn*-1,2-diacylglycerol (Fig. 4). This reaction represents one example of several possible routes for channeling fatty acids produced on PC (such as ALA and punicic acid) into TAG.

A systematic characterization of the recombinant TAG-biosynthetic enzymes was performed. Our investigations identified unique pairs of flax PDAT which may be useful in producing seed oils with enhanced ALA content. A number of lines of evidence were in support of flax containing two novel pairs of PDAT (LuPDAT1/LuPDAT5 and LuPDAT2/LuPDAT4) which are highly selective for ALA-containing substrates. In the presence of exogenously added ALA, yeast (*Saccharomyces cerevisiae*) mutant strain H1246 producing the corresponding recombinant PDAT was able to produce TAG containing up to 90 mol% of ALA (Fig. 5).

In addition, yeast cells co-producing PDAT along with LuFAD2-1 and LuFAD3B generated TAG with ALA as the predominant fatty acid. Normally, the yeast *S. cerevisiae* does not produce PUFA-containing TAG. In addition, seed-specific expression of these PDAT in *A. thaliana* resulted in a signifi-

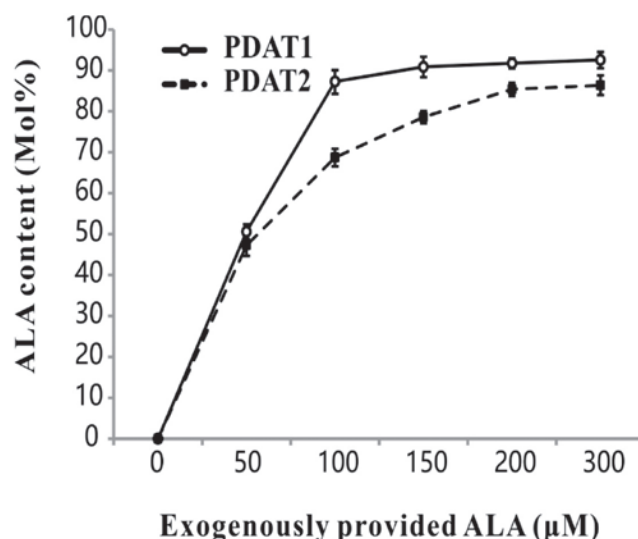


FIG. 5. Recombinant flax phospholipid:diacylglycerol acyltransferase (PDAT) is highly selective for catalyzing the incorporation of α -linolenic acid (ALA) into triacylglycerol. Exogenous ALA was fed to H1246 *Saccharomyces cerevisiae* which produced recombinant flax PDAT. H1246 is a strain of the yeast which is devoid of triacylglycerol synthesis. This figure was originally published in *J. Biol. Chem.*, Pan et al., 2013, 288: 24173–24188. © the American Society for Biochemistry and Molecular Biology.

cant increase in the ALA content of the seed oil. Furthermore, we have shown that the substrate selectivities of these PDAT are not limited to ALA, but extend to other PUFA, including stearidonic acid ($18:4\Delta^{6cis,9cis,12cis,15cis}$), γ -linolenic acid ($18:3\Delta^{6cis,9cis,12cis}$), and eicosapentanoic acid ($20:5\Delta^{5cis,8cis,11cis,14cis,17cis}$), which have broad nutritional value and some industrial applications.

In summary, we have developed genetically engineered *A. thaliana*, canola and flax producing oils enriched in punicic acid. This biotechnology could potentially be extended to other temperate oil-forming crops such as safflower (*Carthamus tinctorius*) and *Camelina sativa*. Oil crops with seed oils enriched in punicic acid could result in high-value specialty oils for various nutraceutical and industrial applications. Additionally, the PUFA-selective PDAT we identified will benefit future metabolic engineering projects aimed at enhancing the accumulation of PUFA-containing TAG in microorganisms and plants. These new developments will add value to the oilseed industry by providing consumers and the growing bio-product industry with a cheaper and more available source of punicic acid and ALA.

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Xue Pan is a post-doctoral fellow with the University of California at Riverside, California, USA. Pan discovered flax phospholipid:diacylglycerol acyltransferases with high selectivity for substrates containing α -linolenic acid during her doctoral research studies at the University of Alberta. She can be contacted at xue.pan@ucr.edu.

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The oil of oregano (*Origanum vulgare*)

Mohamed Fawzy Ramadan and Khaled Elbanna

- Cold-pressed oregano (*Origanum vulgare*) oil is a rich source of essential fatty acids, tocopherols, and phenolic compounds.
- To assess the oil's potential as an antimicrobial and functional ingredient in food and pharmaceuticals, researchers recently examined its radical scavenging activities and antimicrobial properties.
- This article, based on a paper published in *European Food Research and Technology*, summarizes their results.

In the United States alone, food-borne pathogens are responsible for more than 76 million cases of food-borne illness, 5,000 deaths, and 325 thousand hospitalizations annually, while *Candida* species cause systemic “yeast” infections in predisposed persons and patients undergoing long-term antibiotic treatment. Meanwhile, consumers are looking for natural products that support healthier lifestyles; consequently, aromatic plants and herbs are being sought for their biological potential as antioxidant and antimicrobial agents.

Oregano (*Origanum vulgare*, family Lamiaceae) is a popular cooking ingredient recognized for its wide health-promoting and antimicrobial effects. Since ancient times, the flowering herb and its oil have been used in folk medicine, and they are still used today—in the general treatment of coughs and digestive disorders, and as a dietary supplement. The essential oil of oregano contains two main bioactive compounds: carvacrol and thymol.

The growing desire to preserve such natural bioactive compounds in oils has led to an increased interest in cold-pressed oils. Cold-pressing preserves more of these healthful compounds because it does not involve the chemical or thermal treatments that often remove these compounds during processing. Consequently, cold-pressed oils generally contain higher levels of bioactive compounds with health-promoting and functional properties than conventionally processed oils do. This article reports on the composition and functional properties of cold-pressed oregano oil (OO).

BIOACTIVE LIPIDS

Linoleic and oleic acids are the main fatty acids in OO. Together, these two fatty acids account for about 83% of total fatty acids. OO is also high in monounsaturated fatty acids (44%) and polyunsaturated fatty acids (41%), making OO a good source of essential fatty acids and functional compounds for nutraceuticals. Palmitic and stearic are the major saturated fatty acids, together comprising about 15% of total fatty acids. OO is also high in unsaponifiables (23.7 g/kg) and is characterized by high amounts of phenolic compounds (5.6 mg/g, respectively as GAE). α -, β -, γ -, and δ -tocopherols accounted for 180, 60, 650, and 117 mg/100 g OO, respectively. α -, γ -, and δ -tocotrienols levels were 521, 59, and 430 mg/100 g OO, respectively. The amounts of tocopherols found in OO may contribute to the health-promoting traits and the oxidative stability of the oil.

ANTI-RADICAL POTENTIAL

Radical scavenging activities of OO and extra virgin olive oil were compared using 1,1-diphenyl-2-picryl-hydrazyl (DPPH \cdot) and galvinoxyl radicals. OO exhibited stronger anti-radical potential than extra virgin olive oil. After 30 min of incubation with DPPH \cdot radicals, 62% of DPPH \cdot radicals were quenched by OO, while extra virgin olive oil deactivated 40% (Fig. 1.). According to electron spin resonance (ESR) results, OO quenched 50% of the galvinoxyl radicals, and extra virgin olive oil quenched 27% after 30 min of incubation (Fig. 1.). Phenolic compounds and tocopherols are very important for the oxidative stability and biological activities of OO. The higher radical scavenging activities of OO compared to olive oil may be due to the differences in the levels content and profile of unsaponifiables.

ANTIMICROBIAL ACTIVITIES

OO exhibited strong antimicrobial traits against bacterial and yeast strains selected based on their relevance as food or human pathogens (Fig. 2.). OO demonstrated strong antimicrobial activity against food-borne pathogen bacteria (*S. enteritidis*, *L. monocytogenes*, *E. coli*, and *S. aureus*) and food spoilage fungi (*C. albicans*, and *A. flavus*) as well as dermatophyte fungi (*T. mentagrophytes*, and *T. rubrum*). The antibacterial activity against food-borne pathogenic bacteria measured as clear zone diameter (CZD) were 35, 33, 30, and 15 mm for *S. aureus*, *E. coli*, *S. enteritidis*, and *L. monocytogenes*, respectively. OO had high activity against food-borne pathogenic bac-

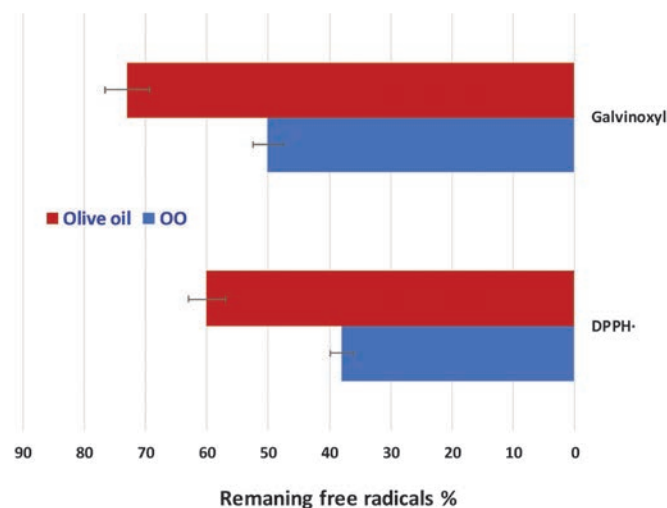


FIG. 1. Antiradical action after 30 min incubation time of OO and extra virgin olive oil on DPPH \cdot radical as measured by changes in absorbance values at 515 nm and on galvinoxyl radical as recorded by ESR

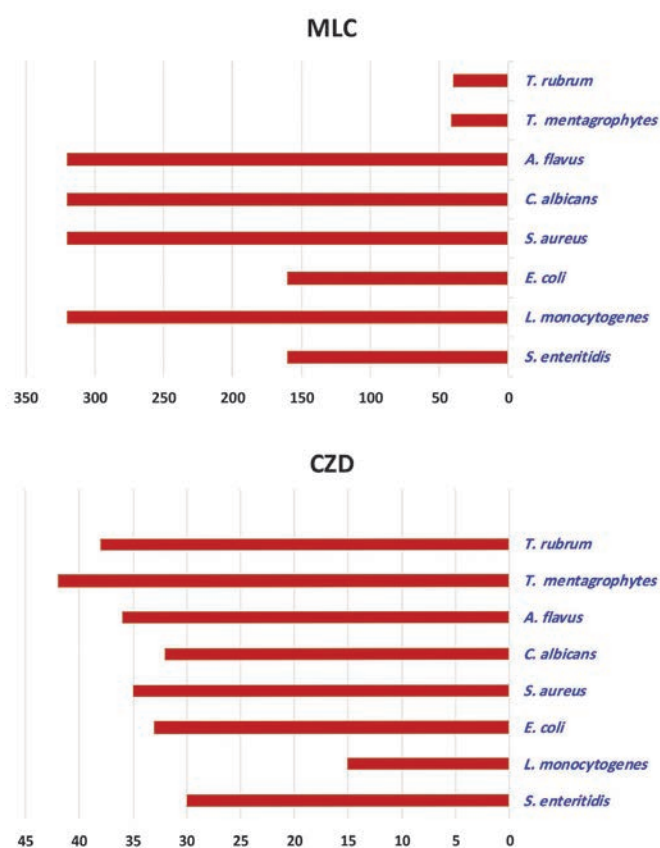


FIG. 2. Antimicrobial activities of OO measured by minimal lethal concentration (MLC, µg/mL) and clear zone diameter (CZD, mm)

teria with minimal lethal concentration (MIC) ranging between 160 to 320 µg/mL.

OO showed high CZD value (36 mm) against *A. flavus* (Fig. 2). It is known that *A. flavus* causes different clinical manifestations of human aspergillosis, such as *cutaneous aspergillosis*, *aspergillar onychomycosis*, *invasive lung aspergillosis*, and *aspergillar sinusitis*. The CZD value recorded for *C. albicans*

was 32 mm, and MLC was 320 $\mu\text{g/mL}$ for both fungi. Among *Candida* species, *C. albicans* is the most associated with serious fungal infection and it exhibited increased resistance to antifungal agents.

Dermatophytic fungi are anthropophilic fungi which cause acute or chronic inflammatory tinea corporis, a superficial fungal infection (dermatophytosis) of the arms and legs. OO exhibited a significant antifungal trait against tested dermatophytic fungi (*T. mentagrophytes* and *T. rubrum*), whereas the CZD values were 38 and 42 mm, respectively.

In addition, OO exhibited low MLC values (40 $\mu\text{g/mL}$). Pictures representing the inhibitory effect of OO against tested dermatophytic fungi (*T. mentagrophytes* and *T. rubrum*) are shown in Fig. 3. It is very important to mention that the antimicrobial potential of OO against tested bacterial and yeast strains recorded the same CZD when left for more than 10 days as an additional incubation period. In addition, no growth was observed when new agar plates or broth media were inoculated with loop from the clear zone area, indicating that the OO have lethal effects. Antimicrobial properties of OO could be explained by the presence of high levels of tocopherols and phenolics.

Finally, we can conclude that OO has a potential antioxidant and antimicrobial activity. The oil could be used in food, cosmetics, and pharmaceutical products to inhibit microbial growth, especially dermatophytic fungi.

Khaled Elbanna is a full professor of agricultural microbiology at Fayoum University, Egypt. His research focuses on antimicrobial and biotechnological products. He has over 40 R&D international publications to his name, and can be reached at kab00@fayoum.edu.eg.

