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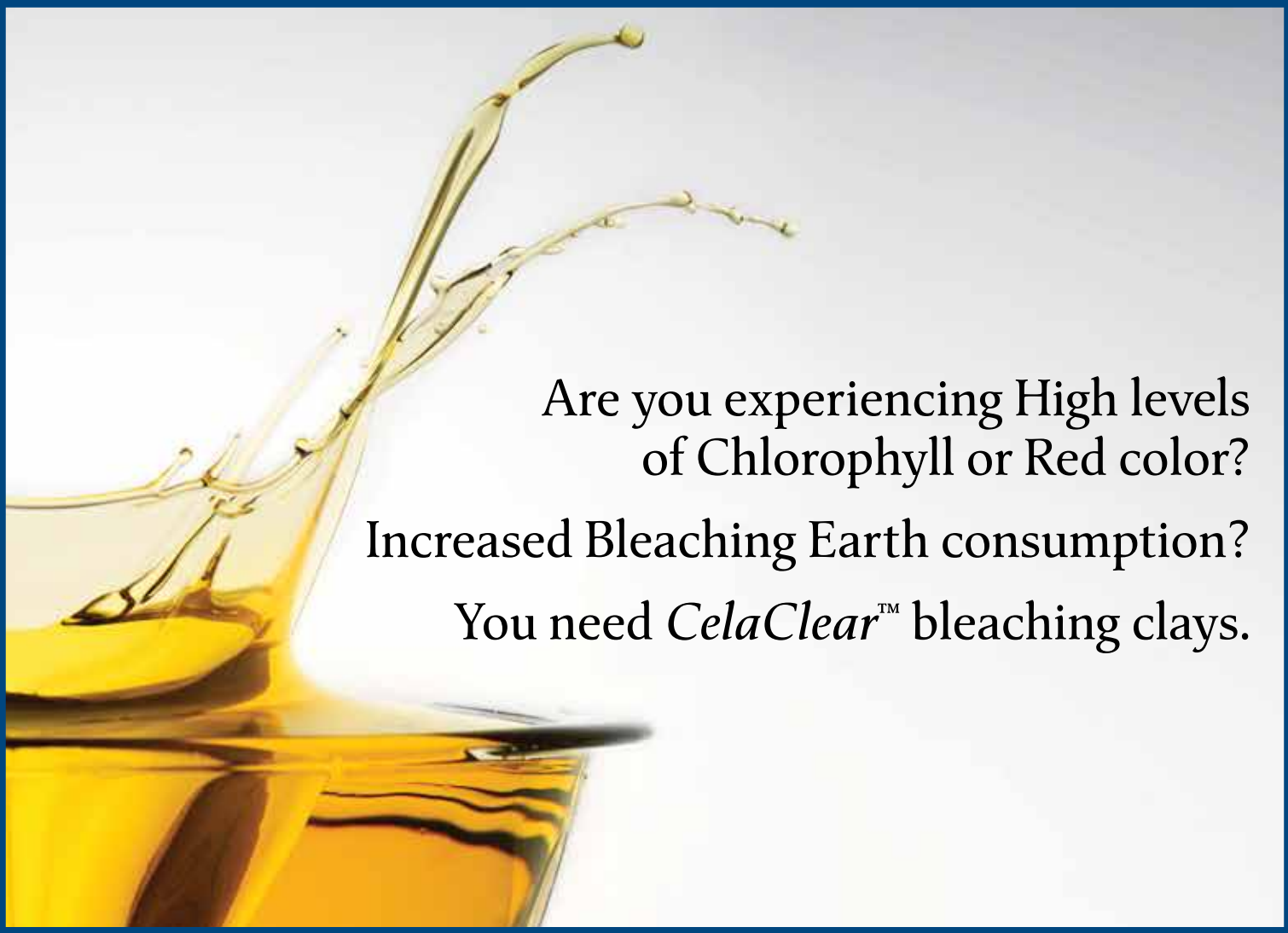

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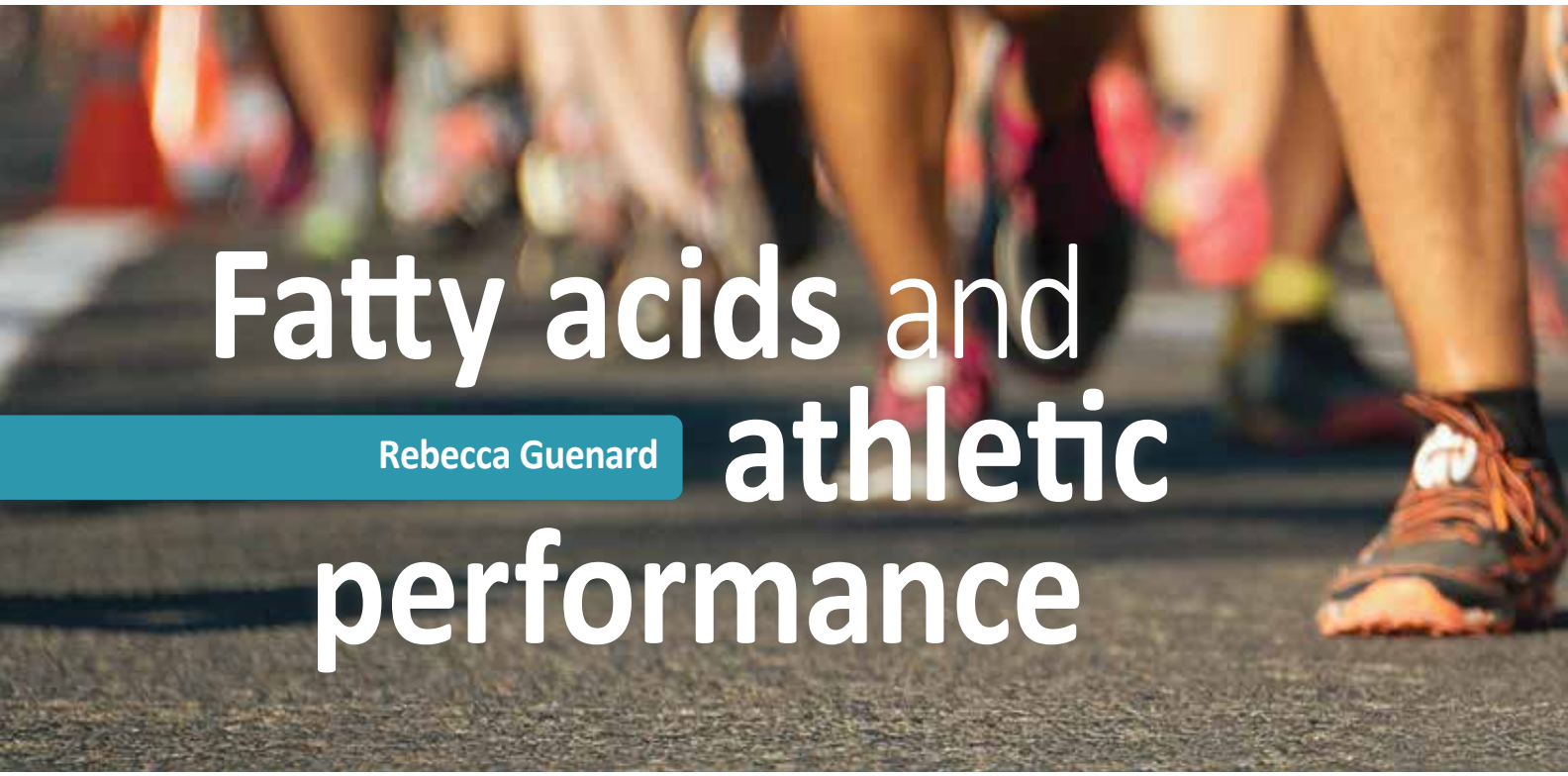


Fatty acids and athletic performance

Rebecca Guenard

- **Omega-3 fatty acids have a solid reputation for contributing positively to the immune system, particularly as anti-inflammatory agents.**
- **Decades of research have failed to produce a direct link between fatty acid consumption and improved athletic performance, though enticing correlations exist.**
- **Related research, on animal models and in human tissue, shows that athletes require fatty acids for very specific functions, from healing brain injuries to opening airways.**
- **However, the question of whether athletes can gain an advantage through supplementation and dietary choices remains unanswered.**

Athletes have myriad nutritional needs. They must stay hydrated and consume enough protein and carbohydrates to build and maintain the muscle strength and energy to perform in their sport. Among all the nutritional factors athletes must consider, how important are fatty acids in maximizing performance?

Fatty acids possess immunomodulatory and anti-inflammatory properties that improve immune function and benefit human health. Consequently, researchers have been investigating whether omega-3 supplements can be used to prevent or treat detrimental inflammatory responses like diabetes and cardiovascular disease. By extension, some scientists developed the hypothesis that omega-3 supplements would improve recovery during athletic training or after competition.

Meta-analyses of a decade's worth of research are inconclusive (Table 1). Depending on the team conducting the review, the conclusion may be that omega-3 supplements benefit athletes (https://doi.org/10.3390/nu11010046) or that they have no measurable effect on healthy, young competitors (https://doi.org/10.1016/j.metabol.2016.10.007).

Simultaneously, mounting research results have failed to produce evidence that omega-3 supplements reduce the incidence of diabetes and cardiovascular disease in the general population. Likewise, most reports conclude that omega-3 supplements do not improve recovery or endurance in athletes. They are undoubtably important despite remaining questions about whether or not athletes can capitalize on fatty acid activity in the body by increasing consumption. In fact, the latest fatty acids research reveals that their influence on athletic performance is likely indirect and definitely complex.

"Athletes have well-regulated and understood diets to try to increase their performance, but the molecular mechanisms behind many of these processes is unknown," says Andrew Tobin, a cellular biology professor at the University of Glasgow, Scotland. Fatty acids, for instance, are more than just an energy source.

"Fatty acids are like food hormones," Tobin says. "When you eat certain foods, they enter the circulatory system and they activate receptor proteins."

Hormones and neurotransmitters are signaling molecules that maintain human homeostasis by evoking a cellular response in organs and tissue. The molecules work by interacting with specific receptors in the cell, the largest and most diverse being the G protein-coupled receptors. This superfamily of receptors participates in virtually all aspects of human physiology. Some endocrine diseases occur because of mutations in these receptors. Considering this and their physiological relevance, G protein-coupled receptors are a popular drug target.

TABLE. 1. A list of omega-3 supplement experiments and their key results. Source: *Nutrients 11*: 46, 2019.

Protocol	Key Results
551 mg eicosapentaenoic acid (EPA) and 551 mg docosahexaenoic acid (DHA) twice daily, during five weeks of pre-season rugby training	Reduced fatigue in countermovement jump tests (*Eur. J. Sport Sci. 18*: 1357–1367, 2018).
24-h exposure with 100 microM EPA in human myotubes	Augmented adaptability and upregulation of specific genes implicated in fatty acid beta-oxidation with global improvement in muscle metabolic flexibility (*J. Lipid Res. 51*: 2090–2104, 2010).
Four-week supplementation with n-3 PUFAs, 1.1 g per day	Significant increase in maximal oxygen uptake (VO2-max) and in endothelial function (*PLoS ONE 10* :e0117494, 2015).
14-days diet enriched with 5% cod liver oil followed by 14 days immobilization	Reduced myosin heavy chain loss during 14 days of hind limb immobilization (*Appl. Physiol. Nutr. Metab. 35*: 310–318, 2010).
Six-months supplementation with 1.8 g EPA, 1.5 g DHA daily	Increased hand grip and muscle strength (*Am. J. Clin. Nutr. 102*: 115–122, 2015).
Three-week supplementation with 3.2 g of EPA and 2.0 g of DHA	Reduced eicosanoids and pro-inflammatory cytokines concentration in the sputum of asthmatic athletes (*Chest 129*: 39–49, 2006).
Six-months supplementation with 3.36 g/day of n-3 PUFAs	Increased muscle mass and strength in older people (*Am. J. Clin. Nutr., 102*: 115–122, 2015).

Free fatty acids from dietary fats act as both an energy source and, surprisingly, as signaling molecules. They bind to G protein-coupled receptors throughout the body that are associated with human metabolism.

WHAT IS THIS THING DOING IN THE LUNG?

Tobin explains that receptors for the long-chain fatty acids reside in the gut, as well as on fat cells and in pancreatic cells. In most cases, they induce activity associated with digestion. "By acting on these cell types, long-chain fatty acids control fat storage and blood glucose levels in the body," he says. So, the food we eat dictates how our body responds to food.

However, Tobin is not interested in how G protein-coupled receptors interact with free fatty acids to manage food; he wants to know the interaction's effect on air. His research group studies the Free Fatty Acid Receptor 4 (FFA4). They were curious to know if the receptor resided beyond the digestive system and were surprised to find large numbers in the lungs. "When you find them in the lung, groups like ours get excited." Tobin says. "We think, what on earth is this thing doing in the lung?"

The group cultured lung tissue to perform experiments that would reveal the receptors' function. They found that FFA4 activated a mechanism similar to asthma, contracting the airway. Stimulating the receptor with a long-chain fatty acid molecule, like linoleic acid, relaxed the smooth muscle and opened the airway.

Then Tobin's group altered the DNA of mice so their cells would not contain FFA4. When the restricted airways of these model mice were stimulated with a long-chain fatty acid molecule, the airways did not open. It was definitive, FFA4 could be targeted to relieve symptoms of asthma.

"This does not mean that taking in a load of corn oil will help you recover from asthma," says Tobin. "We have absolutely no evidence for that." In fact, he is not sure what role diet plays in the process. He says, there is still a question regarding where the endogenous free fatty acids come from to regulate lung function through this receptor. Diet does not make sense as a direct source, because that would mean our breathing would depend on what we eat.

FFA4 AND ATHLETES

For now, Tobin's group is working on developing a treatment for asthma, chronic obstructive pulmonary disease, or other airway diseases by synthesizing a molecular similar to a free fatty acid. They tested the compound on human and mouse models and found it effective in reducing inflammation and inducing muscle relaxation in the lungs.

Along with helping exercise-induced asthma suffers, this research could lead to a better understanding for how fatty acids improve oxygen intake—something that is particularly important for endurance athletes. Tobin says his group is also interested in finding out if these receptors are present in the brain. Meanwhile, they will work on starting drug trials for their compound to help asthma patients who do not respond to current treatments.

If there are FFA4 receptors in the brain, it would indicate that the organ has a built-in ability to reduce inflammation. New research is making it clear that omega-3s do have an impact on how the brain handles inflammation.

OMEGA-3S AND SPORTS-RELATED CONCUSSIONS

When brain tissue is torn or bruised during a concussion, polyunsaturated fatty acids (PUFAs) escape from neural membranes. Animal studies indicate that recovery can depend on the brain's DHA levels.

"Our brain is made of a great deal of fat, and a lot of that fat is omega-3s, the majority of that being DHA," says David Ma, health and nutritional science professor at the University of Guelph in Ontario, Canada. "The principle of structure and function says, if there is a large amount of a certain molecule it has to be doing something."

This Spring, Ma and his graduate student Cody Lust published a review paper on the effects of fatty acids and traumatic brain injury (https://doi.org/10.1139/apnm-2019-0555). His research team found that PUFAs can be beneficial or harmful depending on their composition.

In one of the only studies that analyzed human cerebrospinal fluid of brain injured patients, researchers discovered that higher levels of the omega-6 PUFA, arachidonic acid, resulted in a worse outcome (https://doi.org/10.1016/s0304-3940(03)00803-6). In another study, the author showed that

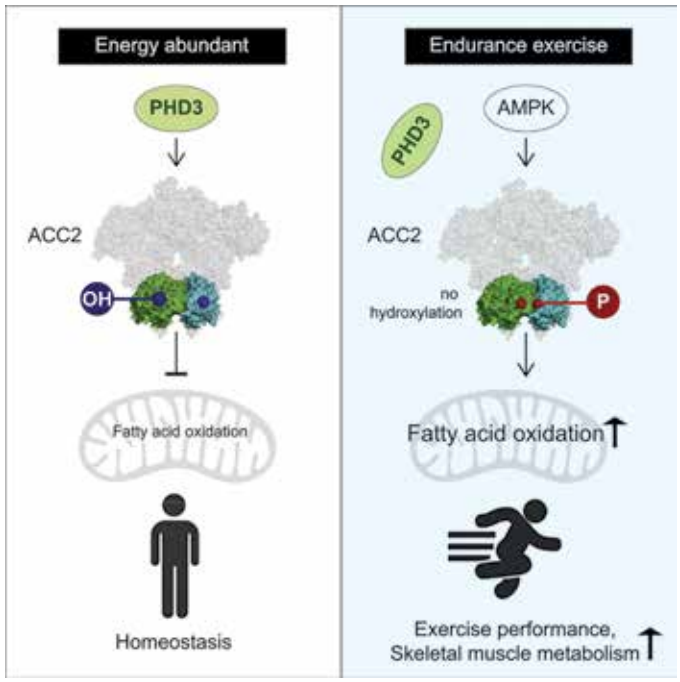


FIG. 1. Tissues maintain homeostasis during changes between sugar and fat as energy sources through a metabolic regulator called acetyl-CoA carboxylase 2 (ACC2). ACC2 can be activated by the AMPK and PHD3 enzymes. In mice with no PHD3 enzyme, fatty acids were oxidized instead of hydroxylated and fueled muscle tissue longer during endurance exercise. Source: *Cell Metabolism 32*: 1–14, 2020.

release of arachidonic acid during injury initiates a proinflammatory response in mice, but when DHA levels were elevated in their tissue—through dietary supplementation or pretreatment—less of the inflammation-causing fatty acid was released (https://doi.org/10.7205/milmed-d-14-00162).

Omega-6 and -3 PUFAs compete for the same enzymes that lead to either a pro- or an anti-inflammatory response. Consuming more DHA means that more neuroprotective compounds are formed, and harmful inflammation is inhibited. However, Ma says that data indicates there are multiple mechanisms to explain why omega-3s help with brain injury recovery. “For our future studies, we want to define clearly which type of omega-3 is better,” he says. “I suspect both EPA and DHA are important, but they probably work in tandem through different mechanisms.”

Other researchers are concentrating on how to increase DHA in human brains to assist recovery. Specific transporters and pathways grant DHA access across the blood brain barrier. Scientists are studying these entry points as possible therapeutic targets for brain injuries (https://doi.org/10.1016/j.biochi.2016.07.011).

Athletes are a unique group, according to Ma. He says more research is needed to understand their precise needs and how adding omega-3s can help them in terms of injury recovery. “When you are talking about athletic performance, typically it brings to mind running faster or jumping higher,” he says. “Injury prevention and management of treatment is part of performance, as well.”

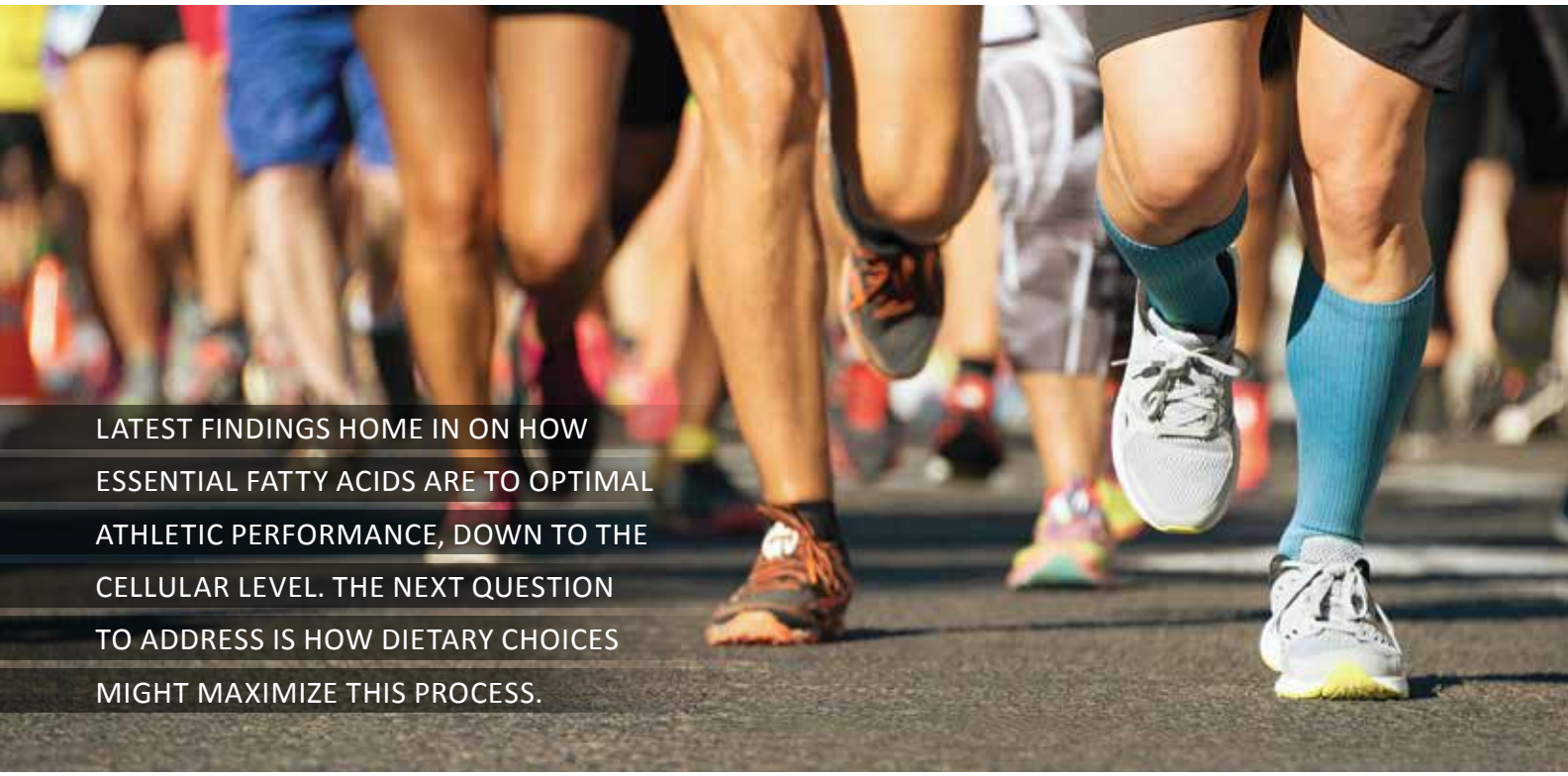
FATTY ACIDS AND ENDURANCE

While a lot of fatty acids research looks at the importance of these molecules in reducing inflammation for athletic recovery, a new study reminds us of their primary role as an energy source. A team of scientists at Harvard in Boston, Massachusetts, USA, discovered that altering the fatty acid metabolism of mice turned them into endurance athletes (https://doi.org/10.1016/j.cmet.2020.06.017).