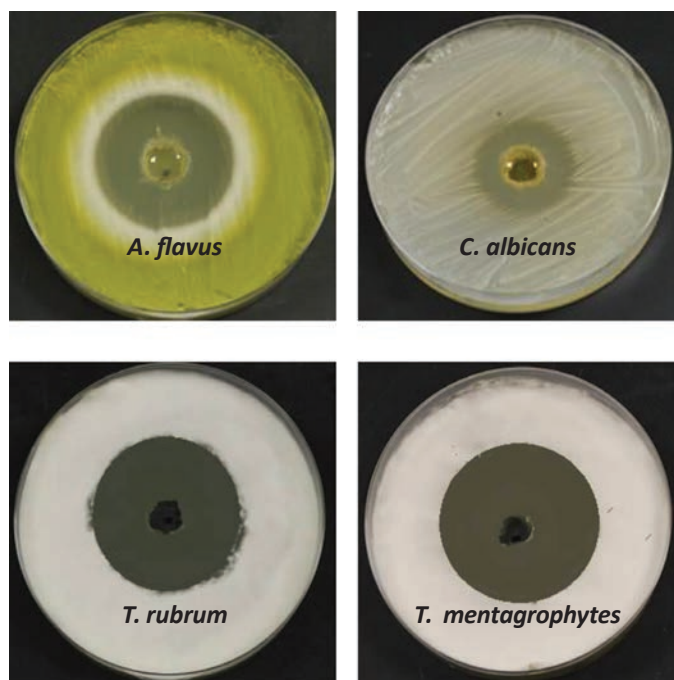


FIG. 3. Antifungal activity of OO against food spoilage and dermatophytic fungi indicated by CZD

Mohamed Fawzy Ramadan is a full professor of food chemistry at Zagazig University, Egypt. His research is focused on the connection between the (micro) structure of food lipids and their biological activity and functionality. His fields of expertise include the development of functional foods and lipids modification, and his research spans several projects—all related to lipid science—from characterizing and modeling the formation and structure of lipids to studying the functional and biological activities of bioactive lipids. He has over 150 R&D international publications to his name, and can be reached at hassanienmohamed@hotmail.com.

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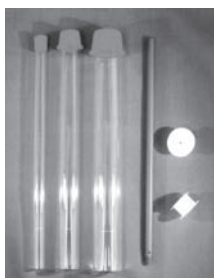
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Commercially available alternatives to palm oil

Nils Hinrichsen

In the last few decades, palm oil has become one of the most important edible oils globally. Because it is solid at room temperature and its fractions deliver a wide range of functional melting profiles, palm oil has played a particularly important role in replacing partially hydrogenated oils (PHOs) in food applications throughout Western Europe. Since PHOs can contain a significant amount of trans-fatty acids, which are considered to be unhealthful [1], their use and application has steadily decreased since the mid-90s. In many countries (and the number is growing) there are legal limits for the level of trans-fatty acids in foods; these have very often led to PHO being replaced by palm oil or palm kernel oil based products, both of which come from the oil palm tree (*Elaeis guineensis*).

- In some countries, there are strong preferences for palm-oil-free products.
- Several palm oil alternatives based on liquid oils, fully hydrogenated fats, and exotic fats have been developed.
- This article describes the advantages and disadvantages of those products and compares them to similar products based on palm oil. It also considers the feasibility of replacing palm products, since sustainable and 3-MCPD/glycidolester-reduced palm-based specialty oils are also available on the market.

On the other hand, palm oil is also under discussion, as it contains a relatively high level of saturated fatty acids (which have been a health issue for many years) compared to liquid oils, such as rapeseed or sunflower oil. Also, the cultivation of palm oil is often connected with issues of sustainability, although significant progress in this area has been made via organizations such as RSPO (Round Table for Sustainable Palm Oil).

The oil yield of palm oil per hectare (ha) is higher than that of any other oil crop, ranging between 4 to 6 million metric tons (MMT)/year and ha (in comparison, rapeseed oil yields 1.5–2.5 MMT/year and ha), yet palm oil has a higher potential to produce 3-MCPD (3-chloro-1,2-propandiol) and glycidolesters in the refining process compared to many other oils [2]. This has led to negative media coverage in certain countries, such as France and Norway, making palm oil an unpopular choice. Meanwhile, there also seems to be a growing interest in palm oil alternatives in other European countries. The pros and cons of each will be discussed in the following paragraphs.

HYDROGENATED ALTERNATIVES

Although partially hydrogenated fats are not an option to replace palm oil in food applications, a fully hydrogenated fat could potentially be used. Trans fatty acids are not an issue, because their levels change during the hydrogenation process (Fig. 1); at full hydrogenation (when almost all double bonds are saturated) a fat does not contain either unsaturated fatty



acids or trans-fatty acids in any significant quantity. The issue with fully hydrogenated fats (except for lauric fats, C12 and C14) is their high melting point (typically $>50^{\circ}\text{C}$); this results in a high “solid fat content” at body temperature, and, consequently, an unpleasant sensory experience. To achieve a melting profile that is appropriate for most food applications (almost liquid at body temperature, plus functionality at lower temperatures), a fully hydrogenated oil can be blended and interesterified with various non-hydrogenated oils to produce a functional and organoleptically acceptable end-product.

By blending liquid oils, such as rape, sunflower, soybean, and so on, with fully-hydrogenated oil(s), a good baking fat blend can be achieved. The melting profile is typically flatter than that of palm oil, especially at lower temperatures. However, in the temperature range between 20 and 40°C , which is critical in the processing of a baking fat, the melting profile is very similar to palm oil. Due to its relatively high amount of liquid oil, the blend will have a level of saturated fatty acids about 15% lower than palm oil, for a similar functioning product.

Another option would be to blend the hydrogenated liquid oil with a lauric fat, such as coconut oil. Using coconut oil in combination with fully hydrogenated oils will produce a much steeper melting profile and a higher solid fat content at 10 and 20°C . Due to the steep melting profiles and pleasant meltdown at body temperature, such blends can give very good results in confectionary fillings. However, as coconut oil and fully hydrogenated liquid oils both have a very high level of saturated fatty acids, the blend will be significantly higher in saturates than palm oil and therefore may be an issue for some customers and/or consumers.

Generally, when fully hydrogenated/saturated fats are used, the overall saturate content is dependent on the choice of additional components (either liquid or coconut oil). If liquid oils are used, the level is normally lower compared to palm oil; if coconut oil is used, the blend will have a significantly higher level of saturated fatty acids. Therefore, the required steep

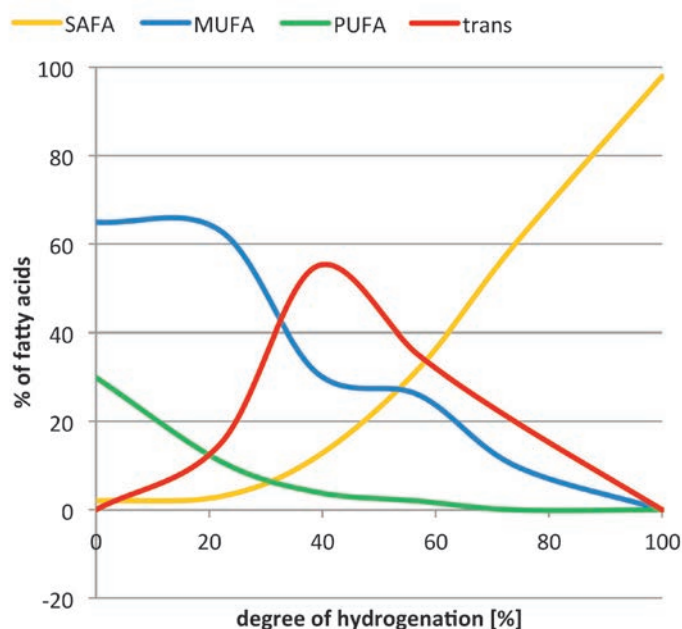


FIG. 1. Changes in trans and saturated fatty-acid levels during the hydrogenation process for a soybean oil

melting profile needed for confectionary fats can typically only be achieved by choosing a blend with a high level of saturated fatty acids. For solid frying fats, margarines, and bakery fat blends, where a rather flat melting profile could be applied, blends of fully hydrogenated oils with liquid oils could be used and have a relatively low level of saturated fatty acids—often even lower than palm oil itself.

Although the abovementioned fully hydrogenated fats do not contain trans-fatty acids, in many countries it is still necessary to label the process of hydrogenation on the food packaging—although the EU commission is reviewing this as part of potential “trans” legislation. If a food should be labeled as “non-hydrogenated” and “palm oil free,” fat blends based on exotic fats might therefore be a better solution.

ALTERNATIVES BASED ON EXOTIC FATS OR COCOA BUTTER

“Exotic fats” are typically fats such as shea, sal, illipé, kokum, and mango kernel. There are many other exotic fats, but these are not available in significant volumes. Exotic fats have in common a relatively high level of symmetrical triglycerides (triglycerides having a saturated-unsaturated-saturated configuration, such as SOS, POS, POP) and therefore need special tempering to crystallize into the desired stable form. Cocoa butter also has a high level of symmetrical triglycerides and, accordingly, a similar, probably even more complex, crystallization behavior; it therefore can also be used as a component in non-palm, non-hydro-fats. Some exotic fats, such as shea, are not used directly, but only after fractionation, with the symmetrical triglycerides being concentrated in the stearin fraction. The triglyceride configuration of some other exotic fats is already adequate for confectionery applications without the need for fractionation.

By blending liquid oils with shea stearin, fat blends can be produced that replace palm mid fractions—a filling fat in confectionary applications, for example. Still, to achieve the required stable crystal configuration, it is necessary to temper the filling, as with chocolate. Especially at room temperature (ca. 20°C), a non-tempered fat with a high level of symmetrical triglycerides will often be too soft. It will also tend to recrystallize during storage and can cause fat bloom. Consequently, a slight amendment of the recipe and processing conditions might still be necessary, as these blends will not have exactly the same melting profile as palm mid fractions (Fig. 2).

Due to their high level of liquid oils, such blends will mostly have a level of saturated fatty acids lower than the originally used palm mid fraction. In the described example, the non-palm fat has a level of saturated fatty acids of 41% compared to a level of 59% in a palm mid fraction.

Blending exotic fats with liquid oils therefore can be a good solution to replace palm-oil-based fats in some confectionary applications, provided equipment to temper the product is available. In many cases, though, the production lines are not equipped with tempering equipment, and an upgrade would be costly. By interesterifying the exotic fats, the symmetrical triglycerides will be partially converted to asymmetrical triglycerides. In this way, a fat can be produced that is based on exotic fats, but does not need tempering due to the reconfiguration of the triglycerides.

Intesterified exotic fats themselves typically have a high solid fat content across a broad temperature range, as well as a melting temperature much higher than body temperature. Used alone and without the addition of liquid oils or coconut oil, they are mostly too solid to be used in foods. Similar to fully-hydrogenated fats, the addition of lauric fats to the interesterification blend with exotic fats gives the best results with respect to the melting profile. A high solid fat content at 10 and 20°C and an almost liquid product at body temperature can be achieved. Again, the use of coconut oil in such an interesterified product will increase the level of saturated fatty acids and result in mostly higher levels compared to palm based products.

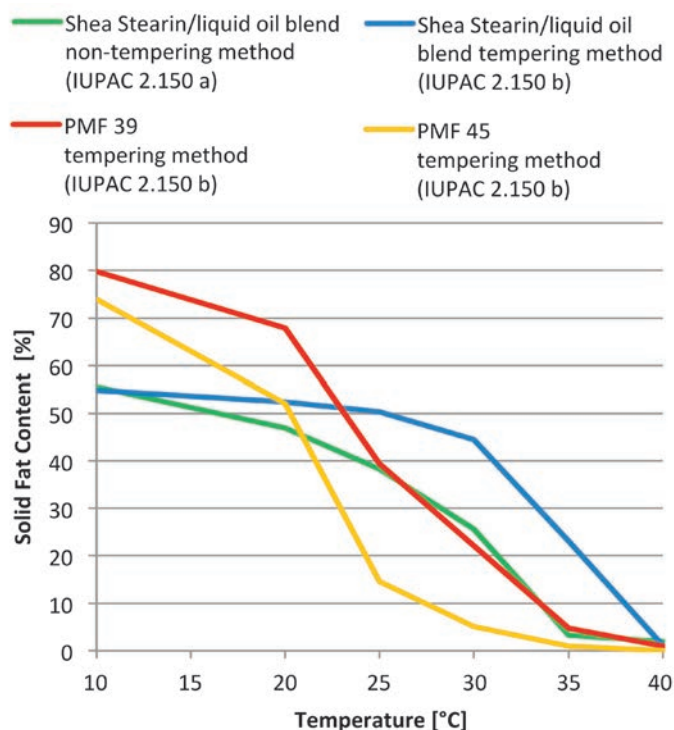


FIG. 2. Solid fat contents of palm oil mid fractions (iodine value 39 and 45) and of a shea stearin/liquid oil blend. The results for the shea stearin/liquid oil blend are shown once measured with a non-tempering method and once with a long-time tempering method.

One of the major drawbacks of non-palm and non-hydro fats based on shea, cocoa butter, or other exotic fats is the high price of the raw materials. While in most cases a specialty fat is not composed exclusively from exotic fats, a replacement by a non-palm solution is significantly more expensive than a palm based product, for the same performance.

Traditionally, shea butter is used in cosmetics and pharmaceutical products, which has given shea a reputation as a non-food oil. In addition, the availability of exotic fats is limited, and the demand is high. Shea is rarely grown on plantations; the nuts are mainly collected in the savannah by local people and sold in small volumes to processing plants. This makes the supply potentially susceptible to political unrests or other unexpected events. Other exotic fat trees such as Illipé only crop at irregular intervals.