During exercise, muscles retrieve glucose from cells and use it for quick bursts of energy. However, the cells carry a limited supply, and when glucose is depleted triglycerides stored in muscle and fat tissue take over. Fats act as a long-term source of energy since they contain double the calories of glucose.

The researchers discovered that an enzyme called prolyl hydroxylase 3 (PHD3) inhibits fat breakdown when cells have an abundance of glucose. “We previously found that PHD3 regulates mitochondrial fatty acid oxidation in a subset of cancers,” the study’s lead researcher, Marcia Haigis, told BioSpace.com (https://tinyurl.com/biospacefattyacids). Muscle function also relies on mitochondria, she says, and that led them to wonder how the PHD3 enzyme was involved in energy use during exercise.

First, they found that mice with low glucose during fasting had less PHD3 activity, because a different enzyme was operating: one that converts fatty acids into fuel. Next, they genetically altered mice so they had no PHD3 enzyme. With the ability to oxidize more fat as an energy source, the mice were able to run longer and further than normal mice (Fig.1).



LATEST FINDINGS HOME IN ON HOW ESSENTIAL FATTY ACIDS ARE TO OPTIMAL ATHLETIC PERFORMANCE, DOWN TO THE CELLULAR LEVEL. THE NEXT QUESTION TO ADDRESS IS HOW DIETARY CHOICES MIGHT MAXIMIZE THIS PROCESS.

In another set of experiments, the researchers narrowed the effect of increased exercise capacity to just eliminating PHD3 in skeletal muscle with the enzyme unaltered in the rest of the body. These findings indicate that increasing the fatty acid use of skeletal muscles is one key to enhancing athletic performance. However, more research is needed to understand why these enzymes work this way and whether other signaling mechanisms are involved.

It is early days for each of the research projects discussed here. In every case, the results of the fatty acid experiments have led to more questions that will be addressed in future experiments. As with previous conclusions about fatty acids, these latest findings were determined through mouse studies. A complementary result is not guaranteed when the human body becomes the test subject. Nonetheless, they home in on how essential fatty acids are to optimal athletic performance, down to the cellular level. The next question to address is how dietary choices might maximize this process.

EVEN EQUINE ATHLETES BENEFIT FROM FATTY ACIDS

Horses have difficulty racing in the fall, because many of them suffer from allergies. The histamine reaction, caused by harvest dust and moldy hay bales, can be so bad that some horses develop seasonal asthma (https://tinyurl.com/horseallergies).

A veterinary medicine professor at Purdue University in West Lafayette, Indiana, USA, studied the feed of athletic horses and developed remedies for limiting the allergic response. He found that haylage, which has a higher omega-3 fatty acid content than dry hay, improved the horses symptoms. In addition, omega-3 supplements helped keep asthmatic horses healthy.

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Where are lubricants headed?

Raj Shah, Mathias Woydt, and Nathan Aragon

Lubricants are highly functionalized products that meet a multitude of requirements simultaneously. Today, they meet demanding technical-oriented specifications. In the future, non-technical criteria, like eco-toxicological properties and/or sustainability, will be added to their functionality on top of technical requirements. With sweeping environmental regulations from organizations such as the European Chemicals Agency (ECHA) and the US Environmental Protection Association (EPA), the growing concern for sustainability, and the advent of completely electric vehicles (EVs), the future of the lubricant industry for the next five to 50 years is sometimes called into question. This article will attempt to shed some light on this very uncertain future.

- **The shift toward sustainability and using green chemistry to derive new base oils made from renewable resources will continue.**
- **Electric vehicles (EVs) will require smaller quantities of highly specialized lubricants as opposed to large quantities of standard lubricants currently used for internal combustion engine vehicles.**
- **As EVs partially replace internal combustion engine vehicles, the volume production of lubricants will decrease, but new specialized EV lubricants will provide more value to companies that develop them. On the other hand, the International Energy Agency (IEA) projects that the global vehicle fleet will grow from 1.3 billion today to 2.3 billion in 2040.**

It would be remiss to discuss the future of lubricants for both automotive and industrial applications without mentioning the shift toward sustainability and using renewable resources (green chemistry) to derive new base oils, in total or in part. Around 1990, the first environmentally acceptable lubricants, or "EALs", appeared in Europe; these new lubricants especially met eco-toxicological properties. Today, some of the important certifications for EALs are:

- European ecolabel as per EC/2018/1702 (3rd revision);
- Second issuance of U.S. Vessel General Permit (VGP); and
- Biolubricants as per EN16807.

EALs with these certifications must have an accelerated, ultimate biodegradation (full mineralization) or short persistence in the environment when spilled. They have very limited toxicities for aquatic species like fish, daphnia, and algae, and they also contain a certain percentage of renewables. The issue of environmental friendliness is thus solved by legislations, test methods, and guidelines. "Biolubes" are available today for almost all classes of lubricants. They operate safely when they are used for their intended purpose.

A class of lubricants called "sustainable lubricants" have different attributes than the EALs. EALs focus on human and environmental toxicology when fluids enter the aquatic environment, whereas sustainable lubricants will consider:

- the carbon dioxide emissions throughout the product's lifecycle; and
- the consumption of resources over the product's lifetime, and the carbon dioxide emissions that are generated as a result.

A visual summary

A poster summary of this topic with additional figures and graphics is available at bit.ly/where-are-lubricants-headed.

The concept of sustainable lubrication has been and will continue to be very important as research shifts away from a heavy reliance on fossil fuels. On average, about 20 million barrels of petroleum were utilized every day last year in the United States alone, and this number will not get smaller unless there is a shift away from fossil fuels [1]. In addition, only about one percent of the petroleum that is extracted goes into the production of lubricants. World consumption is about 42 million tons of finished lubricants [2] annually, compared to about 4,500 million tons of oil [3]. This implies that as we focus on reducing the amount of petroleum that we use, there will be a lower volume of it available for the production of lubricants, so there needs to be a replacement for this loss in volume of petroleum. Sustainable lubricants derived from biomass and other renewable sources have shown to be very promising in addressing this issue and should remain a large focus in the near future.

ANIMAL FATS, VEGETABLE OILS, AND OTHER BIOMASS FEEDSTOCKS

With all these considerations in mind, much research has been focused on the development of bio-lubricants made from animal fats and vegetable oils. The pre-existing base oil alternatives comprise esters, polyalkylene glycols, and more recently bio-olefins, all based on renewables with a requested content of renewables of >25% or >50%. Esters are currently well known to be very useful for industrial applications that require excellent low-temperature properties. Esters that exhibit pour points of lower than -42°C have been synthesized and show viscosity indices that are near commercial lubricants, such as polyalphaolefins (PAOs) and polyolesters (POEs). Saturated, dimer fatty acid esters (DFAEs) are known for their thermal and oxidative stability. A group of researchers have synthesized DFAEs by combining dimer acid with different types of alcohols, and the approach does not use a solvent or any catalyst [4]. Different types of polyglycols have been known to have very high viscosity indices and lower pour points [5]. Reducing the amount of energy loss due to friction has been and will continue to be a focus regarding emissions reduction. A study done in 2017 used a very accurate high-load setup to compare the ability of two hydrocarbon-based lubricants versus a polyalkylene glycol-based lubricant to effectively lower friction. They found that the polyalkylene glycol-based lubricant led to 25% less friction than either of the two hydrocarbon lubricants [6].

Other promising feedstocks for base oils of bio-lubricants are microalgae and hydroxy-fatty acids [7]. The use of hydroxy fatty acids was confirmed to provide solid anti-wear and friction reduction properties by a study done in 2017 [8]. A different study done a year before this displayed the anti-friction and wear characteristics of bio-oils derived from microalgae [9]. This research toward developing more biodegradable and eco-friendly base oils for lubricants is very likely to take precedence very soon as environmental regulations become more stringent. The most important thing to know when considering this research focus is that lubricants can no longer be developed with the idea of purely fulfilling technical functions. Going forward, they will need to fulfill the standard technical requirements with the addition of eco-toxicological properties and/or sustainability criteria on top.

Another research area that has gained momentum in recent years and will likely continue is the investigation of additives containing nanomaterials. These have been known to increase the anti-wear and extreme pressure properties of various lubricants. They are also known to improve the properties of bio-lubricants and are considered environmentally friendly [9]. Some researchers have seen that graphene and carbon nanotubes used as additives can lower friction and wear coefficients in different types of lubricants [10,11].

HOW ELECTRIC VEHICLES WILL CHANGE THE GAME

A very important consideration in the direction of the lubricant market is the advent of electric vehicles (EVs), or the electrification of the powertrain/drivetrain. EVs require very specialized lubricants as opposed to the standard lubricants for internal combustion engine (ICE) vehicles. It has been predicted that the demand for automotive lubricants will not rise anymore due to the EV market. The largest demand for EVs are in both the European and Chinese markets, so the development of efficient EV lubricants will be of great value in the coming years in these areas. Large companies such as Royal Dutch Shell and TOTAL S.A. have already released their own lines of dedicated lubricants for EVs, so it seems that these companies and others that follow would be better positioned for the upcoming change in the global lubricants market [11,12].

An analysis done by McKinsey and Company [2] predicts much of the same thing. They say that looking ahead to 2035, the volume production of lubricants will decrease but the value can expand. This makes sense considering it has been posited that battery EVs need 50–70% less lubricants than ICE vehicles. This analysis also specifically predicts that in about five years, the global automotive lubricants demand will max

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out, after which the demand will decrease in the European and American markets but continue to grow in the Chinese market. As for the industrial lubricant sector, demand will continue to grow but will slow down after the next five years. Based on their analysis, McKinsey and Company predict that the value in the global lubricant market will increase by 44% in the next 15 years due to more advanced formulated synthetic lubricants and with the increased demand for industrial applications. This is consistent with the projected increase in stock of the vehicle market by the International Energy Agency (IEA) from 1.3 billion vehicles today to about 2.3 billion by 2040. However, McKinsey and Company point out that a 35% decrease in this projected value could occur due to battery technology becoming more affordable and continued tight regulations relating to the environment [12].

Looking even further ahead to the next 50 years is much harder to predict, but some scientists have given it a try based on past trends. Roland Larsson made a presentation at the 6th World Tribology Congress in 2017, where he predicted that lubricants will assuredly move toward renewable materials and tribo-modelling more into the future. The need for lubricants to be more renewable is very clear, and Larsson has proposed glycerol as a possible bio-lubricant. Glycerol is a by-product from biodiesel production and is widely available today. He also discusses the use of glycerol aqueous solutions to lower friction coefficient and possibly replace rapeseed oils [13]. The development of tribo-modelling, which optimizes tribology in the entire system, is very important, because developing viable lubricants that are sustainable can be challenging [14]. In conclusion, the development of lubricants made from renewables will take precedence for use in most automotive and industrial applications. Additionally, new market demands for EVs will require new dedicated lubricants that can provide much value to any companies that are willing to take on the research and development for them.

Raj Shah is a Director at Koehler Instrument Company in New York, where he has worked for the last 25 years. With a PhD in Chemical Engineering from Penn State University and a Fellow from the Chartered Management Institute, London, Raj has been an active member of AOCS for the last 2 decades. An Adjunct Professor at State University of New York, Stony Brook, Shah has been elected a Fellow by his peers at STLE, AIC, NLGI, INSTMC, CMI, IChem E, The Energy Institute, and The Royal Society of Chemistry. He is also a Chartered Scientist with the Science Council, a Chartered Petroleum Engineer with the Energy Institute, A Chartered Chemist with the Royal Society, and a Chartered Engineer with the Engineering council, UK. He has over 225 publications and is a co-editor of various books related to lubricants. He can be reached at rshah@koehlerinstrument.com. More information on Dr. Shah can be found at https://www.che.psu.edu/news-archive/2018/Alumni-Spotlight-Raj-Shah.aspx.

Mathias Woydt is managing director of MATRILUB (Materials-Tribology-Lubrication). He has more than 33 years of experience in R&D and has more than 325 publications and 50 plus priority patents filed. He is also the vice-president of the German Society for Tribology.

Nathan Aragon is part of a thriving internship program at Koehler instrument company, and a student of chemical engineering at Stony Brook University, New York, where Shah is the chair of the external advisory board of directors.

Canola enhanced with long-chain omega-3 becomes a reality

Malcolm Devine, Xue-Rong Zhou, and Surinder Singh

- A novel form of canola containing significant amounts of docosahexaenoic acid (DHA), other long-chain polyunsaturated fatty acids (LC-PUFAs), and elevated alpha-linolenic acid (ALA) in the seed oil has been developed. Multiple field trials have confirmed the crop to be a reliable source of these essential fatty acids.
- Aquaculture research in Norway and Australia, and trials on commercial farms in Chile, have demonstrated that this LC omega-3 canola oil is an effective ingredient in aquafeeds, with significant sustainability and nutritional benefits.
- Various model studies and a human clinical trial have shown that LC omega-3 canola oil is a safe and effective source of LC-PUFAs, confirming it as an important new source of DHA for animal and human nutrition.

Pelagic species (anchovy, menhaden, and others) have been the major source of the essential long-chain polyunsaturated fatty acids (LC-PUFAs) for many years. However, the increasing demand for these fatty acids, supported by a multitude of studies showing beneficial effects on heart and brain health, has led to overfishing of many source species. Consequently, there has been a concerted effort by several groups to develop a land-based source, and many publications describe the production of EPA (eicosapentaenoic acid; C20:5n-3) and/or DHA (docosahexaenoic acid; C22:6n-3) in oilseed crops. Here we describe the development of a canola crop with high levels of DHA and ALA (α-linolenic acid; C18:3n-3), the first such crop to obtain regulatory approval in any jurisdiction (Petrie, *et al.*, 2020).

A collaboration between Nuseed Pty Ltd., the Commonwealth Scientific and Industrial Research Organization (CSIRO), and the Australian Grains Research and Development Corporation (GRDC) has resulted in the successful development of canola that produces fish oil-like levels of DHA in the seed oil ("LC omega-3 canola"). A set of seven transgenes coding for enzymes required to biosynthesize DHA from oleic acid (OA, 18:1 n-9) were arrayed on an *Agrobacterium* T-DNA vector. These include a Δ12-desaturase and ω3-/Δ15-desaturase from yeast, and a Δ6-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase and Δ4-desaturase from microalgae

(see Figure 1). This gene set was introduced into canola via *Agrobacterium*-mediated transformation to create LC omega-3 canola. Although canola has its own Δ12-desaturase and Δ15-desaturase enzymes, introduction of the extra copies of a yeast Δ12-desaturase and ω3-/Δ15-desaturase converted OA to maximal levels of ALA, the precursor for DHA biosynthesis. LC omega-3 canola oil consistently contains ca. 20% ALA and 9–10% DHA, with small amounts of other ω3 LC-PUFAs (Table 1). The high ALA content, combined with the DHA and other ω3 LC-PUFAs, results in a decreased ω6:w3 ratio, from ca. 2.0 in conventional canola oil to 0.2 in LC omega-3 canola oil, thereby providing additional health benefits (e.g., Simopoulos, 2016).

Molecular characterization and genome sequencing confirmed that the deregulated LC omega-3 canola event contained two T-DNA insertions in chromosomes A05 and A02. The A05 locus contained two complete but linked copies of the T-DNA, while the A02 T-DNA insert contained the genes coding for Δ6-desaturase, Δ5-elongase, Δ5-desaturase and ω3-/Δ15-desaturase but not the Δ4-desaturase, Δ12-desaturase or Δ6-elongase genes. No vector backbone sequence from the binary vector used for transformation was inserted in the LC omega-3 canola genome. Stable integration of the T-DNA insertions was confirmed over multiple generations, with no decrease in DHA content of the oil.

Multiple field trials over several years in Australia, the United States, and Canada have shown that the DHA trait does not affect any of the crop's agronomic characteristics, other than a small (3–4%) reduction in seed oil content (Petrie *et al.*, 2020). Based on multiple field trials with the T_5 to T_7 generations, the average grain yield was equal to that of the untransformed check variety. LC-PUFA levels ranged between 9–12% in these trials, with a typical fatty acid profile shown in Table 1. The agronomic characteristics of the crop (yield, disease resistance, etc.) are being continuously improved in Nuseed's canola breeding program, and a larger acreage has been produced for downstream commercial applications (Fig. 2, page 18).

The long-term stability of the oil was tested by storing seed at 4°C, 24°C, and 32°C for 6 months. Fatty acid profiles and DHA levels in the seed oil were not significantly affected by storage under these conditions, and no significant differences

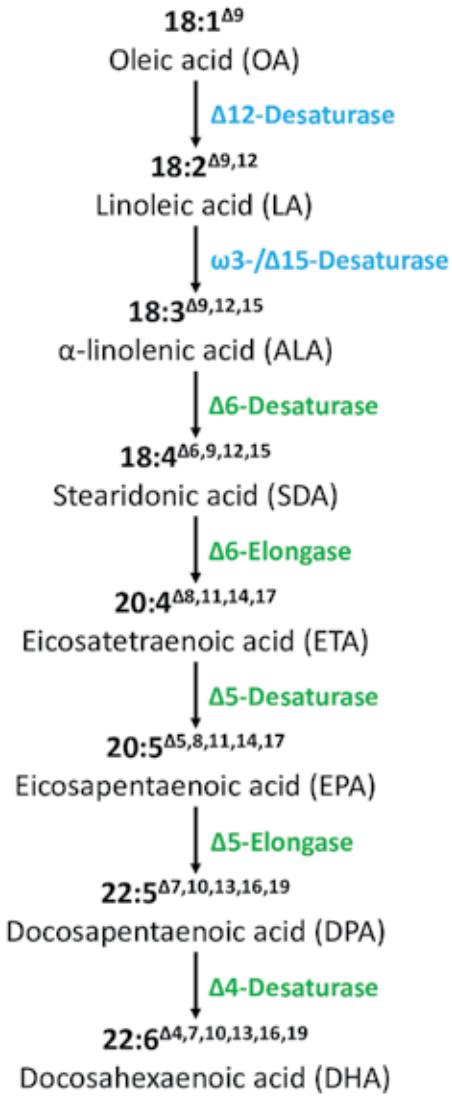

FIG. 1. Biosynthesis of DHA from oleic acid in LC omega-3 canola. The chemically synthesized genes coding for these enzymes based on yeast (blue) or algal (green) sequences were constructed in a T-DNA vector under the control of seed-specific promoters. Details of the genes and regulatory elements can be found in Petrie, *et al.* (2020).

TABLE 1. Fatty acid composition (%) in the seed oil of field-grown LC omega-3 canola and the untransformed parental line, AV Jade (from Petrie, *et al.*, 2020)

	Fatty acid content (%)													
					Novel ω3 fatty acids									
	OA ω9	LA ω6	ALA ω3	GLA ω6	SDA ω3	ETrA ω3	ETA ω3	EPA ω3	DPA ω3	DHA ω3	∑EPA, DPA, DHA	Total ω3	Total ω6	ω6:ω3
LC omega-3 canola	42.0	7.0	20.0	0.4	2.2	0.6	1.3	0.5	1.0	9.7	11.1	35.3	7.4	0.2
AV Jade	59.1	19.3	9.5	-	-	-	-	-	-	-	-	9.6	19.4	2.0

FIG. 2. LC omega-3 canola growing in Washington State, USA, in 2017.

were observed in seed germination or viability compared to wild-type seed (Petrie, *et al.*, 2020).

Measurements from multiple field trials demonstrated that the novel proteins were detected only in mature and developing seeds of LC omega-3 canola, and not in non-seed tissues—a property of the seed-specific promoters driving the transgenes (Colgrave, *et al.*, 2019a). *In vitro* protein digestibility analysis with simulated gastric fluid assay and tryptic peptide markers showed that the enzymes were readily degraded (Colgrave, *et al.*, 2019b). Combined with bioinformatic comparison with known protein allergens and toxins, these results support the novel proteins as non-allergenic and non-toxic. Phenotypic, agronomic, and compositional analysis confirmed LC omega-3 canola is comparable to conventional canola except for the intended changes in seed oil profile.

A major use of LC omega-3 canola oil will be as an ingredient in aquaculture feeds, alleviating the pressure on dwindling oceanic feed stocks. Research conducted by Nofima (the Norwegian Institute of Food, Fisheries and Aquaculture) and CSIRO demonstrated the safety and efficacy of LC omega-3 canola oil in juvenile Atlantic salmon in fresh water (Ruyter, *et al.*, 2019) and in larger fish in sea water (Ruyter, in prep.). More recently, several large-scale, full life-cycle production pilot studies have been conducted in collaboration with aquafeed producers and commercial Atlantic salmon farms in Chile. Fish weight gain and feed conversion ratios were excellent when fish oil was partially replaced (30–60%) with LC omega-3 canola oil. Fillet quality was also excellent, with EPA and DHA levels in the fillets equal to those in fish raised on traditional fish oil diets. Of note, salmon survival was higher in all three farm trials when LC omega-3 canola oil was included in the feed.