LIQUID OILS AS ALTERNATIVES

A very obvious way to replace palm oil would be to use liquid oils, such as rapeseed, sunflower, or soy. Due to their liquid aggregate state, liquid oils cannot replace all of the palm fractions needed for every application. Still, in applications where only small volumes of fat are used—bread, wafers, and release agents, for example—or where liquid fractions of palm oil (oleins) are applied, a replacement of palm by liquid oils is often possible.

In frying applications, palm fractions such as palm olein IV 64 or palm olein IV 56 can very often be replaced by liquid oils. Still, here the choice of the right liquid oil is also important. Most liquid oils have a significant lower level of saturated fatty

acids and a higher level of polyunsaturated fatty acids than palm oil. This gives them a positive reputation with respect to health, but on the other hand, a lower stability in terms of oxidative degradation. Linoleic acid is about 40 times more reactive than oleic acid and linolenic acid is about 2.4 times more reactive than linoleic acid [3]. Therefore, oils with a high level of saturated and/or monounsaturated fatty acids have a higher stability against oxidation than oils with a high level of fatty acids with two or more double bonds. Fig. 3 shows the rancimat stability index of various oils. Due to its high level of saturated and monounsaturated fatty acids, palm oil and its fractions have a relatively high rancimat stability index and are therefore ideal for frying applications in which the fat is typically used at high temperatures of about 180°C. These high temperature conditions promote oxidation of frying fats, especially in less stable oils.

Conventional rapeseed oil and sunflower oil have a high level of polyunsaturated fatty acids and therefore oxidize relatively quickly in the frying process. From the oxidation, aldehydes and other breakdown products arise, some of which may also be regarded as unhealthful [4]. The use of liquid oils that contain a high level of oleic acid is more appropriate for replacing palm oil in frying applications. High oleic sunflower and high oleic rapeseed oil would both be good solutions, as they have high levels of monounsaturated fatty acids and a rancimat stability index only slightly lower than palm oil or palm olein, due to their elevated levels of oleic acid and reduced levels of both linoleic and linolenic acid.

3-MCPD- AND GLYCIDOLESTERS

3-MCPD and glycidolesters are contaminants that are formed during the refining process, especially during the deodorization step. Various researchers demonstrated that in traditionally processed palm oil, the levels of both 3-MCPD and glycidolesters are significantly higher than in many other edible oils/fats [2, 5]. However, such contaminants are not confined only to palm. Amounts of 3-MCPD- and glycidolesters also occur in the refining process of almost all other oils and fats—albeit at generally lower levels, and 3-MCPD- and glycidolester levels in palm oil can be partially mitigated by applying newly developed process steps.

As illustrated above, it is possible to replace palm oil with liquid oils, blends with exotic fats, or blends with fully hydrogenated liquid oils. Such substitutions will present some technological challenges, but these are not insurmountable. Still, the question remains whether the exclusion of palm oil is actually fully viable or necessary. Fats such as coconut oil and cocoa butter, which may have a perceived health advantage over palm oil, have similar or even higher levels of saturated fatty acids. Also, sustainable palm oil is already commercially available, and efforts are being made to improve its sustainability. With improved refining techniques, it is possible to produce palm oil and palm oil fractions with lower levels of 3-MCPD and glycidolesters. Admittedly, such mitigation will incur additional costs, but non-palm oil alternatives—especially those based on exotic fats—will have an even higher cost.

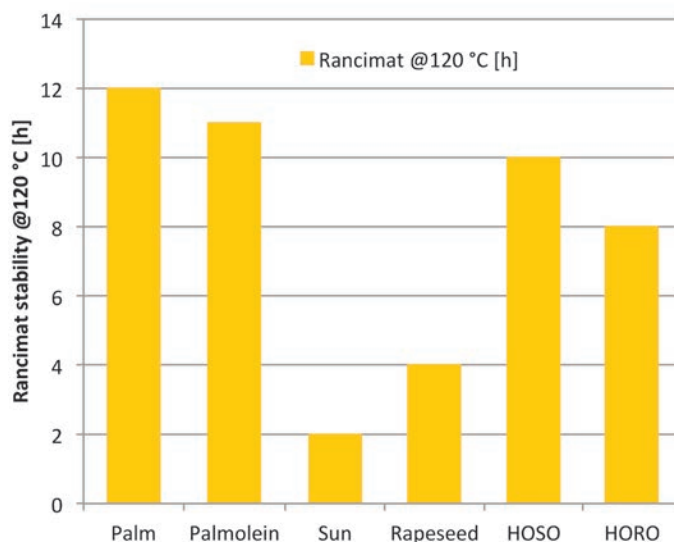


FIG. 3. Rancimat stability at 120°C of different oils and fats. Fats with a higher level of saturated fatty acids (palm oil/palmolein) and with a high level of oleic acid (high oleic sunflower oil (HOSO)/high oleic rapeseed oil (HORO)) show significantly higher values than sunflower and rapeseed oil, which have high levels of fatty acids with two or more double bonds.

The good news is that technologies for the production of non-palm alternatives for virtually all product types exist, but sustainable and 3-MCPD- and glycidolester-mitigated palm oils are also becoming more and more available. Therefore, customers will have a choice of solutions to meet their individual requirements.

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Avocado: characteristics, health benefits, and uses

Patrícia Fonseca Duarte, Marcia Alves Chaves, Caroline Dellinghausen Borges, and Carla Rosane Barboza Mendonça

- Avocados are rich in protein, potassium, and unsaturated fatty acids. They also contain fat-soluble vitamins lacking in other fruits.
- Their oil has a fatty acid composition similar to olive oil, and compounds present in the lipid fraction, such as omega fatty acids, phytosterols, tocopherols, and squalene, are recognized for their health benefits.
- This article reviews the characteristics, health benefits, current applications, and potential uses of this major tropical fruit and its oil.

In 2011, world avocado production reached 4.4 million tons, increasing about 20% from 2007 to 2011. Mexico is the largest avocado producer, accounting for 25% of world production, followed by Chile with 8.5% [FAO, 2013]. The increasing productivity has occurred due to advances in post-harvest technologies, reduction in trade barriers, strong health-related claims, and increased incentives and cultivated areas in the producing countries [Almeida and Sampaio, 2013].

Brazil is the 9th largest avocado producer, with 160,400 tons harvested on 10,750 hectares, in 2011. Avocado is the 17th most-produced fruit in the country. São Paulo state is the largest producer (47.5%), followed by Minas Gerais (19.0%), and Paraná (11.2%) [Almeida and Sampaio, 2013].

CULTIVATION

The avocado tree, which is native to Mexico and Central America, belongs to the *Lauraceae* family, the genus *Persea*, and comprises two subgenera: *Persea* and *Eriodaphne*. The avocado grown commercially belongs to two species of the genus *Persea*, being divided into three botanical varieties: *Persea americana* Miller var. *Drymifolia* (Mexican species); *Persea americana* Miller var. *American* (West Indian species); and *Persea nubigena* Miller var. *Guatemalensis* (Guatemalan species) [Koller, 1992].

The main climatic requirements of the avocado tree are related to temperature and rain fall, and vary by race. Trees of the Antillean race (common), which grow in the lowland regions of South America and Central America, bear large fruits that are piriform in shape, have a low oil content (less than 8%), and are resistant to temperatures as low as -2°C. Varieties of the Guatemalan race, which grow in the highlands of Central America, bear round fruits that mature late, have a higher oil content (8–20%), and are resistant to temperatures as low as -4°C. In contrast, the Mexican races, which grow in the highlands of Mexico and the Andes, are characterized by small fruits with a high oil content (greater than 20%). They are also the most resistant to low temperatures (down to -6°C) [Almeida and Sampaio, 2013; HORTIBRASIL, 2013].

The avocado tree has a very high fruit yield, reaching a production of 138 kg seven years after planting. It is a perennial plant and can be grown in rough locations, where it will not compete with the annuals that are adapted to flat lands [Bleinroth and Castro, 1992].

The fruits of the Quintal variety (a hybrid of the Antillean and Guatemalan races) are large (500–800 g) and piriform. They have green-smooth skin, yellow flesh, and medium to large seeds, which are relatively loose within the fruit. They are the most-consumed variety in Brazil, and their lipid fraction is comprised of approximately 63% omega-9 fatty acids [Koller, 1992]. The avocado of the Hass variety is the most-exported worldwide, with an average size of 180 to 300g [Borges and Mello, 2011].

Avocado growth and development is intense and differs from that of other fruit species, as the fruit completes maturation post-harvest, when major changes in metabolism and an increasing respiratory rate result in a high production of ethylene. These rapid changes make the fruits highly perishable, and much is wasted. In this sense, avocado pulp processing contributes to the avocado's best use—by converting the fruit into storable food and oil products with less waste [Kluge *et al.*; SENAI, 2006; Rocha, 2008].

FRUIT CARE AND CONSERVATION

In addition to perishability, factors such as mechanical damage; compression and cut; and physiological, chemical, and biochemical changes can cause changes in the color, aroma, taste, and texture of the fruit [Sanches *et al.*, 2008].

Although these changes may not be immediately apparent when the fruit is intact, the pulp may be partially or completely dark when the fruit is ripe [Bleinroth and Castro, 1992; Sanches *et al.*, 2008]. Consequently, refrigerated packaging (4–7°C) is recommended to delay rot development post-harvest. Wax application can also improve the appearance and increase the shelf life of intact fruits by reducing the transpiration rate and metabolic activity [Darvas *et al.*, 1990].

Unfortunately, cold storage can negatively affect lipid concentration, and the longer the storage time at low temperatures, the lower the fat content of the fruit will be [Tremocoldi, 2011]. Kluge, *et al.*, showed that fruits of the Quintal variety can be stored for 14 days at 7°C and 85–90% relative humidity, then stored commercially at room temperature for 3 to 4 days.

Enzymatic browning catalyzed by the enzyme polyphenol oxidase (PPO) and the degradative reactions of peroxidase (POD) are other factors that depreciate the commercial value of the avocado. The activity of these enzymes in various fruits and vegetables leads to considerable economic losses, as well as a reduction in nutritional and sensory quality. In some avocado varieties, PPO activity increases after climacteric fruit ripening, with differences among varieties of the same species [Luiz *et al.*, 2006]. To control enzymatic browning reactions, acidulants such as citric acid may be added to preserve the fruit and to enable its use in food products [Lugo *et al.*, 2006].

Whereas avocado is a climacteric fruit, the plant hormone ethylene can be controlled with chemical compounds. The ethylene action inhibitor 1-methylcyclopropene, is one compound that has been used to extend shelf life. It can be applied in a gaseous state, and is effective for 24 hours at 20°C and at concentrations ranging from 30nL L⁻¹ to 500nL L⁻¹ [Pereira *et al.*, 2007].



NUTRITIONAL AND PHYSICOCHEMICAL CHARACTERISTICS

Avocado is consumed in various forms in northern South America, Central America, and Mexico: as puree salads, seasoned with salt, pepper, vinegar, and other condiments—as well as in the preparation of other dishes [Koller, 1992]. In Brazil, the ripe fruit is mixed together with sugar, honey, and liqueurs, and consumption is influenced heavily by its sensory and nutritional characteristics [Luiz *et al.*, 2007].

The pulp content in several varieties is between 52.9 and 81.3%, relative to fruit mass [Tango *et al.*, 2004]. High lipid and low carbohydrate levels remain in avocado pulp after water removal, thus conferring a high dry matter content to the product. Therefore, avocado is considered to be one of the few cultured fruits with a lipid fraction as the major component [Tremocoldi, 2011], accounting for as much as 25% of the fruit portion [Hierro *et al.*, 1992; Abreu *et al.*, 2009]. The pulp contains 67 to 78% moisture, 13.5 to 24 % lipids, 0.8 to 4.8% carbohydrate, 1.0 to 3.0% protein, 0.8 to 1.5% ash, 1.4 to 3.0% fiber, and has an energy density between 140 and 228kcal [Soares and Ito, 2000]. The nutritional value of avocado is four

times that of any other fruit except the banana. The avocado not only contains proteins (1 to 3%), but also significant levels of fat-soluble vitamins (Francisco and Baptistella, 2005), folic acid, and appreciable amounts of calcium, potassium, magnesium, sodium, phosphorus, sulfur, silicon, and vitamins E, B1, B2, and D [Dembitsky *et al.*, 2011].

The fruit is exceptionally high in potassium (339mg 100g⁻¹) compared to other fruits. Potassium regulates muscle activity and protects the body from cardiovascular diseases [Cancian *et al.*, 2008]. Avocados are also a source of glutathione, a powerful antioxidant that acts on potentially carcinogenic compounds [Wang *et al.*, 2012].

BIOACTIVE COMPOUNDS

Avocado also contains substantial amounts of bioactive compounds such as phytosterols—especially in the lipid fraction, the main representative being β -sitosterol [Salgado *et al.*, 2008b; Santos *et al.*, 2014b]. Diets rich in phytosterols can lead to the reduction of total cholesterol and low-density lipoprotein (LDL) cholesterol [Lottenberg, 2002]. A 17% average decrease in blood cholesterol levels was observed in a study in Mexico of 45 volunteers who consumed avocado once a day for one week [Borges and Melo, 2011].

Phytosterol is a substance of vegetable origin whose structure is very similar to cholesterol. Its mechanism of action in the body involves the inhibition of intestinal cholesterol absorption and decreased hepatic cholesterol synthesis. According to Brufau *et al.* (2008), it acts on total plasma cholesterol and LDL cholesterol without affecting high-density lipoprotein (HDL) and blood triglycerides. Replacing saturated by unsaturated fats has also been shown to promote a decrease in total cholesterol and LDL and an increase in HDL levels [Salgado *et al.*, 2008a].