In parallel, trials have been conducted to demonstrate safety and efficacy of LC omega-3 canola oil for human consumption. A rat feeding study showed excellent uptake of DHA from this oil into serum, red blood cells, and various organs, with no negative health effects. A recently completed randomized, double-blind, placebo-controlled human clinical trial confirms LC omega-3 canola oil as a safe source of DHA. Supplementation with LC omega-3 canola oil resulted in a notable improvement in the omega-3 index. Again, the novel oil was well tolerated by all subjects, with no adverse effects from consumption of LC omega-3 canola oil.

In addition to the health and nutritional benefits of this oil, producing essential fatty acids through a renewable, land-based source provides sustainability advantages by reducing pressure on ocean resources and decreasing environmental impact. Regulatory approval has been obtained for cultivation of the crop in Australia, the United States and Canada; for human consumption of the oil in Australia, New Zealand and Canada; and for animal consumption in Australia, New Zealand and Canada (fish only). A food and feed consultation is progressing in the United States, with similar assessments pending approval in several other countries. Collectively, the

evidence demonstrates that oil from LC omega-3 canola will be an important new source of essential LC-PUFAs for human and animal health.

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Xue-Rong Zhou is a Senior Principal Research Scientist at CSIRO Agriculture and Food, Australia. He has conducted research in regulation, biochemical analysis, and metabolic engineering of plant lipids for decades, with molecular, biochemistry, lipid analysis, lipid synthesis, lipidomics, and deregulation expertise. He can be contacted at Xue-Rong.Zhou@csiro.au.

Surinder Singh is a Chief Research Scientist at CSIRO Agriculture and Food. He has decades of experience in developing new oilseed crops through genetic engineering and has over 100 granted patents in this field. He is the CSIRO lead in the LC omega-3 canola project. He can be contacted at Surinder.Singh@csiro.au.

The authors acknowledge funding from the GRDC and the contribution of many colleagues and collaborators in this project.

Further reading

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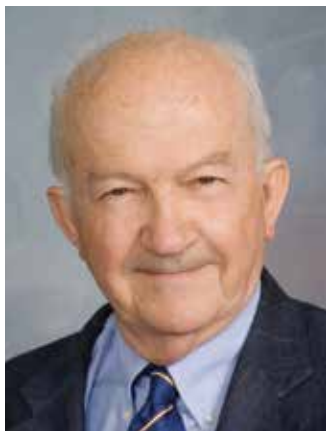
Giants of the past: Edwin Frankel (1928–2019)

G.R. List

Edwin (Ed) Frankel was born in Egypt, but came to America as a young man. He enrolled at Michigan State University in East Lansing, where he received a BS degree in 1950. From 1950–1956 he earned MS and PhD degrees at the University of California, Davis. Ed had a passion for lipid oxidation, and in 1956, he began his career as a research chemist at the US Department of Agriculture–Agricultural Research Service USDA–ARS Northern Regional Laboratory in Peoria Illinois, USA.



From 1956–1960, he published several papers, including ones on the purification and characterization of fatty acid hydroperoxides. In 1960, Ed accepted a position as a group leader in the Food Products Division of Proctor & Gamble, Co., in Cincinnati, Ohio. He quickly discovered, however, that industrial research results were proprietary, and publications in the open literature were discouraged. In 1962, he returned to the Peoria Lab, where freedom to publish was not only encouraged but essential for upward mobility. Research priorities in government, however, changed from basic to applied. In the late 1960s–1970s, the USDA redirected research from edible uses to industrial utilization. Thus, Ed's interest in lipid oxidation was temporarily put on hold. By 1976, his research on modification of fatty acids included an oxo process for hydroformylation of fatty acids with a rhodium catalyst. Exotic hydrogenation catalysts were further developed and explored for fatty acid modification. After a very productive period of applied research, Ed was free to return to lipid oxidation. From 1976 until his retirement from USDA in 1989, Ed and his research group, made numerous contributions to our understanding of the breakdown of hydroperoxides and their products.

After a long and productive career in government and industry, Ed started a third career in academia as an adjunct professor in the Department of Food Science and Technology, UC, Davis. It was here that he was able to pursue his love of lipid oxidation. Ed became the most highly cited author in agricultural chemistry, with about 700 papers, nearly 33,500 citations, and an exceedingly high g index and h index (80 and 175, respectively). These stats equaled or exceeded those of many Nobel Laurates. Ed's top 10 publications received over 11,000 citations ranging from 640–2,300. Among his outstanding works was the discovery that phenolic compounds in red wine inhibit oxidation of low-density lipoproteins in humans. He further characterized the principal phytochemicals in California wines responsible for their antioxidant properties. Ed's other studies showed that green tea, berries, rosemary, and certain fruits also inhibited oxidation of low-density lipoproteins. The methodology to assess oxidation in complex lipid and biological systems was a major obstacle in his research. Ed subsequently developed sensitive methods to determine the effectiveness of antioxidants. One seminal publication showed that the oxidation of bulk oils and lipid emulsions proceed by different mechanisms and depend on their hydrophilic or lipophilic character. This discovery led to the concept of interfacial oxidation in which the interaction of antioxidants and lipids in emulsions have a profound effect on the stability of foods where antioxidants and prooxidants were distributed differently in colloidal systems. This pioneering work opened a whole new field in oxidation research.

Ed authored several reviews on lipid oxidation including his classic book, *Lipid Oxidation* (1998, 2nd Edition 2005). In 2007, *Antioxidants in Food and Biology: Facts and Fiction* by Ed Frankel was published by Elsevier. A reviewer commented, "This is a well-written and informative text

written by one of the top investigators in the field of lipid oxidation. It would be a nice addition to any food scientist's library, especially those scientists with a substantial interest in lipid oxidation." In addition to research, Ed taught graduate courses on food chemistry and oxidation. For many years, Ed organized and presented lectures at AOCS short courses, and he also gave invited lectures in Australia, Denmark, Sweden, Finland, and England.

Ed received numerous honors and awards from both the USDA and various technical societies. His AOCS awards include Fellow (initial class, 1999), Alton Bailey Medal (1985), Steven S. Chang Award (1999), and the Pelick AOCS Research Award (2007). International recognition came from The Society of Chemical Industry (UK) and The Finnish Chemical Society, which he addressed as a keynote speaker in 1992. In 1990, he was the International Lecturer for the Society of Chemical Industry (SCI) in London, UK. SCI later honored him with the Lewkowitch Lecture in 2002. The German Fat Society (DGF) honored him with the Kaufmann Memorial Lecture at the World Congress held in the Hague, The Netherlands, in 1995.

In 2006, the AOCS Lipid Oxidation and Quality Division (AOCS) held a symposium in honor of Professor Frankel's 50-year career in lipid oxidation. The division established the annual "Edwin Frankel Award for Best Paper Lipid Oxidation and Quality," which recognizes papers published in AOCS journals.

In 1980, the Fats and Oils Group at the Peoria lab was reorganized due to several retirements. My new supervisor was Ed Frankel. While Ed could be difficult at times, he was not a micro manager, but gave subordinates considerable freedom to carry out their assigned research. His appointment came at a critical time in my career. For my promotion evaluation, a detailed report had to be submitted to a peer panel. Ed was extremely generous and made numerous suggestions to improve it. His edits were very helpful and resulted in my promotion to senior scientist.

Ed was someone who thought out of the box and believed the literature contained much unchallenged dogma that necessitated researchers to look critically at other approaches to problem solving. This could only be achieved by becoming familiar with the patent literature as well as published research papers. Failure to do so would only result in duplication and low-quality papers that lacked new information or novelty.

Ed strongly believed that young scientists should strive for quality rather than quantity when publishing their research findings. Of course, advancement in academia and government too often depends largely on the numbers of peer reviewed journal articles rather than their impact.

Ed and his wife Barbara celebrated 69 years of marriage together and have five children and five grandchildren.

G.R. List is currently working as a consultant after retiring from the US Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, in Peoria, Illinois, USA. He can be reached at grlist@telstar-online.net.

Information

Here is a short list of significant publications by Edwin Frankel that are recommended for further reading:

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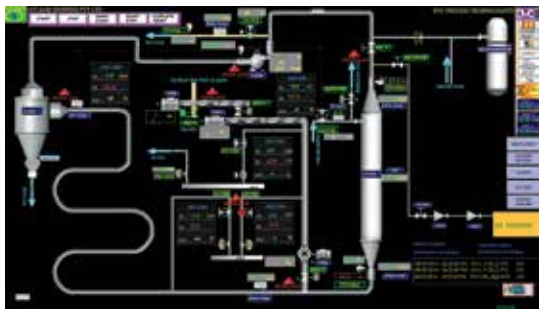

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Chemical composition and thermal behavior of Brazil nut (*Bertholletia excelsa*) oil

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

- **Brazil nut oil is high in unsaturated fatty acids, vitamins, and minerals.**
- **The oil is liquid at room temperature, and its moisturizing properties are ideal for dry skin and applications in creams, balms, body lotions, and other cosmetics.**
- **Edible Brazil nut oil is a colorless premium oil with a pleasant almond flavor and taste that make it suitable for use in cakes, biscuits, other bakery products, and emulsions such as mayonnaise.**
- **This article looks at the production, nutrition, chemical composition, thermal characteristics, and functionality of this exotic oil.**

Brazil nut is a native tree of the tropical regions of northern South America. It is common in the rainforests of the Amazon and the Orinoco, in Brazil, Bolivia, Peru, Ecuador, Colombia, and Venezuela (Kluczkovski, *et al.*, 2015; Krist, 2020). The fruits of this tree are capsules (known as "ouriços" in Brazil), with a very hard woody shell and a spherical or slightly flattened shape. Each fruit shell contains 15 to 30 triangular seeds about 4 cm long, with hard shells (Fig. 1, page 24) (Botelho, *et al.*, 2019). These seeds, or "nuts", are 63–70% oil and have a protein content of about 15–20% (Chunhieng, *et al.*, 2008). Brazil nuts are important sources of protein; oil; fiber; complex B1, B2, and B3 vitamins; pro vitamins A and E; and minerals, such as calcium, magnesium, iron, potassium, sodium, and selenium that have anticarcinogenic effects (Sartori, *et al.*, 2020).

FIG. 1. Brazil nuts from fruit to oil

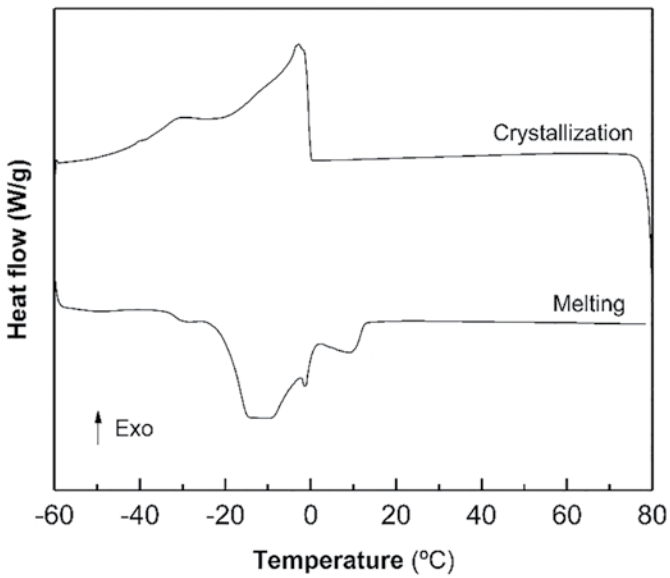
To extract the oil of Brazil nut, the seeds are submitted to solvent extraction or pressing. The quality of pressed oil is better than that of the solvent extracted oil, because it contains more fat-soluble vitamins and other lipophilic bioactive compounds (Krist, 2020). Brazil nut oil is a liquid clear, yellowish oil with a pleasant smell (Fig. 1). It does not contain carotenoids or chlorophyll. A few complete studies of Brazil nut oil have been conducted. This work evaluated the chemical composition and the thermal characteristics of this exotic raw material.

Because of its unique fatty acids and triacylglycerol (TAG) composition, Brazil nut oil is liquid at room temperature. This gives it emollient properties that are useful in the cosmetics industry. The results of our evaluation of the TAG distri-

TABLE 1. Triacylglycerols—TAG (%) according to the number of carbon (NC) in the Brazil nut oil

TAG	NC	Brazil nut oil (%)
PPS	50:0	0.73
PPO	50:1	2.41
PPLn	50:3	2.31
PSS	52:0	0.54
PSO	52:1	3.61
POO	52:2	5.98
PSLn	52:3	3.46
POLn	52:4	11.45
PLnLn	52:6	5.48
SSO	54:1	1.35
SOO	54:2	4.48
OOO	54:3	6.23
SOLn	54:4	8.58
OOLn	54:5	14.18
SLnLn	54:6	4.10
OLnLn	54:7	13.58
LnLnLn	54:9	4.33

NC: Number of carbon; P: palmitic acid, O: oleic acid, Ln: linolenic acid, S: stearic acid.



Crystallization	Brazil nut
T_i (°C)	0.38±0.16
T_{p1} (°C)	-1.98±0.71
ΔH_1 (J/g)	34.59±1.88
T_F (°C)	-52.71±3.82

Melting	Brazil nut
T_i (°C)	-36.03±0.51
T_{p1} (°C)	-13.01±0.99
ΔH_1 (J/g)	59.08±2.51
T_F (°C)	15.36±1.79

FIG. 2. Crystallization and melting curves and thermal parameters of Brazil nut oil. T_i: Initial temperature of crystallization/melting; T_{p1}: peak temperature; T_F: final temperature of crystallization/melting; ΔH_1: enthalpy of peak 1.

TABLE 2. Fatty acid composition of Brazil nut oil

Fatty acids (%)	Brazil nut oil
C 12:0 – Lauric acid	0.10±0.00
C 14:0 – Miristic acid	0.14±0.00
C 16:0 – Palmitic acid	14.81±0.05
C 16:1 – Palmitoleic acid	0.36±0.00
C 17:0 – Margaric acid	0.09±0.01
C 17:1 – cis-10-heptadecenoic acid	0.03±0.00
C 18:0 – Estearic acid	11.10±0.05
C 18:1 – Oleic acid	36.69±0.12
C 18:2 – Linoleic acid	35.13±0.02
C 18:2 – trans t-Linoleic acid	0.07±0.00
C 18:3 – Linolenic acid	0.10±0.01
C 18:3 – trans t-linoleic acid	0.05±0.07
C 20:0 – Araquidic acid	0.31±0.00
C 20:1 – Eicosenoic acid	0.07±0.00
C 22:0 – Behenic acid	0.48±0.057
C 24:0 – Lignoceric acid	0.04±0.01
Saturated fatty acids	**27.08±0.45**
Unsaturated fatty acids	**72.92±0.45**
S. I.	194±5.44
I.I. (I_2/100g)	93±2.64

S.I: Saponification index; I.I: iodine index.

bution (Table 1) showed a greater quantity of TAG containing linoleic (Ln - C18:2), oleic (O - 18:1), and palmitic fatty acids (P - C16:0)—mostly OOLn (14.18%), OLnLn (13.58%), and POLn (11.45%)—due to a predominance of these fatty acids (Table 2). These values, along with the iodine (93gI_2/100g) and saponification index (194 mg KOH/1g), are in line with the results of previous studies by Funasaki, *et. al.* (2012).

Fatty acid and triacylglycerol composition have a direct correlation with the physical properties. Brazil nut oil is liquid even under low temperatures. This is confirmed by thermal analysis, which showed low initial temperatures of crystallization and melting, with only one peak of energy release in both cases (Fig. 2).

Because the oil is highly unsaturated and therefore susceptible to oxidation, the peroxide index and free fatty acids were evaluated. The values were similar to the quality of a crude oil, with a peroxide index of 8.33 ± 2.87mEq/kg and free fatty acids of 0.28 ± 0.2%, indications of good storage and high quality (Santos, *et al.*, 2013).

For use in foods, Brazil nut oil is considered a premium oil. It is colorless and has a pleasant almond flavor and taste that make it suitable for use in cakes, biscuits, other bakery products, and emulsions such as mayonnaise. As a cosmetic ingredient, thanks to its moisturizing properties, Brazil nut oil is ideal for dry skin, application in creams, balms, and body lotions (Guerra, *et al.*, 2017; Kluczkovski, *et al.*, 2015; Krist S., 2020; Pereira, *et al.*, 2019).

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Short-chain fatty acids improve poststroke recovery via immunological mechanisms

Rebecca Sadler and Arthur Liesz, *et al.*

- **Stroke alters the gut microbiome which, in turn, has considerable impact on stroke outcome.**
- **Short-chain fatty acid (SCFA) supplementation in live mice improved recovery of affected limb motor function, altered contralesion cortex connectivity associated with SCFA-dependent changes in spine and synapse densities, and had a substantial impact on microglial activation.**
- **These results identified SCFAs as a missing link along the gut–brain axis and as a potential therapeutic to improve recovery after stroke.**

Stroke induces a multiphasic pathophysiological cascade, which consists of an initial excitotoxicity followed by a longer neuroinflammatory phase within the brain. Moreover, stroke can be regarded as a systemic disease that also affects remote organ function, including the lung, heart, immune system, and intestinal function. Recently, it has been shown that a dysbiotic gut microbiota is correlated with a worsened outcome in patients, and that these changes are evident up to three weeks after hospitalization. We have previously demonstrated an important role of the gut microbiome on stroke outcome in proof-of-principle experiments using germ-free and recolonized mice. Further experimental studies in rodent stroke models have identified a key role for the immune system, particularly brain-invading lymphocytes originating from the intestinal immune compartment, in mediating along the gut–brain axis.

The gut microbiome produces a large number of bioactive metabolites which may affect brain function via modulating the immune system or afferent neuronal pathways. In particular, the metabolite group of short-chain fatty acids (SCFAs) acetate, butyrate, and propionate have been shown to readily cross the blood–brain barrier and affect brain function in development, health, and disease. For

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example, SCFA treatment improved the disease course in experimental autoimmune encephalitis by promoting anti-inflammatory mechanisms and reducing axonal damage. In mouse models of chronic stress, mice that received SCFA treatment exhibited significant improvements in antidepressant and anxiolytic behaviors, which was accompanied by reduced plasma corticosterone levels and differential gene regulation.

More recently, the role of SCFAs in modulating the immune system has been studied in great detail. Through these investigations, they have been shown to play a role in the polarization of T cells in the intestinal immune compartment and inducing anti-inflammatory T-cell subset. Other studies have shown a critical role for microbiota-derived SCFAs in the maturation of microglial cells, the brain's resident immune cells. However, SCFAs can also have far-reaching pleiotropic effects beyond the immune system, including a direct effect on neuronal function through their potent function as histone deacetylase inhibitors. Accordingly, the importance of SCFA function has been implicated in neurodegenerative diseases and even in postischemic neurogenesis.

Despite the key contribution of the gut microbiome to stroke outcome and the identification of SCFA as one of the microbiome's primary bioactive mediators, the role of SCFAs and their potential therapeutic use for poststroke recovery in the chronic phase after brain ischemia have not yet been investigated. In this study, we comprehensively investigated the effect of SCFA administration on poststroke recovery using advanced behavior analyses, *in vivo* wide-field calcium imaging, transcriptomic studies, and histological analyses to study and link SCFA-mediated recovery mechanisms from the molecular level up to behavior.

SCFA SUPPLEMENTATION IMPROVES RECOVERY AND CORTICAL REORGANIZATION AFTER STROKE

We previously showed that stroke induces dysbiosis of the gut microbiome with the hallmark of reduced bacterial diversity. To test the impact of poststroke gut dysbiosis on the metabolic function of the microbiome, we performed targeted analysis of plasma samples for SCFA concentrations after fMCAo stroke and sham surgery in mice by mass spectrometry. This analysis revealed significantly reduced plasma SCFA concentrations 3 d after fMCAo stroke surgery compared with sham-operated mice. Therefore, we hypothesized that supplementation of SCFA

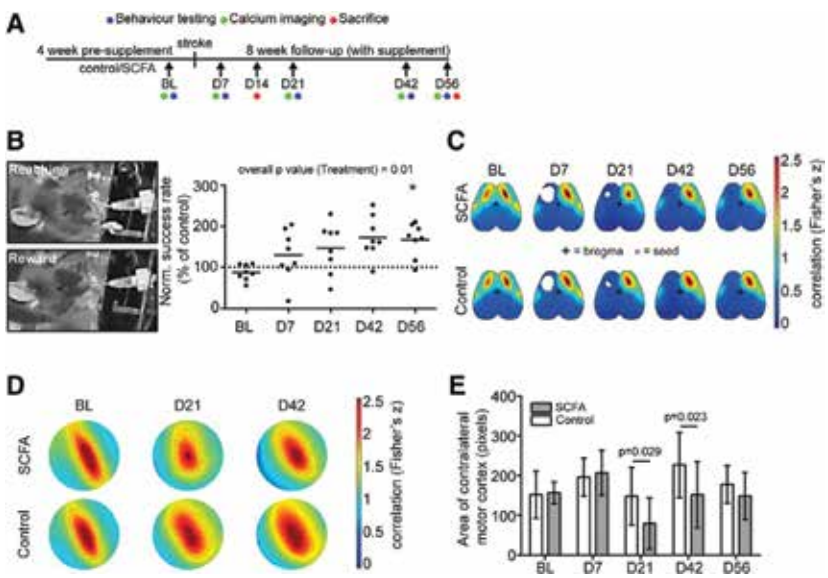

FIG. 1. SCFA supplementation improves recovery after stroke. A. Schematic diagram illustrating the timeline of SCFA supplementation and analysis time points. BL, Baseline; D, day (after stroke). B. Representative images obtained during the lever pull test of trained mouse successfully reaching for the lever (left, above) and obtaining the peanut oil reward (left, below). Right, Normalized success rate for lever pulls by the affected (contralesional) forelimb. Relative values are shown per time point normalized to the mean of the control group. N = 8 per group. Horizontal line indicates mean. Two-way repeated measure ANOVA with Holm–Sidak's post hoc test. C. Topographic depiction of seed-based functional connectivity of both hemispheres at indicated time points of SCFAs and control-treated mice. Seed is placed in the homotypic contralesional region to the ipsilesional lesion area (i.e., the contralesional motor cortex). Color code represents Fisher's z correlation between the seed and every other pixel in the cortex. D. Enlarged images of the contralesional motor cortex (region homotypic to the infarct lesion). Area highlighted with dotted line represents the highly connected functional motor cortex area (pixels with Fisher's z values > 2.25). E. Quantification of highly correlated (Fisher's z > 2.25) area of the contralesional motor cortex in control (open bars) and SCFA-treated mice (gray bars). N = 15 per group. Multiple t tests per time point with Holm–Sidak's correction for multiple testing.