The β -sitosterol in avocados has a positive effect on immunity, which can assist in the treatment of diseases such as cancer, HIV, and infections. In the case of cancer, β -sitosterol suppresses carcinogenesis. In HIV, it strengthens the immune system [Bouic, 2002]. The compound enhances lymphocyte proliferation and natural killer cell activity against invading microorganisms (Bouic *et al.*, 1996). In addition, studies have shown that β -sitosterol activity aids weight loss by reducing compulsive eating binge and fat accumulation in the abdominal region [Senai, 2006; Murta, 2013].

The health effects of sterols and stanols have been the subject of several studies. Some authors have demonstrated a 25% reduction in the risk of coronary heart disease with the consumption of 2g of such compounds per day. Such compounds can be incorporated into margarines, spreads, and vegetable oils via esterification—without affecting vitamin solubility [Turatti, 2002].

The avocado oil variety Margarida contains a greater diversity of sterols, with β -sitosterol representing 71.8% of the total. Margarida avocado oil also has lower cholesterol levels (0.3%) than other varieties in which cholesterol can be as high as 2.3% [Salgado *et al.*, 2008b]. Santos *et al.* (2014b) investigated oil from the Fortuna avocado that had been extracted with petroleum ether and subjected to drying under forced



air (40°C). Of the total phytosterols, 87.6% was β -sitosterol, 12.41% campesterol, and 0.04% stigmasterol.

Avocado also contains the carotenoid lutein, which helps protect against prostate cancer and eye diseases such as cataracts and macular degeneration [Johnson, 2005].

AVOCADO OIL

Avocado pulp has a high lipid content (5 to 35%), which is mostly made up of unsaturated fatty acids (60–84%) [Borges and Melo, 2011]. The avocado varieties with lower core and shell percentages are the most suitable for oil extraction due to higher pulp yields, for which the Quintal variety stands out [Tango *et al.*, 2004].

The high moisture content in fresh pulp is the main obstacle to obtaining avocado oil, as it negatively affects extraction yields and production costs. Thus, the varieties most suitable for oil extraction based on 18% lipids and low moisture levels in the pulp are: Hass, Fuerte, and Glória, followed by Collinson, Anaheim, Itzamna, Wagner, Ouro Verde, Carlsbad, and Mayapan [Tango *et al.*, 2004].

Although some authors have reported a yield of 59% in oil extracted from fleshy pulp when using hexane as a solvent, this value decreased to 12% when acetone was used (Abreu *et al.*, 2009).

Santos *et al.* (2013) evaluated the extraction yields of Fortuna avocado oil as a function of the drying process (freeze-drying or air flow: 40 to 70°C) and the extraction method (pressing and solvent) of a pulp containing 5 to 6.5% moisture. The authors reported oil contents between 25 and 33% by cold pressing, and between 45 and 57% by solvent extraction. The freeze-drying method achieved a higher oil yield than oven drying under forced air did. Enzyme-assisted aqueous extraction has emerged as an alternative and environmentally friendly extraction process [Abreu *et al.*, 2009].

The small avocado oil volume currently produced by some countries is used in its raw form by the pharmaceutical and

cosmetics industries, as its unsaponifiable fraction is responsible for regenerative properties of the epidermis. Avocado oil is easily absorbed by the skin and readily absorbs scents, characteristics that are of great value to the cosmetics industry. In addition, avocado easily forms the emulsions needed in the manufacture of fine soaps [Tango *et al.*, 2004].

Compared to other vegetable oils, avocado oil has high levels of monounsaturated fatty acids (oleic and palmitoleic acids), low polyunsaturated fatty acids (linoleic acid), and relatively high levels of saturated fatty acid (palmitic and stearic acids). Fatty acid composition is influenced by the cultivars, stage of maturity, anatomical region of the fruit, and geographic location for plant growth [Tango *et al.*, 2004].

Rocha (2008) has reported that avocado oil from the varieties Wagner, Fortuna, Hass, and Fuerte had higher levels of monounsaturated fatty acid (MFA) ranging from 59 to 72% of total fatty acids; followed by saturated fatty acids (SFA) ranging from 17 to 23%; and polyunsaturated fatty acids (PUFA) ranging from 10% to 14%.

Santos *et al.* (2014a) determined the fatty acid profile of Fortuna avocado, evaluating the effect of the pulp drying process (freeze-drying or air circulation: 40 and 70°C) and oil extraction method (solvent or pressing). The authors reported that oleic fatty acid represented more than half of the total fatty acids of this raw material, together with substantial amounts of unsaturated linoleic and palmitoleic acids. They also verified that the dehydration of the pulp can affect the

fatty acid profile since the oil extracted from the lyophilized pulp contained higher levels of unsaturated fatty acids. No significant effects were observed with respect to the extraction method.

Avocado oils from the Northrop, Duke, Wagner, Quintal, and Fuerte varieties are characterized by having more than 63% oleic acid, while oils from the Rincon, Barker, Waldin, Prince, and Panchoy varieties had less than 50% of this fatty acid. Palmitic acid content ranged between 15.38 and 32.37% in oils from different varieties. Therefore, the avocado variety affected the levels of palmitic acid and oleic acid; varieties with high oleic acid levels had low palmitic acid levels and *vice versa* [Bleinroth and Castro, 1992]. In addition to their fatty acid composition, these oils contain other bioactive minor components such as tocopherols, squalene, β -sitosterol, campesterol, and cycloartenol acetate, all of which have positive effects on health [Dembitsky *et al.*, 2011; Santos *et al.*, 2014b].

Pure avocado oil can be used as a substitute for olive oil, while olive oil and avocado oil can be combined to replace other oils in olive oil mixtures. Avocado oil for salad dressings should be submitted to winterization to eliminate saturated triglycerides, which can cloud oil stored at low temperatures [Salgado *et al.*, 2008b].

Although lipid extraction generates large accumulations of pulp residues in the processing industries, the high fiber content of these by-products make them suitable for use in the

Soap Manufacturing Technology, Second Edition

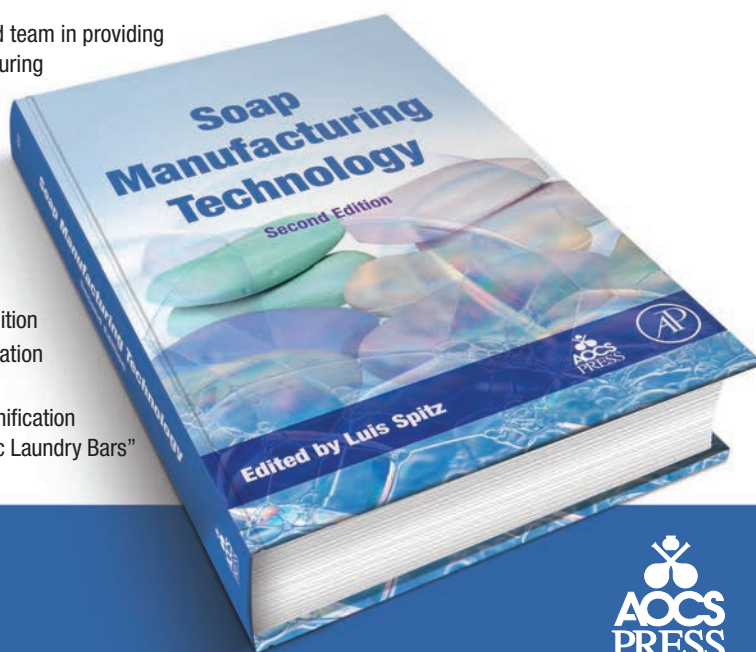
Edited by Luis Spitz

May 2016 | ISBN 978-1-630670-65-8

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preparation of flour for fiber-rich cookies, breads, pasta, and other bakery products. [Chaves *et al.*, 2013].

APPLICATIONS AND FORMS OF PULP

The processed products of avocado pulp include paste, puree, and guacamole. Guacamole is a fruit pulp seasoned with salt, onion, lemon, pepper and tomato that is produced in an artisanal way and marketed by some US companies [Daiuto *et al.*, 2011].

The sensory quality of guacamole made from avocados of the Hass variety, without chemical additives and stored under refrigeration, was evaluated based on the type of packaging used. Greater consumer acceptance was observed for the product stored in containers with a gas barrier than those stored in a polyethylene package [Daiuto *et al.*, 2011]. Although these authors have also considered treating the guacamole with heat to inactivate polyphenol oxidase, the application of heat can result in bitterness and off-flavors, and changes the texture, contributing to a mashed appearance.

Chaves *et al.* (2013) studied avocado pulp from the Margarida variety that had been dehydrated and defatted by cold pressing to partially replace wheat flour and butter, respectively, in whole grain crackers. The authors reported that the flour from avocado pulp exhibited characteristics similar to those of conventional flour and whole wheat flour. The biscuits had higher mineral and fiber levels, with good sensory acceptance.



Notice of Annual Business Meeting

AOCS' annual business meeting will be held on Monday, May 1, 2017 at 10:30 am at the Rosen Shingle Creek Hotel, Orlando, Florida, USA. Routine business of the Society will be conducted.

Held in conjunction with the

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Meat derivatives can also be supplemented with avocado pulp to reduce saturated fats, and vegetable oils and fats can be incorporated into emulsified meat products to enhance their fatty acids balance [Kayaardi and Gök, 2004; Lugo *et al.*, 2006]. The replacement of animal fats by vegetable oils in meat products has been studied with positive effects on the chemical, physical and sensory characteristics of the products, but with negative effects on water activity and texture [Lugo *et al.*, 2006].

Products that contain high levels of vegetable oil, like avocado, are sensitive to oxidation, resulting in rancidity and hence production of undesirable flavors and loss of quality during storage. Elez-Martinez *et al.* (2005) demonstrated the ability to control oxidative rancidity in processed avocado puree with the use of α -tocopherol and ascorbic acid.

Several preservation methods have been studied to obtain a stable avocado pulp, including pasteurization, drying, oil extraction, freezing, and freeze-drying [Palou *et al.*, 2000; Soliva *et al.*, 2001; Soliva-Fortuny *et al.*, 2004]. Use of microwave heating and copper chloride to preserve color of mashed avocado has been also investigated [Guzmán *et al.*, 2002]. Furthermore, chemical reducing agents, sequestrants, acids, nitrogen atmosphere and vacuum [Soliva *et al.*, 2001], and high hydrostatic pressure treatment have been studied [Jacobo-Velázquez and Hernández-Brenes, 2012].

BY-PRODUCTS

Avocado seeds are underutilized and represents a large portion of the fruit. Thus, use of this by-product could significantly increase the value from edible oil production. Unfortunately, the seeds contain phenolic compounds that exhibit toxicity, which limits their use. Studies have shown that if ethanol is used to extract the phenolic compounds, the seeds can be used in feed for monogastric animals [Ichimaru *et al.*, 1982].

Avocado leaves are a pharmaceutical ingredient widely used in extracts for therapeutic purposes, and also as teas in folk medicine [Vendruscolo and Mentz, 2006], mostly for their diuretic properties [Wright *et al.*, 2007].

Finally, phytochemicals, such as orhamnetin, luteolin, rutin, quercetin, and apigenin have been isolated from avocado leaves. Such compounds can help prevent the progress of various diseases related to oxidative stress [Owolabi *et al.*, 2010].

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Partial hydrogenation, *sans trans*

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

Laura Cassiday

On June 18, 2015, the US Food and Drug Administration determined that partially hydrogenated oils (PHOs) are no longer generally recognized as safe (GRAS) for use in human food because they contain trans fatty acids, which have been linked to cardiovascular disease. As a result, food companies have faced the challenging task of developing alternative oils and fats that mimic the functionality of PHOs in foods. After June 18, 2018, PHOs may be used in foods in the United States only as additives subject to the FDA's approval. But what if PHOs that lack harmful trans fats could be produced? A recent paper by Ximena V. Yépez at Purdue University (West Lafayette, Indiana, USA) and Kevin M. Keener at Iowa State University (Ames, USA; formerly at Purdue) demonstrates a technique to generate partially hydrogenated soybean oil without trans fatty acids through high-voltage atmospheric cold plasma (HVACP) hydrogenation (<http://dx.doi.org/10.1016/j.ifset.2016.09.001>, 2016). However, this pilot method still needs to be optimized and expanded to the industrial scale, note Keener and other experts.

Traditional hydrogenation of soybean oil involves reacting the unsaturated oil with hydrogen gas in the presence of a nickel catalyst. On the catalyst's surface, hydrogen atoms attach to carbon atoms in C=C double bonds, under conditions of high temperature (150–235 °C) and moderate pressure (1–3 atm). Most polyunsaturated fatty acids are reduced to mono-unsaturated fatty acids, which converts the liquid oil into a solid or semi-solid fat. The hydrogenation can be controlled by varying the reaction conditions, resulting in a PHO with the desired functional properties. Unfortunately, during the reaction some of the remaining C=C double bonds in the fatty acids are converted from the cis to the trans isomer. Trans fatty acids in the diet raise LDL, or “bad,” cholesterol, while lowering HDL, or “good,” cholesterol.

In their study, Yépez and Keener investigated the use of HVACP to produce partially hydrogenated soybean oil lacking *trans* fatty acids. “Atmospheric plasmas are basically ionized gases at atmospheric pressure,” says Keener. “In our case, we used a high voltage (90 kV) to ionize hydrogen gas, creating

highly energized hydrogen species.” The researchers actually used a mixture of hydrogen and nitrogen (HN) gas because the blend is less flammable than pure hydrogen gas. The atomic hydrogen species generated by HVACP can attach to C=C double bonds in fatty acids, similar to the traditional hydrogenation process. However, HVACP does not require high temperatures or a catalyst, so trans fatty acids are unlikely to form, says Keener.