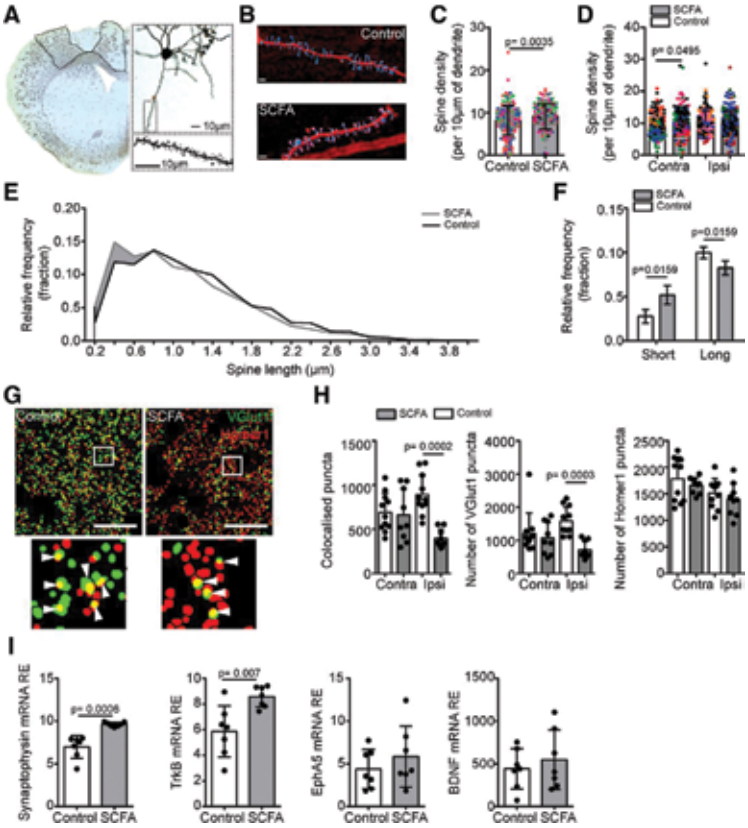

FIG. 2. Poststroke neuronal plasticity is altered by SCFA treatment. A. Representative images of Golgi-Cox stained brain sections 14 d after PT stroke. Dotted area in overview image represents perilesional cortical area used for spine analysis. Magnifications show representative pyramidal neuron and high-magnification image as used for spine analysis. Top right, Cortical pyramidal neuron. Bottom right, Example of spines identified on dendrite. Scale bar, 10 µm. B. 3D reconstruction for a dendrite section with spines as used for further quantification of spine densities and lengths. Scale bar, 2 µm. C. Quantification of pyramidal spine density per 10 µm of dendrite in the cortex of control and SCFA-treated naive mice (no stroke induction). Each color represents a different mouse, and each dot indicates a different dendrite. Five neurons per hemisphere analyzed in total for 4 or 5 mice per group (Mann–Whitney U test). D. Quantification of spine density per 10 µm of dendrite in the perilesional and contralesional cortex at 14 d after stroke (Kruskal–Wallis test with Dunn's correction for multiple comparisons). E. Histogram of the relative frequency (fraction) of spines found at different lengths 14 d after PT lesion in the perilesional cortex. Bin width is 0.2 µm. F. Quantification of short (0.2 µm) and long (1.4 µm) spines in control (open bars) and SCFA (gray bars) treated mice. N = 4 or 5 per group. Mann–Whitney U test. G. Representative particle images of presynaptic terminals by VGlut1 (green), postsynaptic densities by Homer1 (red), and nuclei with DAPI (blue) of the cortex from control and SCFA-treated mice, as used for quantification of colocalized presynaptic and postsynaptic particles (puncta). Scale bar, 20 µm. Arrowheads indicate colocalized (yellow) puncta. H. Synapse counts were quantified as colocalized VGlut1 and Homer1 puncta. Quantification for colocalization (left) and for single markers (middle and right) revealed significantly changed synapse counts as a result of the reduced number of VGlut1+ terminals in the perilesional cortex. contra, Contralesional hemisphere; ipsi, ipsilesional hemisphere. N = 3 or 4 mice (3 sections per mouse). Statistical analysis was performed with Kruskal–Wallis test with Dunn's multiple comparison correction. I. Relative expression (RE) of mRNA for key molecules involved in synaptic plasticity (left), synaptophysin (left middle), TrkB (right middle), and EphA5 (right) BDNF from the perilesional cortex in control (open bars) and SCFA (gray bars) treated mice. N = 7 per group. Mann–Whitney U test.

would increase circulating SCFA concentrations and potentially induce therapeutic effects within the chronic poststroke recovery period. To test this hypothesis, we supplemented mice for 4 weeks with drinking water containing either a mix of acetate, butyrate, and propionate or control drinking water with matched sodium chloride concentration. SCFA supplementation did not affect body weight or overt behavior of the animals.

We performed PT stroke surgery after 4 weeks of SCFA supplementation and assessed poststroke motor deficits of the affected forelimb with an automated lever pull test while animals received further SCFA supplementation during the complete survival period (Fig. 1. A,B, page 27). Mice receiving SCFA supplementation performed significantly better compared with control-treated animals (Fig. 1B). A two-way ANOVA for repeated measurements revealed an overall *p* value of 0.01 for the treatment effect, and a significant difference (after correction for multiple comparisons) between treatment groups at 56 d after stroke (Fig. 1B). To analyze cortical network plasticity as the morphological surrogate of behavioral recovery, we used Thy1-GCaMP6s mice and performed resting-state *in vivo* calcium to record cortical wide-field fluorescence from a neuronal based calcium reporter. Using the homotypical contralesional region of the cortex, we performed seed-based correlation analysis, indicating the connectivity strength (*z* score) of this area to every other pixel in the cortex (Fig. 1C).

Previous research using fMRI in stroke patients has indicated that the homotypical contralesional region receives less neuronal inhibition from the stroked hemisphere, leading to disinhibition of the contralesional hemisphere. To analyze the size of highly connected homotypical contralesional regions (i.e., the contralesional motor cortex), we measured the area of pixels with a *z* score > 2.25 (Fig. 1D). We observed a significantly reduced area of the contralesional motor cortex in SCFA- compared with control-treated mice at D21 and D42 (Fig. 1E). In contrast, SCFA treatment did not significantly affect the primary infarct area by *in vivo* imaging and histological analyses with infarct volumetry in three different focal stroke models after stroke. These results indicated that, while SCFA supplementation did not affect the initial lesion development, SCFAs improved behavioral stroke outcome and modulated cortical network plasticity at later stages after stroke.

SCFA SUPPLEMENTATION MODULATES POSTSTROKE SYNAPTIC PLASTICITY

After an ischemic brain injury, the entire cortex undergoes rapid functional and morphological reorganization, including neuronal dendritic plasticity, which allows adult neurons to form new connections. This process is correlated with improved functional connectivity after a cortical lesion. To analyze underlying morphological plasticity of the observed functional recovery in

behavior and cortical connectivity, we performed Golgi-Cox staining of brain sections to investigate the effects of SCFA supplementation on dendritic spine density of pyramidal cells (Fig. 2. A,B). We initially performed this analysis in the brains of naive animals that received either SCFA or control treatment, which revealed a higher pyramidal cortical spine density in the SCFA-treated mice (Fig. 2C). Next, we quantified brains at 14 d after PT stroke to capture a time period before the behavioral and cortical connectivity improvements were evident. Correspondingly, we observed that, after stroke, SCFA supplementation was associated with a significantly higher spine density in the homotypic contralesional region; however, these differences were not observed in the ipsilesional hemisphere (Fig. 2D) and were independent of infarct volume. Moreover, the analysis of spine length distribution revealed that SCFA treatment induced a shift toward shorter spine lengths, specifically in the ipsilateral, but not contralesional, hemisphere (Fig. 2. E,F). To further investigate synaptic plasticity under control of SCFA supplementation, we assessed synaptic density using costaining for VGlut1 (presynaptic) and Homer1 (postsynaptic) (Fig. 2G). We detected a significant reduction of the number of synapses (i.e., colocalized VGlut1 and Homer1 puncta) in the perilesional cortex of SCFA-supplemented mice (Fig. 2H). Interestingly, the difference in synapse counts was exclusively driven by the number of presynaptic VGlut1 puncta while Homer1-positive puncta remained unaffected.

Additionally, this pattern was also mirrored in the mean size of VGlut1 and Homer1 puncta. Finally, we analyzed the transcriptional regulation of key factors involved in synaptic plasticity. We observed that SCFAs significantly increased the expression of the presynaptic vesicle molecule synaptophysin and the BDNF receptor TrkB. BDNF itself, or the receptor tyrosine kinase EphA2, was not affected by SCFA supplementation. These results indicate effects of SCFA on morphological, synaptic plasticity, which could potentially precede the effects observed on functional recovery at later time points after stroke.

BRAIN TRANSCRIPTOMIC ANALYSIS INDICATES MICROGLIA AS THE MAIN CELLULAR TARGET OF SCFA

To determine whether the observed effects of SCFA were either directly mediated on neuronal function or affecting other cerebral cell populations, we took an unbiased approach to investigate the effects of SCFA on gene expression in the peri-infarct cortex. For this, we first depleted the gut microbiome of mice as the main SCFA source by administration of antibiotics, followed by supplementation of SCFA. Plasma gas chromatography-mass spectrometry quantification confirmed an increase of SCFA after supplementation in drinking water. Fourteen days after surgery, the infarct and peri-infarct regions were isolated for RNA sequencing, identifying 18 upregulated and 20 downregulated genes in SCFA supplemented mice.