The researchers placed 10 mL soybean oil between the two electrodes in the plasma chamber (Fig. 1). The sample was flushed with HN gas and then treated with HVACP for up to 12 hours. The 12-hour treatment reduced the iodine value (IV) of the soybean oil from 133 to 92, indicating increased fatty acid saturation. For comparison, the traditional soybean oil hydrogenation process requires 1–4 hours to achieve a similar IV reduction. The soybean oil increased in viscosity after HVACP treatment, another sign of increased saturation.

The 12-hour HVACP treatment reduced the content of polyunsaturated fatty acids (PUFAs) in the soybean oil

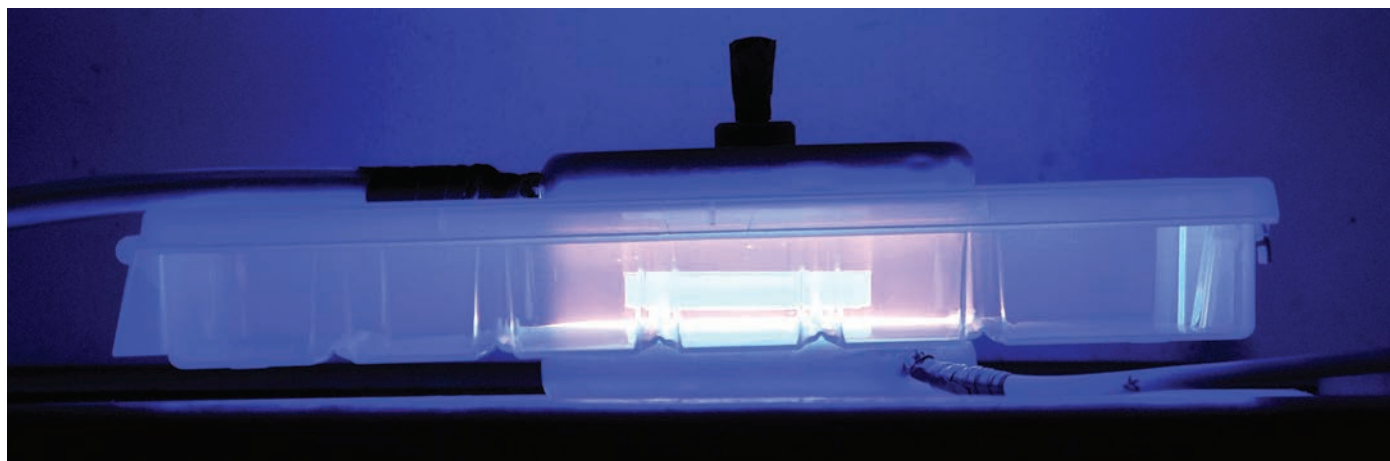


FIG. 1. During the HVACP treatment, soybean oil was reacted with hydrogen atoms generated by applying a high voltage to HN gas.

Credit: Ximena Yépez

by 16.2%, while monounsaturated fatty acids increased by 4.6%, and saturated fatty acids increased by 12%. When the researchers analyzed the fatty acid composition by gas chromatography, they found that linolenic (18:3) and linoleic (18:2) acids significantly decreased, whereas oleic (18:1), stearic (18:0), and palmitic (16:0) acids significantly increased.

In traditional hydrogenation of soybean oil, trans fatty acids form from the geometrical isomerization of oleic and linoleic acid. Yépez and Keener did not detect any of these trans fatty acids in PHO produced by HVACP. However, the researchers did observe a minor chromatographic peak that did not correspond with any of the standards. The researchers are now trying to identify this by-product with NMR. “It could be, for instance, that there’s one hydrogen and one nitrogen atom added to the double bond,” says Keener. “We’re actually seeing this in some cases where we used a hydrogen/nitrogen gas combination.” However, Keener emphasizes that the new compound is only 1.9% of the total oil content after the 12-h HVACP treatment—about 20 times less abundant than the trans fatty acids formed in the traditional procedure.

In addition to the mystery compound, it is possible that other unintentional products, such as polymerized fatty acids, may be formed during the process, says Albert Dijkstra, a consultant to the oils and fats industry based in St. Eutrope-de-Born, France. Polymerization products may not elute from the GC column, and thus would not be detected. Dijkstra notes that a similar situation occurs when fat is used for deep frying: The 16:0 content appears to increase, while the PUFA content decreases. “But in fact the 16:0 content stays the same, and the PUFA content goes down even further than they thought,” says Dijkstra. “They are forming unknown oligomers of unknown dietary effects.” Keener acknowledges that they cannot currently rule out fatty acid polymerization, but his group intends to explore this issue with NMR.

“This is quite an interesting process, but the sample size of 10 mL is a factor of a million below what would be a useful commercial quantity,” says Alan Paine, product specialist at Desmet Ballestra in Brussels, Belgium. “The paper describes a small-scale curiosity, but this is what electricity was when Faraday started lecturing about it in the early 1800s, so you never know.”

Information

Yépez, X. V. and Keener, K. M. (2016) “High-voltage atmospheric cold plasma (HVACP) hydrogenation of soybean oil without *trans*-fatty acids.” *Innovative Food Sci. Emerg. Technol.* 38: 169–174. <http://dx.doi.org/10.1016/j.ifset.2016.09.001>

Keener is optimistic that the procedure could be scaled up for industrial PHO production. “There are a couple of aspects that make it very favorable for scalability,” says Keener. “One is the low power requirement. It’s a few hundred watts of energy, and the initial changes to the oil seem to be fairly quick. There’s a small amount of oil that almost immediately starts to show increased viscosity, and then the amount builds over time.” Keener says that the efficiency of the process could be improved by increasing the contact time between the oil and the plasma, perhaps with a spray or bubbling system or a falling film. “The two priorities for commercialization are building a bigger electrode system and increasing the contact time,” says Keener. “Both of those are engineering challenges, but I think solvable.” Another advantage of the HVACP hydrogenation is that it can be conducted at ambient temperature and pressure, without a catalyst.

When scaled up, HVACP hydrogenation should be comparable in cost to traditional hydrogenation, estimates Keener. Currently, a popular alternative to partial hydrogenation is fully hydrogenating palm oil, and then interesterifying it with a liquid oil to produce *trans*-free fats with properties similar to PHOs. But sustainable sourcing of palm oil can be challenging and costly, notes Keener. On the other hand, soybean oil is produced and readily available in the United States. “The HVACP-produced oil would be able to compete with palm oil because it has similar characteristics,” he says. “It could particularly be useful for baking and some frying situations where there’s high temperature.”

Olio is produced by Inform’s associate editor, Laura Cassiday. She can be contacted at laura.cassiday@aocs.org.

US retailer Target to eliminate PFCs, phthalates, parabens

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

Kelly Franklin

US retailer Target plans to phase out phthalates, flame retardants, perfluorinated chemicals, and parabens from a variety of product categories in both its own brands and the products it sells. The announcement is part of the company's new chemicals policy that will also require its suppliers to provide full ingredient disclosure for some product categories.

The policy comes shortly after Target named removing "unwanted chemicals" from products among its 2020 responsible sourcing aspirations.

The company's first goals under the program are:

- **chemical management:** removing perfluorinated chemicals (PFCs) and potentially carcinogenic or harmful flame retardants from textiles—including such products as clothing, carpets, and upholstered furniture—by 2022. And, eliminating phthalates, NPEs, propyl-paraben, butyl-paraben, formaldehyde, and formaldehyde-donors from all beauty, baby care, personal care, and cleaning products by 2020. The initial goal covers only the latter products' formulations, but may be extended to their packaging;
- **transparency:** for suppliers to disclose all chemical ingredients to Target—including fragrances—in beauty, baby care, personal care, and cleaning products by 2020; and
- **innovation:** investing up to \$5 million in green chemistry innovation by 2022.

Jennifer Silberman, chief sustainability officer, described the strategy as "one of the most comprehensive in the US retail industry, including all Target-owned and national brand products and operations, not just formulated products." And, by using its "size, scale, and expertise," it expects to make significant progress.



CHEMICALS POLICY

The company says it set its chemical management goals around addressing unwanted chemicals with the biggest potential health impact. This included consideration of how prevalent they are in products, and which product categories are priorities to consumers.

Beyond specific substance phase-outs, Target's broader chemicals management policy calls for working with business partners to implement policies and practices that "facilitate the management of chemicals through the supply chain and across our operations."

This will include using hazard profiles to prioritize substances for restriction in products and processes, with an emphasis on identifying which have the greatest impact on workers and customers.

Full transparency of ingredients between Target and its brands, said Silberman, is an essential part of the process in order to identify where substances of concern occur. A long-term goal is full material disclosure across all products and processes across its operations.

CHANGES AND INNOVATION

Target also recognizes that safer alternatives may not be readily available for all necessary applications, which is why it included the "innovation" investment of up to \$5 million.

"Safer alternatives may not exist today, so therefore we are absolutely committed to pursuing new approaches to chemical development," Silberman told *Chemical Watch*.

While the company is continuing to explore projects and partners to work with, it has already begun this work with the Green Chemistry and Commerce Council's (GC3) Preservatives Challenge, and has been having productive conversations with possible partners, said Silberman.

"Innovation in this space needs a lot of resources and needs a lot of vested interest, and it's our commitment to ensure that we're looking at the full value chain of how safer alternatives can be created" in developing a green chemistry approach.

"Ultimately it's in the best interest for all of our stakeholders to have the greatest amount of alternatives," added Silberman, "which is part of the reason why we felt the innovation goal was essential to expedite and catalyze that investment."

Mike Schade, Mind the Store campaign manager at NGO Safer Chemicals Healthy Families, told *Chemical Watch* that Target's new commitments have made "important improvements that up the ante for the entire retail sector." He hopes its efforts will "help drive a race to the top among the nation's largest retailers."

Schade added that retailers investing in green chemistry "can help accelerate the transition" to healthier products.

Kelly Franklin is editor, North America, for Chemical Watch.

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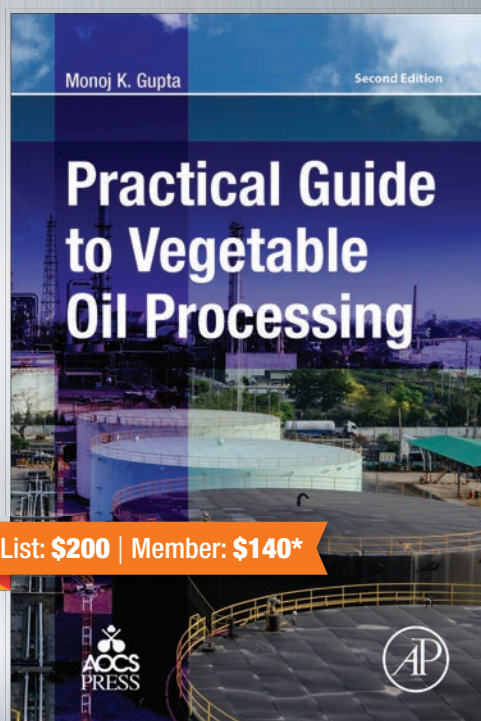
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Argentina: lipids as excipients in pharma

Leslie Kleiner

Lipids are widely used in pharmaceutical, personal care, and even in some nutraceutical applications. To better understand their role as excipients, and to gain insight into pharma R&D in Argentina, I interviewed Alejandro Ledesma, PharmD, Market Development Manager at Ferromet SA, Buenos Aires, Argentina.


Q: How are lipids commonly used for drug delivery in pharmaceutical applications?

Lipids are one of the biggest and widest organic-compound families, and are among the first family of compounds to be used therapeutically. From basic moisturizing-agents to sustained release functionality, lipids are extremely versatile and can be used in many applications. Some of these applications are oral formulations, such as soft/hard gelatin capsules, solid tablets, and liquid formulations. Lipids can be used as fillers, solubility enhancers, wetting agents, surfactants, binders, lubricants, disintegrates, sustained release agents, taste masking, and bioavailability enhancers, among other applications. Other examples are topical formulations, such as creams, lotions, and ointments, in which lipids are commonly used in the oil phase. A further example is that of parenteral products, in which lipids primarily function as solvents.

Q: What are excipients, and what are some common lipid-based excipients?

The best way to define excipients is by stating what they are not: An excipient is everything in a pharmaceutical formulation that is not the “Active Pharmaceutical Ingredient-drug”(API). Excipients may vary depending on the pharmaceutical dosage form (aerosols, capsules, tablets, and so on). For example, a sweetener might be essential in a syrup, while most likely will be useless in a suppository. Nevertheless, there are four rules all excipients must comply with: They must be (1) chemically stable; (2) non-toxic; (3) inert; and (4) innocuous. Ideally, they should also be economical and have acceptable organoleptic properties.

Lipids are widely used either as the almost sole component of a formula (e.g., suppositories and ointments) or as a technical aid (e.g., as lubricants). The most common uses of lipid-based excipients are in the oil phases of creams and emulsions, or as emulsifiers, skin penetration enhancers, solubilizers, or binder ingredients in other applications.



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



Q: In Argentina, are health and beauty products regulated by the same agency that regulates drugs?

Yes; in August 1992, a decentralized agency from the National Public Administration was created by decree 1490/92: the ANMAT, National Administration of Drugs, Foods and Medical Devices (Administración Nacional de Medicamentos, Alimentos y Tecnología Médica, www.anmat.gov.ar). The ANMAT is under the authority of the Ministry of Health, and according to the official website, it was created to “efficiently fulfill the process of authorization, registration, standardization, vigilance, and monitoring of the products used in the human medicine, food, and cosmetic fields” so as to “guarantee that drugs, foodstuff, medical devices at the reach of the public be compliant in terms of efficacy (fulfillment of the therapeutic, nutritional or diagnostic objective), safety (high benefit/risk ratio), and quality (by meeting the citizens’ needs and expectations)” [1].

Q: In Argentina, what are areas of interest in pharmacy R&D?

According to recent studies [2], Argentina’s pharmaceutical industry is the 4th biggest in LATAM (behind Brazil, Mexico, and Venezuela). In Argentina, 230 labs operate locally, and there are over 190 active manufacturing sites listed; combined, they employ over 160,000 people (both directly and indirectly). Despite the fact that the 20 most-used APIs concentrate 30% of the market, over 2,000 drugs are currently used (mainly imported), and almost 70% of local consumption is supplied with local products.

As pretty much everywhere in the world, pharmacy products in Argentina can be divided into two categories: prescription drugs and “over the counter” (OTC) products (medicines sold directly to consumers, without a professional prescription). Prescription drugs are high-added-value products, but their sales volumes are smaller when compared to the OTC

Further reading

- [1] http://www.anmat.gov.ar/webanmat/institucional/que_es_la_anmat_en.asp
- [2] Escenario y perspectivas de la Industria Farmacéutica Nacional – CILFA (02 de Julio, 2015)

segment. In this case, R&D is mainly focused on formulating proper pharmaceutical vehicles and dosage forms for new molecules introduced to the market, and exploring new, updated, and enhanced excipients that could improve the general performance of the final product. Now, when we talk about OTC products, we’re talking about high-volume and low-margin medicines, for which customer usually have plenty of options and brands. Since such products are not prescribed by a professional, and mass advertising is allowed, consumers usually select well-publicized (and therefore, “known”) and low-cost products. This leads to two major tendencies: cost reduction, and product innovation/differentiation. While the first is self-explanatory, the second involves launching new versions of already marketed products with a modified form (soft gelatin capsules instead of tablets, for example), different flavors, packaging improvements, and other features that distinguish the relaunched product from similar ones on the market.

Q: What is the nutraceutical market like in Argentina?

As in other parts of the world, there is increasing interest in preventing rather than treating illness. Prevention efforts are primarily focused on educating people about healthful lifestyles that lead to improved wellbeing disease prevention. The health industry has responded by creating a new market segment comprised of health/ dietary supplements and nutraceutical products. Because such products are neither food nor drugs, they are not yet fully regulated. Vitamins, minerals, omega-3 fatty acids, CLA (conjugated linoleic acids), and herbal extracts, are just some of the products in this segment. This market is growing steadily and, as users gain experience and knowledge, is being pushed toward higher-quality standards that more closely resemble those of pharmaceutical products than those used in the food industry. Nutraceuticals, are not expected to replace prescription drugs nor OTC’s, but rather complement them. One thing is for sure, they are here to stay, and we will have to keep an eye on them.

Latin America Update is produced by Leslie Kleiner, R&D Project Coordinator in Confectionery Applications at Roquette America, Geneva, Illinois, USA, and a contributing editor of *Inform*. She can be reached at LESLIE.KLEINER@roquette.com.



PATENTS

Labile esters of agrochemicals for controlled release and reduction of off-site movement

Morgenstern, D.A., Monsanto Technology LLC, US9402396, August 2, 2016

The present invention relates to esters of carboxylic acid agrochemicals comprising a labile protecting group and having formula (I). Certain of the esters of carboxylic acid agrochemicals do not undergo hydrolysis to a significant degree in the dark, but are cleaved to regenerate the parent carboxylic acid agrochemical when exposed to light. Others of the esters of carboxylic acid agrochemicals undergo hydrolysis under both light and dark conditions. The present invention further relates to methods for the controlled release of a carboxylic acid agrochemicals, and to methods of controlling unwanted plants comprising applying to the unwanted plants an ester of a carboxylic acid agrochemical.

Oxidized and partially hydrogenated oil or fat

Hanajiri, T., *et al.*, J Oil Mills Inc., US9351502, May 31, 2016

An oil or fat composition described herein has a hydrogenated aroma and flavor as a conventional oil or fat composition and a long lasting hydrogenated aroma, while having trans-fatty acid content reduced to the same extent as that in a general liquid salad oil, and the starting material thereof. The oxidized and partially hydrogenated oil or fat contains C18:2 trans-isomer from 10 to 60 weight percent on the total constituent fatty acid content basis and has peroxide value from 8 to 350 meq/kg. A ratio by weight of the C18:2 trans-isomer content to C18:1 trans-isomer content in all the constituent fatty acids can be 0.3 to 1.8.

Plasticizer for low-temperature unwind with weight retention during heat aging

Laufer, C.H., *et al.*, Dow Global Tech LLC, US9403965, August 2, 2016

The present disclosure is directed to a plasticizer, polymeric compositions containing the plasticizer, and conductors coated with the polymeric composition. The plasticizer includes (i) an epoxidized fatty acid methyl ester, (ii) an epoxidized natural oil, and (iii) an epoxidized tallate ester. Polymeric compositions containing a polymeric resin and the plasticizer exhibit a weight loss less than 50 mg/cm² after exposure to 100°C for seven days. Conductors coated with the polymeric composition (containing the plasticizer) pass the low temperature unwind test of UL719 and exhibit a weight loss less than 50 mg/cm² after exposure to 100°C for seven days.

Dispersant for spilled oil

Lochhead, R.Y., *et al.*, Univ. Southern Mississippi, US9352291, May 31, 2016

A method for dispersing oil spilled into the aqueous environment comprising the treatment of the spilled oil with an oil dispersant composition comprising a phospholipid and a polysaccharide derivative is disclosed.

Oil/fat composition comprising α -linolenic acid

Homma, R. and M. Yuumi, Kao Corp, US9392806, July 19, 2016

Provided is a fat or oil composition that may reduce degradation odor to a less extent at the time of cooking even though comprising α -linolenic acid in a large amount, and is capable of improving the taste and flavor of the cooked food. The fat or oil composition comprises the following components (A) and (B): (A) a fat or oil whose constituent fatty acids comprise from 10 to 50 mass percent of α -linolenic acid; and (B) 0.05 to 1.8 mass percent of a free type triterpene alcohol.

High moisturizing cleansing composition containing a lamellar phase

Jung, C.J., *et al.*, Amorepacific Corp, US9399007, July 26, 2016

The present invention relates to a lamellar structure which stabilizes oil and to a cleansing composition comprising the lamellar structure. More particularly, the present invention relates to a lamellar structure, which stabilizes oil to be used in a cleansing composition so that high-content oil is converted to a lamellar phase and contained in a composition. The present invention also relates to a cleansing composition which comprises the lamellar structure which enables the composition to contain high-content oil in a stable manner and to thus provide superior moisturizing ability.

Process for refining glyceride oil and purifying triglyceride oil obtained by such process

Bin Md Noor, A., Sime Darby Malaysia Berhad, US9428440, August 30, 2016

The present invention relates to a process for refining glyceride oil by size exclusion chromatography to obtain a triglyceride enriched fraction. The process comprises passing a glyceride oil through a size exclusion column packed with porous particles having a mass weighted mean particle size of 20 to 1,000 μ m and an average pore size of 10 to 150 Å without using any solvent and collecting an eluate fraction enriched in triglyceride. The process may further comprise passing a solvent through the size exclusion column after the eluate fraction enriched in triglyceride is collected to obtain a partial glyceride enriched fraction. The process can suitably be used to produce triglyceride enriched fraction having a triglyceride content that is close to 100%.

Natural oil-based gels, applications, and methods of preparation

Tanner, J.T., Ethox Chemicals LLC, US9376647, June 28, 2016

The present invention relates to novel non-aqueous gels of natural oils and their derivatives and provides a novel process for the gelation of natural oils and their derivatives that does not require the addition of gellants or the irreversible heat bodying of the oil. The gels and method of the present invention are applicable to a wide range of natural oils, and the method is easily tailored to provide thermoreversible gels of any desired viscosity. The natural oil based gels of the present invention and the method of their preparation have many advantages over the prior art. The natural oil based gels provided have exemplary properties and find use in a variety of applications.

Biodiesel emulsion for cleaning bituminous coated equipment

Reinke, G., *et al.*, Ergon Asphalt and Emulsions Inc., Alm Holding Co, US9394507, July 19, 2016

Methods of cleaning equipment, such as hand tools dirtied by bituminous mixture. A biodiesel emulsion comprising biodiesel, water and emulsifier(s), is applied to the surface of the equipment for a period of time (e.g., at least about 15 min) and optionally agitated. The biodiesel emulsion produces cleaning properties comparable to straight biodiesel, at a cost reduction, due to the replacement of biodiesel with water.

Acetylated polyol hydroxystearate plasticizers and plasticized polymeric compositions

Chaudhary, B.I. and S. Sczekalla Beate, Dow Global Technologies LLC, US9394425, July 19, 2016

Plasticizers having an acetylated polyol hydroxystearate and plasticized polymeric compositions containing such plasticizers. Such plasticized polymeric compositions can be employed in forming various articles of manufacture, such as coated conductors.

Methods for analysis of isomeric lipids

Campbell, J.L. and Y. Leblanc, Dh Technologies Dev Pte Ltd., US9360455, June 7, 2016

A method for analyzing a sample that contains a plurality of lipid isomers is described that involves forming one or more lipid metal ion adducts and transporting the one or more lipid metal ion adducts through a differential mobility spectrometer to cause separation of the one or more lipid metal ion adducts from each other. The lipid isomers can be chosen, for example, from fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids, and prenol lipids. Particular examples include phosphatidylcholine regioisomers such as 1-palmitoyl-2-oleoyl-sn-phosphatidylcholine (POPC) and 1-oleoyl-2-palmitoyl-sn-phosphatidylcholine, and triacylglycerols containing palmitic and oleic acid groups. The metal chosen can

include a cationization reagent that contains sodium, potassium, silver or lithium.

Process for preparing a carboxylic acid from a diol or from an epoxide by oxidative cleavage

Lemaire, M., *et al.*, Univ. Claude Bernard Lyon, Oleon, Interoleagineuse D Assistance Et De Dev S I A Soc, Centre Nat Rech Scient, US9359280, June 7, 2016

A process for preparing a carboxylic acid by oxidative cleavage of at least one vicinal diol, or an epoxide, wherein the reaction is carried out in the presence of a catalyst and of an oxidizing agent and in the absence of solvent.

Ultrasonic and megasonic method for extracting palm oil

Goodson, J.M. and K.L. Teong, Megasonic Sweeping Inc., US9388363, July 12, 2016

A process for extracting palm oil includes an ultrasonic horn press and a megasonic clarifier. The ultrasonic horn press uses ultrasonic vibrations to rupture the palm fruit. After pressing and filtering the palm oil from the ultrasonic horn press, the megasonic clarifier applies megasonic vibrations to clarify the palm oil. The ultrasonic horn press and megasonic clarifier significantly reduce the use of water and minimizes pollution as compared to conventional processes.

Fabric conditioning composition

Schramm, C.J., Jr. and K. Truong, Colgate Palmolive Co., US9441188, September 13, 2016

A fabric conditioning composition comprising an emulsion of particles in an aqueous vehicle, the particles comprising (a) fatty acid triglyceride, and (b) a water swellable cationic polymer.

Phospholipid-based powders for drug delivery

Weers, J.G., *et al.*, Novartis Ag, US9439862, September 13, 2016

Phospholipid based powders for drug delivery applications are disclosed. The powders comprise a polyvalent cation in an amount effective to increase the gel-to-liquid crystal transition temperature of the particle compared to particles without the polyvalent cation. The powders are hollow and porous and are preferably administered via inhalation.

Patent information is compiled by Scott Bloomer, a registered US patent agent with Archer Daniels Midland Co., Decatur, Illinois, USA. Contact him at scott.bloomer@adm.com.



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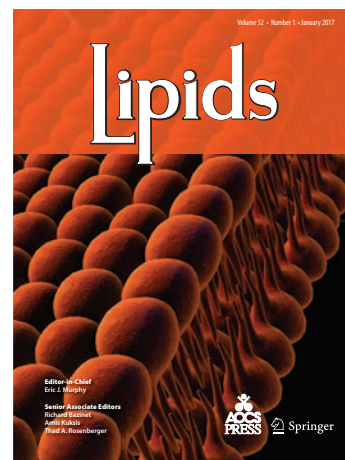
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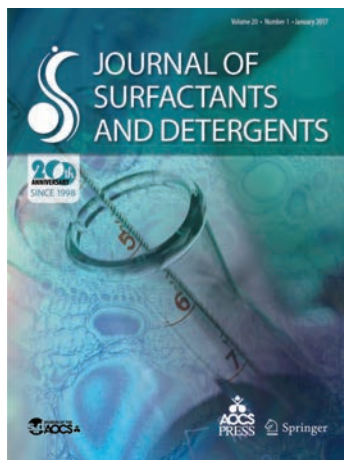
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EXTRACTS & DISTILLATES

Extraction and liquid chromatography—tandem mass spectrometry detection of 3-monochloropropanediol esters and glycidyl esters in infant formula

Leigh, J.K. and S. MacMahon, *J. Agric. Food Chem.* 64: 9442–9451, 2016, <http://dx.doi.org/10.1021/acs.jafc.6b04361>.

A method was developed for the extraction of fatty acid esters of 3-chloro-1,2-propanediol (3-MCPD) and glycidol from infant formula, followed by quantitative analysis of the extracts using liquid chromatography–tandem mass spectrometry (LC-MS/MS). These process-induced chemical contaminants are found in refined vegetable oils, and studies have shown that they are potentially carcinogenic and/or genotoxic, making their presence in edible oils (and processed foods containing these oils) a potential health risk. The extraction procedure involves a liquid–liquid extraction, where powdered infant formula is dissolved in water and extracted with ethyl acetate. Following shaking, centrifugation, and drying of the organic phase, the resulting fat extract is cleaned-up using solid-phase extraction and analyzed by LC-MS/MS. Method performance was confirmed by verifying the percent recovery of each 3-MCPD and glycidyl ester in a homemade powdered infant formula reference material. Average ester recoveries in the reference material ranged from 84.9 to 109.0% (0.6–9.5% RSD). The method was also validated by fortifying three varieties of commercial infant formulas with a 3-MCPD and glycidyl ester solution. Average recoveries of the esters across all concentrations and varieties of infant formula ranged from 88.7 to 107.5% (1.0–9.5% RSD). Based on the validation results, this method is suitable for producing 3-MCPD and glycidyl ester occurrence data in all commercially available varieties of infant formula.

Factors affecting tocopherol concentrations in soybean seeds

Carrera, C.S. and P. Seguin, *J. Agric. Food Chem.* 64: 9465–9474, 2016, <http://dx.doi.org/10.1021/acs.jafc.6b03902>.

Soybean seeds contain several health-beneficial compounds, including tocopherols, which are used by the nutraceutical and functional food industries. Soybean tocopherol concentrations are, however, highly variable. Large differences observed in tocopherol concentrations among soybean genotypes together with the relatively simple biosynthetic pathway involving few genes support the feasibility of selecting for high-tocopherol soybean. Tocopherol

concentrations are also highly influenced by environmental factors and field management. Temperature during seed filling and soil moisture appear to be the main factors affecting tocopherol concentrations; other factors such as soil fertility and solar radiation also affect concentrations and composition. Field management decisions including seeding date, row spacing, irrigation, and fertilization also affect tocopherols. Knowledge of factors affecting soybean tocopherols is essential to develop management strategies that will lead to the production of seeds with consistent target concentrations that will meet the needs of the nutraceutical and functional food industries.

Encapsulation of pancreatic lipase in hydrogel beads with self-regulating internal pH microenvironments: retention of lipase activity after exposure to gastric conditions

Zhang, Z., F. Chen, R. Zhang, Z. Deng, and D.J. McClements, *J. Agric. Food Chem.* 64: 9616–9623, 2016, <http://dx.doi.org/10.1021/acs.jafc.6b04644>.

Oral delivery of lipase is important for individuals with exocrine pancreatic insufficiency; however, lipase loses activity when exposed to the highly acidic gastric environment. In this study, pancreatic lipase was encapsulated in hydrogel beads fabricated from alginate (gel former), calcium chloride (cross-linker), and magnesium hydroxide (buffer). Fluorescence confocal microscopy imaging was used to map the pH microclimate within the hydrogel beads under simulated gastrointestinal tract (GIT) conditions. The pH within buffer-free beads rapidly decreased when they moved from mouth (pH 6.3) to stomach (pH <4), leading to a loss of lipase activity in the small intestine. Conversely, the pH inside buffer-loaded beads remained close to neutral in the mouth (pH 7.33) and stomach (pH 7.39), leading to retention of lipase activity in the small intestine, as shown by pH-stat analysis of lipid digestion. The presence of the encapsulated buffer also reduced bead shrinkage under gastric conditions.

High quality, good health: the case for olive oil

Bernardini, E. and F. Visioli, *Eur. J. Lipid Sci. Technol.* 119: 15005052, 2017, <http://dx.doi.org/10.1002/ejlt.201500505>.

The healthful properties of olives and their main derivatives are being actively investigated. In this review, we briefly appraise and descriptively review the most recent evidence on the purported correlations between olive oil consumption and health, and we provide some bibliometric analyses that show how fast the field of “olive components and health” is indeed moving. Based on accumulated findings, we expect olive oil (and related products) research to further grow in the next few years, when more olive-derived nutraceuticals and functional foods will enter the market and when targeted basic mechanism research will further clarify the manifold mechanisms of action of olive (poly)phenols. Practical applications: Our findings underscore the need to produce and

consume high quality olive oil, whose “minor components” are endowed with healthful properties.

Executive functions and the ω -6-to- ω -3 fatty acid ratio: a cross-sectional study

Sheppard, K.W. and C.L. Cheatham, *Am. J. Clin. Nutr.* 105: 32–41, 2017, <http://dx.doi.org/10.3945/ajcn.116.141390>.

The ω -6 (n -6) to ω -3 (n -3) fatty acid (FA) ratio (n -6: n -3 ratio) was previously shown to be a predictor of executive function performance in children aged 7–9 y. We aimed to replicate and extend previous findings by exploring the role of the n -6: n -3 ratio in executive function performance. We hypothesized that there would be an interaction between n -3 and the n -6: n -3 ratio, with children with low n -3 performing best with a low ratio, and those with high n -3 performing best with a high ratio. Children were recruited on the basis of their consumption of n -6 and n -3 FAs. The executive function performance of 78 children aged 7–12 y was tested with the use of the Cambridge Neuropsychological Test Automated Battery and a planning task. Participants provided blood for plasma FA quantification, and the caregiver completed demographic and activity questionnaires. We investigated the role of the n -6: n -3 ratio in the entire sample and separately in children aged 7–9 y (n = 41) and 10–12 y (n = 37). Dietary and plasma n -6: n -3 ratio and n -3 predicted performance on working memory and planning tasks in children 7–12 y old. The interaction between dietary n -6: n -3 ratio and n -3 predicted the number of moves required to solve the most difficult planning problems in children aged 7–9 y and those aged 10–12 y, similar to results from the previous study. There was also an interaction between the plasma n -6: n -3 ratio and n -3 predicting time spent thinking through the difficult 5-move planning problems. The n -6: n -3 ratio and n -3 predicted executive function performance differently in children aged 7–9 y and in those aged 10–12 y, indicating different optimal FA balances across development. The n -6: n -3 ratio is an important consideration in the role of FAs in cognitive function, and the optimal balance of n -6 and n -3 FAs depends on the cognitive function and developmental period studied.

Sex differences in the effect of fish-oil supplementation on the adaptive response to resistance exercise training in older people: a randomized controlled trial

Da Boit, M., *et al.*, *Am. J. Clin. Nutr.* 105: 151–158, 2017, <http://dx.doi.org/10.3945/ajcn.116.140780>.

Resistance exercise increases muscle mass and function in older adults, but responses are attenuated compared with younger people. Data suggest that long-chain n -3 polyunsaturated fatty acids (PUFAs) may enhance adaptations to resistance exercise in older women. To our knowledge, this possibility has not been investigated in men. We sought to determine the effects of long-chain n -3 PUFA supplementation on resistance exercise training-induced increases in muscle mass and function and whether

these effects differ between older men and women. Fifty men and women [men: n = 27, mean \pm SD age: 70.6 ± 4.5 y, mean \pm SD body mass index (BMI; in kg/m^2): 25.6 ± 4.2 ; women: n = 23, mean \pm SD age: 70.7 ± 3.3 y, mean \pm SD BMI: 25.3 ± 4.7] were randomly assigned to either long-chain n -3 PUFA (n = 23; 3 g fish oil/d) or placebo (n = 27; 3 g safflower oil/d) and participated in lower-limb resistance exercise training twice weekly for 18 wk. Muscle size, strength, and quality (strength per unit muscle area), functional abilities, and circulating metabolic and inflammatory markers were measured before and after the intervention. Maximal isometric torque increased after exercise training to a greater (P < 0.05) extent in the long-chain n -3 PUFA group than in the placebo group in women, with no differences (P > 0.05) between groups in men. In both sexes, the effect of exercise training on maximal isokinetic torque at 30, 90, and 240° s^{-1} , 4-m walk time, chair-rise time, muscle anatomic cross-sectional area, and muscle fat did not differ (P > 0.05) between groups. There was a greater (P < 0.05) increase in muscle quality in women after exercise training in the long-chain n -3 PUFA group than in the placebo group, with no such differences in men (P > 0.05). Long-chain n -3 PUFAs resulted in a greater decrease (P < 0.05) than the placebo in plasma triglyceride concentrations in both sexes, with no differences (P > 0.05) in glucose, insulin, or inflammatory markers. Long-chain n -3 PUFA supplementation augments increases in muscle function and quality in older women but not in older men after resistance exercise training.

Branched-chain fatty acid composition of human milk and the impact of maternal diet: the Global Exploration of Human Milk (GEHM) Study

Dingess, K.A., *et al.*, *Am. J. Clin. Nutr.* 105: 177–184, 2017, <http://dx.doi.org/10.3945/ajcn.116.132464>.

An understudied component of the diet, branched-chain fatty acids (BCFAs) are distinctive saturated fatty acids that may have an important influence on health. Human-milk fatty acid composition is known to differ worldwide, but comparative data are lacking on BCFAs. We tested the hypotheses that concentrations of BCFAs in human milk differ between populations and are associated with maternal diet. We surveyed the BCFA composition of samples collected as part of a standardized, prospective study of human-milk composition. Mothers were enrolled from 3 urban populations with differing diets: Cincinnati, Ohio; Shanghai, China; and Mexico City, Mexico. Enrollment was limited to healthy mothers of term singleton infants. We undertook a cross-sectional analysis of milk from all women with samples at postpartum week 4 (n = 359; ~120 women/site). Fatty acids were extracted from milk by using a modified Bligh-Dyer technique and analyzed by gas chromatography. Statistical analysis was performed by ANOVA and Tobit regression. For Cincinnati mothers, 24-h diet recalls were analyzed in relation to the individual BCFA concentrations measured in milk samples. Total BCFAs in milk differed by site, with the highest concentration in Cincinnati followed by Mexico City and Shanghai (mean \pm SE: 7.90 ± 0.41 , 6.10 ± 0.36 , and 4.27 ± 0.25 mg/100 mL, respec-

tively; $P < 0.001$). Site differences persisted after delivery mode, maternal age, and body mass index were controlled for. The individual concentrations of *iso*-14:0, *iso*-16:0, *iso*-18:0, *anteiso*-15:0, and *anteiso*-17:0 also differed between sites. Milk concentrations of *iso*-14:0 and *anteiso*-15:0 were associated with maternal intake of dairy; *iso*-16:0 was associated with maternal intakes of dairy and beef. BCFA concentrations in milk at 4 wk postpartum differed between mothers from Cincinnati, Shanghai, and Mexico City. Variations in human-milk BCFAs are influenced by diet. The impact of BCFAs on infant health warrants investigation.

Genetic variation of carotenoids, vitamin E and phenolic compounds in provitamin A biofortified maize

Muzhingi, T., *et al.*, *J. Sci. Food Agric.* 97: 793–801, 2017, <http://dx.doi.org/10.1002/jsfa.7798>.

Biofortified maize is not only a good vehicle for provitamin A carotenoids for vitamin A deficient populations in developing countries but also a source of vitamin E, tocochromanols and phenolic compounds, which have antioxidant properties. Using high-performance liquid chromatography and a total antioxidant performance assay, the present study analyzed the antioxidant variation and antioxidant activity of 36 provitamin A improved maize hybrids and one common yellow maize hybrid. The ranges of major carotenoids in provitamin A carotenoids biofortified maize were zeaxanthin [$1.2\text{--}13.2\ \mu\text{g g}^{-1}$ dry weight (DW)], β -cryptoxanthin ($1.3\text{--}8.8\ \mu\text{g g}^{-1}$ DW) and β -carotene ($1.3\text{--}8.0\ \mu\text{g g}^{-1}$ DW). The ranges of vitamin E compounds identified in provitamin A carotenoids biofortified maize were α -tocopherol ($3.4\text{--}34.3\ \mu\text{g g}^{-1}$ DW), γ -tocopherol ($5.9\text{--}54.4\ \mu\text{g g}^{-1}$ DW), α -tocotrienol ($2.6\text{--}19.5\ \mu\text{g g}^{-1}$ DW) and γ -tocotrienol ($45.4\ \mu\text{g g}^{-1}$ DW). The ranges of phenolic compounds were γ -oryzanol ($0.0\text{--}0.8\text{ mg g}^{-1}$ DW), ferulic acid ($0.4\text{--}3.6\text{ mg g}^{-1}$ DW) and *p*-coumaric acid ($0.1\text{--}0.45\text{ mg g}^{-1}$ DW). There was significant correlation between α -tocopherol and *cis* isomers of β -carotene ($P < 0.01$). Tocotrienols were correlated with α -tocopherol and γ -oryzanol ($P < 0.01$). Genotype was significant in determining the variation in β -cryptoxanthin, β -carotene, α -tocopherol and γ -tocopherol contents ($P < 0.01$). A genotype \times environment interaction was observed for γ -tocopherol content ($P < 0.01$).

Corn oil intake favorably impacts lipoprotein cholesterol, apolipoprotein, and lipoprotein particle levels compared with extra-virgin olive oil

Maki, K.C., *et al.*, *Eur. J. Clin. Nutr.* 71: 33–38, 2017, <http://dx.doi.org/10.1038/ejcn.2016.169>.

Corn oil (CO) and extra-virgin olive oil (EVOO) are rich sources of unsaturated fatty acids (UFA), but UFA profiles differ among oils, which may affect lipoprotein levels. The objective of this study was to assess the effects of CO versus EVOO intake on fasting lipoprotein and subfraction cholesterol levels, apolipoprotein (apo) A1, apo B, and low-density lipoprotein particle

concentrations in men and women. As part of a weight maintenance diet, men and women were provided with food items prepared with 54 g per day of CO or EVOO (21-day treatment, 21-day washout) in a randomized, double-blind, controlled-feeding, crossover trial. Fasting lipoprotein cholesterol and related variables were determined with density gradient ultracentrifugation. Among the 54 completers, CO reduced total cholesterol, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), non-high-density lipoprotein cholesterol (non-HDL-C), apo B and LDL particle concentration to a greater extent compared with EVOO intake. Changes in LDL-C and VLDL-C contributed to the larger reduction in non-HDL-C with CO compared with EVOO intake (-0.39 mmol/l vs -0.04 mmol/l ; $P < 0.001$). The larger reduction in LDL-C by CO intake was attributable to changes ($P < 0.05$) caused by CO vs EVOO in large LDL₁₊₂-C (-0.22 mmol/l) and intermediate-density lipoprotein cholesterol (-0.12 mmol/l). HDL-C responses did not differ between treatments, but apo A1 increased more with EVOO compared with CO intake (4.6 versus 0.7 mg/dl , respectively, $P = 0.016$). CO intake reduced atherogenic lipoprotein cholesterol and particle concentrations to a larger extent than did EVOO, which may have implications for cardiovascular disease risk.

Dietary sphingomyelin attenuates hepatic steatosis and adipose tissue inflammation in high-fat-diet-induced obese mice

Norris, G.H., *et al.*, *J. Nutr. Biochem.* 40: 36–43, 2017, <http://dx.doi.org/10.1016/j.jnutbio.2016.09.017>.

Western-type diets can induce obesity and related conditions such as dyslipidemia, insulin resistance and hepatic steatosis. We evaluated the effects of milk sphingomyelin (SM) and egg SM on diet-induced obesity, the development of hepatic steatosis and adipose inflammation in C57BL/6J mice fed a high-fat, cholesterol-enriched diet for 10 weeks. Mice were fed a low-fat diet (10% kcal from fat) ($n = 10$), a high-fat diet (60% kcal from fat) (HFD, $n = 14$) or a high-fat diet modified to contain either 0.1% (w/w) milk SM ($n = 14$) or 0.1% (w/w) egg SM ($n = 14$). After 10 weeks, egg SM ameliorated weight gain, hypercholesterolemia and hyperglycemia induced by HFD. Both egg SM and milk SM attenuated hepatic steatosis development, with significantly lower hepatic triglycerides (TGs) and cholesterol relative to HFD. This reduction in hepatic steatosis was stronger with egg SM supplementation relative to milk SM. Reductions in hepatic TGs observed with dietary SM were associated with lower hepatic mRNA expression of PPAR γ -related genes: *Scd1* and *Pparg2* in both SM groups, and *Cd36* and *Fabp4* with egg SM. Egg SM and, to a lesser extent, milk SM reduced inflammation and markers of macrophage infiltration in adipose tissue. Egg SM also reduced skeletal muscle TG content compared to HFD. Overall, the current study provides evidence of dietary SM improving metabolic complications associated with diet-induced obesity in mice. Further research is warranted to understand the differences in bioactivity observed between egg and milk SM.

A naturally occurring mixture of tocotrienols inhibits the growth of human prostate tumor, associated with epigenetic modifications of cyclin-dependent kinase inhibitors p21 and p27

Huang, Y., *et al.*, *J. Nutr. Biochem.*: 155–163, 2017, <http://dx.doi.org/10.1016/j.jnutbio.2016.10.019>.

Tocotrienols, members of the vitamin E family, have three unsaturated bonds in their side chains. Recently, it has been suggested that the biological effects of tocotrienols may differ from that of tocopherols. Several *in vitro* studies have shown that tocotrienols have stronger anticancer effects than tocopherols. VCaP cell line used in this study is from a vertebral bone metastasis from a patient with prostate cancer. Eight-week-old male NCr(–/–) nude mice were subcutaneously injected with VCaP-luc cells in matrigel and then administered a tocotrienol mixture for 8 weeks. The tocotrienol mixture inhibited the growth of human prostate tumor xenografts in a dose-dependent manner. The concentrations of tocotrienols and their metabolites were significantly increased in treatment groups. Tocotrienols inhibited prostate tumor growth by suppressing cell proliferation, which was associated with the induction of the cyclin-dependent kinase (CDK) inhibitors p21 and p27. In addition, tocotrienol treatment was associated with elevated H3K9 acetylation levels at proximal promoter regions of p21 and p27 and with decreased expression of histone deacetylases. Tocotrienols inhibited human prostate tumor growth, associated with up-regulation of the CDK inhibitors p21 and p27. Elevated expression of p21 and p27 could be partly due to the suppressed expression of HDACs.

Lipid class specific quantitative analysis of n-3 polyunsaturated fatty acids in food supplements

Kutzner, L., *et al.*, *J. Agric. Food Chem.* 65: 139–147, 2017, <http://dx.doi.org/10.1021/acs.jafc.6b03745>.

Supplementation products containing n-3 PUFA from marine sources serve a large market. Although the amount of eicosapentaenoic acid and docosahexaenoic acid in the products is provided by the manufacturer, no or little information is available on their lipid pattern. Therefore, we quantitatively analyzed the fatty acid pattern in the lipid fractions triglycerides, phospholipids, ethyl esters, and free fatty acids in supplementation products by means of solid phase extraction and gas chromatography. Twelve products from the European and U.S. markets containing fish, krill, algal, or plant oil were analyzed. Total n-3 PUFA content ranged from 68 g/100 g fat (fish oil) to 42 g/100 g fat (algal oil) to 17 g/100 g fat (krill oil). On the basis of the n-3 PUFA containing lipid class, the supplements can be separated dominantly in ethyl ester, re-esterified triglyceride, triglyceride, and phospholipid containing products. Algae-based products contained natural triglycerides, krill oils a complex mixture of phospholipids, triglycerides, and free fatty acids, and fish oil products either ethyl esters, re-esterified triglycerides, or triglycerides. Even products of the same class and

source showed distinct differences in their lipid pattern. A specification of the lipid composition of n-3 PUFA products would allow distinguishing the different (qualities of) supplements.

Industrial Applications

Oilseed pressing and vegetable oil properties and upgrading in decentralised small-scale plants for biofuel production

Buffi, M., *et al.*, *Int. J. Oil, Gas Coal Technol.* 14: 1–2, 2017 <http://dx.doi.org/10.1504/IJOGCT.2017.081104>.

Vegetable oils became a very popular bioliquid for renewable power and transport applications. At local level, the short chain production of vegetable oils is currently important in terms of sustainability and use of rural area. This present work investigates the development of vegetable oils production chain in the Tuscany territory, focusing also to valorisation of the co-products (i.e., oil cake). The work is based on the study of the cultivation of rapeseed and sunflower, and the creation of an extraction plant based on cold-screw pressing technology, in order to obtain a raw vegetable oil suitable as biofuel. The German DIN 51623 standard was adopted to investigate the quality of vegetable oils obtained from different oil crops. Vegetable oils quality was evaluated by comparing the oil produced by a bench press at lab scale, comparing the results in terms of process yields and lipid fraction recovered. Due to the high phosphorous content of rapeseed oil, out of the DIN limits, an experimental procedure to remove P content and restore the oxidation stability of the oils was performed. The results showed a substantial P reduction by adding 2 wt% of P-removing additive. The oil cake properties were evaluated in view of its potential use for bioenergy production, in particular in terms of metals content.

The effect of microwave pretreatment on some physico-chemical properties and bioactivity of black cumin seed oil

Bakhshabadi, H., *et al.*, *Crops Prod.* 97: 1–9, 2017, <http://dx.doi.org/10.1016/j.indcrop.2016.12.005>.

In the current study, different processing times including 90, 180, and 270 s and microwave powers including 180, 540, and 900 W were applied for optimizing of the extraction process. After microwave pre-treatments, the oil seeds were extracted with screw press with different rates (11, 34, and 57 rpm), then parameters including extraction efficiency, oxidative stability, peroxide, and acidity index, DPPH free radical scavenging activity as well as the refractive index of the extracted oil were studied. Statistical analysis and process optimization was performed with the use of *response surface methodology (RSM)*. The results revealed that enhancement in the microwave power and the processing time increased extraction efficiency, acidity index and oil peroxide value, but it decreased the oxidative stability value of the achieved oil. The

achieved results also showed up that the studied parameters had no significant impacts on the refractive index; moreover the extraction efficiency was reduced with an enhancement in the rotational rate of the screw press. According to the process optimization results, it might be stated that with applying processing time for about 185.44 S, microwave pretreatment of 718.65 W and screw-rotation speed of the press of 11 rpm, the desired outcomes are reached.

Microalgae biorefinery: high-value products perspectives

Chew, K.W., *et al.*, *Bioresource Technol.* 229: 53–62, 2017, <http://dx.doi.org/10.1016/j.biortech.2017.01.006>.

Microalgae have received much interest as a biofuel feed-stock in response to the uprising energy crisis, climate change and depletion of natural sources. Development of microalgal biofuels from microalgae does not satisfy the economic feasibility of overwhelming capital investments and operations. Hence, high-value co-products have been produced through the extraction of a fraction of algae to improve the economics of a microalgae biorefinery. Examples of these high-value products are pigments, proteins, lipids, carbohydrates, vitamins, and anti-oxidants, with applications in cosmetics, nutritional, and pharmaceuticals industries. To promote the sustainability of this process, an innovative microalgae biorefinery structure is implemented through the production of multiple products in the form of high value products and biofuel. This review presents the current challenges in the extraction of high value products from microalgae and its integration in the biorefinery. The economic potential assessment of microalgae biorefinery was evaluated to highlight the feasibility of the process.

Production of liquid biofuels (biodiesel and bioethanol) from brown marine macroalgae *Padina tetrastromatica*

Ashokkuma, V., *et al.*, *Energ. Convers. Manage.* 135: 351–361, 2017, <http://dx.doi.org/10.1016/j.enconman.2016.12.054>.

In this study, an integrated biomass conversion concept of producing liquid biofuels from brown marine macroalga *Padina tetrastromatica* was investigated. The algal biomass was collected from the Mandapam coastal region and processed under laboratory. Various parameters were studied to extract crude lipids from the biomass. A kinetic study was conducted for extracting the lipids from the biomass, which follows the first order kinetics and the lipid yield was 8.15 wt.%. The activation energy; $E_a = 34.314 \text{ kJ mol}^{-1}$ and their thermodynamic parameters were determined. Since the crude algal lipids contain high amount of free fatty acids, a sequential transesterification technique was examined and 7.8% of biodiesel (78 mg/g algal biomass) yield was obtained. The biodiesel was analyzed by ^1H and ^{13}C -NMR spectroscopy and the conversion yield was estimated. Further, the biodiesel fuel properties were investigated and found that all the features fit the required ASTM D6751 specification limits. The residual biomass after lipid extraction was further explored for bioethanol production through the anaerobic fermentation process. The ethanol yield obtained after saccharification and fermenta-

tion were estimated and 161 mg/g residue biomass was reported. The theoretical yield of conversion of hydrolysate to bioethanol was estimated and found to be 83.4%. Therefore, this study demonstrates that macroalga *P. tetrastromatica* biomass has great potential to produce liquid biofuels such as biodiesel and bioethanol.

Obtaining fatty acids from *Mortierella isabellina* using supercritical carbon dioxide and compressed liquefied petroleum gas

Sallet, D., *et al.*, *J. Supercrit. Fluids* 122: 79–87, 2017, <http://dx.doi.org/10.1016/j.supflu.2016.12.005>.

The objective of this article was to provide the extraction yields, the kinetic parameters, and the fatty acids compositions in the oil obtained from freeze-dried cells of *Mortierella isabellina*. The oil was obtained by supercritical CO_2 (SFE- CO_2) and compressed liquefied petroleum gas (LPG) extractions in different conditions of temperature and pressure. According to the kinetic curves, the mass transfer rates, and the yields of extract, 80 °C/250 bar (SFE- CO_2) and 40 °C/20 bar (LPG) were the most favorable conditions. The highest yield was 4.45 wt.% (LPG). According to the fatty acids composition, SFE- CO_2 provided a slightly higher performance than compressed LPG. The highest concentration of fatty acids was 361 mg/g oil at 40 °C/150 bar. Stearic, *cis*-10-pentadecanoic, *cis*-10-heptadecanoic, oleic, linoleic, and linolenic acids were the major fatty acids extracted. These results highlight the possibility of using pressurized fluids to break the cells for increasing the fatty acids extraction from a lipids-rich fungal biomass.

Synthetic Biology

Genetic engineering approaches to enhance oil content in oilseed crops

Savadi, S., *et al.*, *Plant Growth Regul.*, 2016, <http://dx.doi.org/10.1007/s10725-016-0236-1>.

Oilseed crops play an important role in the agricultural economy. Apart from being an integral component of human diet and industrial applications, they are also gaining importance as replacement to fossil fuels for meeting the energy needs. The last two decades have been marked by several important events in genetic engineering and identification of gene targets for enhancing seed oil content in oilseed crops, and will aid the successful development of new generation high yielding oil crops. Specifically, genetic engineering has shown real breakthrough in enhancing oil content in oilseed rape, camelina, soybean, and maize. Moreover, ongoing research efforts to decipher the possibilities of genetic modifications of key regulators of oil accumulation along with physiological and biochemical studies to understand lipid biosynthesis will set a platform to produce transgenic oilseed crops with enhanced oil content. In this review, we briefly describe different genetic engineering approaches explored by different researchers for enhancing oil content. Further, we discuss a few promising and potential approaches and challenges for engineering oil content in oilseed crops.

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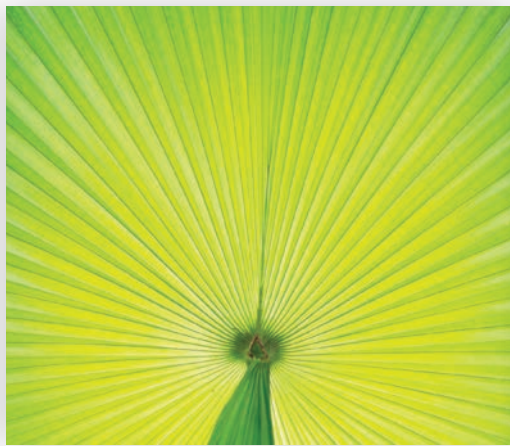
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