The list of the top upregulated genes hinted at a role for microglia as we found numerous genes that have previously been reported to be involved in microglial function and/or activation, such as Ctsd, various complement molecules, Tyrobp, and Laptm5. To ascertain which cell type SCFA supplementation was mainly regulating in the brain, we took the 38 differentially regulated genes and compared them with an existing RNA-Seq database, which lists the fragments per kilobase of exon model per million reads found in astrocytes, neurons, oligodendrocytes, microglia, and endothelial cells (www.brainrnaseq.org). From the total number of fragments per kilobase of exon model per million read values of the 38 differentially regulated genes, we discovered that the vast majority of gene reads from the significantly regulated genes were associated with microglial cells. Additionally, we performed an ingenuity pathway analysis on the 38 differentially regulated genes and found that the complement system was the top pathway and highly upregulated in mice supplemented with SCFA. Within the brain, the complement pathway is critical for microglia activation and has been associated with synaptic pruning by microglia.

SCFAs MODULATE MICROGLIAL ACTIVATION AND IMMUNE CELL COMPOSITION

Based on the transcriptomic data's indication that microglia are the effector of SCFA-mediated poststroke recovery, we performed more in-depth analyses of microglial activation and the inflammatory response to stroke. As a surrogate of microglia activation, we performed Iba-1 immunohistochemistry of the cortex (Fig. 3A) and assessed microglia morphology using automated analysis. Fourteen days after stroke, cortical microglia from SCFA-supplemented mice displayed a significantly more ramified and less spherical (i.e., less activated) morphology compared with controls (Fig. 3B). Additionally, the total number of microglia were significantly reduced in SCFA- compared with control-treated mice (Fig. 3C).

These results indicate a modulation of the microglial response to stroke by SCFAs. Therefore, we further explored microglial function by assessing classical histological markers for microglial activation and polarization. We observed a significant reduction of CD68 expression in Iba-1$^+$ microglia (Fig. 3D), whereas Arginase-1 and iNOS were not significantly regulated by SCFA supplementation (Fig. 3E). Aside from evoking a microglial response, stroke induces invasion of peripheral immune cells into the brain parenchyma, which can deteriorate stroke outcome. In particular, lymphocyte counts in brains after photothrombosis are still elevated 14 d after surgery. Additionally, cytokines secreted by brain-invading lymphocytes can modulate microglial activation. Therefore, we performed flow cytometry of brain homogenates (Fig. 3F) and observed a significant reduction in cerebral lymphocyte invasion in SCFA-treated animals compared with controls (Fig. 3G).

The invasion of peripheral lymphocytes to the ischemic brain depends on the number of circulating lymphocytes, the expression of cerebral endothelial adhesion molecules, and the chemokine gradient derived from the injured brain tissue. In accordance with our nontargeted transcriptomic analysis, which did not reveal significant regulation of chemokines or adhesion molecules by SCFAs, also a targeted PCR analysis of key adhesion molecules and tight junction proteins involved in

SCFAs DERIVED FROM THE GUT MICROBIOME ARE CAPABLE OF IMPROVING POSTSTROKE RECOVERY VIA THE MODULATING EFFECTS OF BRAIN-INVADING LYMPHOCYTES ON MICROGLIAL FUNCTION.

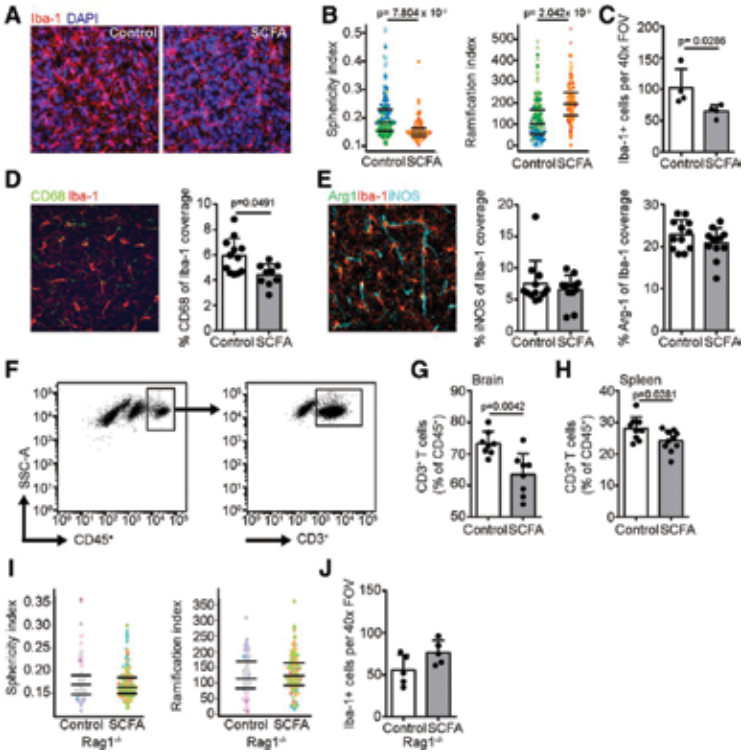

FIG. 3. Modulation of poststroke neuroinflammation by SCFA depends on peripheral lymphocytes. A. Representative maximum intensity projections of microglial staining using Iba-1 (red) and DAPI (blue) in the ipsilesional hemisphere 14 d after PT stroke with either control (left) or SCFA (right) supplementation. B. Microglial morphology was analyzed in 3D using an automated analysis algorithm in the ipsilesional cortex of mice 14 d after stroke, which revealed significantly reduced sphericity (left) and increased number of branch nodes (right) as markers of reduced microglial activation by SCFA compared with control treatment. Each symbol represents one microglia. Different colors group together microglia from the same mouse. C. Number of microglia found per 1 high-power (40×) FOV in the perilesional cortex. D. Coexpression coverage analysis in the ipsilateral hemispheric cortex for CD68 and Iba-1 expressed as percentage of Iba-1 from a maximum intensity projection. Representative immunofluorescence image (red represents microglia; green represents CD68) (left) and quantification (right). E. Coexpression coverage analysis in the ipsilateral hemispheric cortex for iNOS and Arginase1 (Arg1) with Iba-1 expressed as percentage of Iba-1 coverage area from a maximum intensity projection. Representative immunofluorescence image (red represents microglia; green represents Arg1; cyan represents iNOS) (left) and quantification (right). N = 3 mice per group and 3 images per hemisphere. In contrast to the effects of SCFA on microglia function, endothelial cells were unaffected by the SCFA treatment. F. Representative gating strategy for flow cytometric analysis of T cells (CD45+CD3+). SCFA supplementation significantly decreased the frequency of T cells in (G) brains and (H) spleens 14 d after stroke. N = 9 per group. Quantification of (I) sphericity (left) and ramification index (right) and (J) absolute cell counts of microglia 14 d after stroke in the perilesional cortex of Rag1–/– mice. In contrast to WT mice (compare with B,C), SCFA treatment did not affect microglia activation in lymphocyte-deficient Rag1–/– mice. All statistical analyses in this figure were performed using the Mann–Whitney U test.

poststroke lymphocyte recruitment at the blood–brain barrier did not show a significant regulation by SCFA. In contrast, we detected a significant reduction of systemic T-cell counts by SCFA supplementation in the spleen (Fig. 3H), a secondary lymphatic organ that well characterizes the systemic immune response after stroke.

These findings suggest that SCFAs may primarily affect lymphocytes already in the peripheral immune compartments, which then might secondarily mediate changes in the cerebral immune milieu after brain invasion. Therefore, we next aimed to test this hypothesis by investigating the effects of SCFA on microglia in the absence of lymphocytes. For this, we used lymphocyte-deficient *Rag1*$^{-/-}$ mice, which were given SCFA supplementation or control drinking water. In contrast to lymphocyte-competent WT mice, SCFA supplementation in *Rag1*$^{-/-}$ neither affected microglia morphology, nor reduced microglial cell counts indicating a key role of lymphocytes for mediating the SCFA effects on microglia.

SCFAs: LIKELY THE MISSING LINK IN THE PATHOPHYSIOLOGICAL FUNCTION OF THE GUT–BRAIN AXIS IN STROKE AND POSTSTROKE RECOVERY?

This study demonstrates that SCFAs, critical metabolites derived from the gut microbiome, are capable of improving poststroke recovery via the modulating effects of brain-invading lymphocytes on microglial function. Results from our study support this conclusion through several key findings. First, stroke lowers blood SCFA concentrations. Second, SCFA supplementation combats the deleterious effects of this poststroke response, which we demonstrated by associated SCFA supplementation with improved behavioral recovery, changes in cortical network connectivity which are generally associated with improved stroke outcomes, and changes in histological markers of synaptic plasticity. Third, we indicated a potential mechanism for the observed improvements in the recovery of SCFA-treated animals by finding changes in microglial function, which were dependent on circulating lymphocytes. Consequently, these findings indicate that SCFAs affect peripheral lymphocytes, maturation, or egress from primary lymphatic tissue, and lymphocytes then indirectly mediate the SCFA effects on the brain micromilieu either by their overall reduction in cerebral invasion or polarization of the secreted cytokine profile.

These novel findings introduce SCFA as the most likely missing link in the pathophysiological function of the gut–brain axis in stroke and poststroke recovery, for which the microbiota-derived effector molecules were so far unknown. Our use of a novel imaging modality, *in vivo* wide-field calcium imaging, provided a highly sensitive tool for assessing cortical network plasticity after stroke (Cramer, *et al.*, 2019). This tool

allowed us to perform analyses of network plasticity by comparable analytical approaches to fMRI in patients, with the exception of using a genetically encoded reporter for direct neuronal activation instead of the blood flow surrogate marker (BOLD) used in MRI (Cramer, *et al*., 2019). The analysis of connectivity within the cortical network provides information about the dynamic changes in defined cortical areas under resting-state conditions, which gave us a unique ability to measure even the more subtle effects of SCFA supplementation on poststroke plasticity.

In human stroke patients, interhemispheric resting-state connectivity is significantly weakened following stroke. Specifically, it is thought that inhibitory projections from the lesioned hemisphere to the homotypic contralesional hemisphere are attenuated, leading to a disinhibition of the homotypic area in the contralesional hemisphere.

We confirmed this effect with our optogenetic imaging approach, by observing an increase in the contralesional motor cortex area as an indicator of reduced transhemispheric inhibition. This "blooming" effect of the contralesional motor cortex was significantly ameliorated by the SCFA treatment at chronic time points after stroke, which could indicate an improvement of interhemispheric connectivity and thereby reestablish inhibition of the contralesional hemisphere.

Together, the results of this study identified SCFAs as critical metabolites derived from the gut microbiome affecting T-cell function and thereby indirectly modulating the neuro-regenerative milieu. This expands our current understanding of the mechanisms along the gut–brain axis in acute brain injury and recovery where poststroke dysbiosis affects the production of key microbiota-derived metabolites, their impact on immunological homeostasis, and finally the capability for efficient functional recovery. The efficacy of SCFAs for promoting recovery in an experimental stroke model on a functional as well as morphological level opens up novel therapeutic possibilities for improving recovery of human stroke patients. Future studies should validate the proregenerative effect of SCFAs on poststroke recovery before further translational development. Based on the findings from this study and indications for efficacy and similar modes of actions in primary autoimmune brain disorders, it is well conceivable that SCFA supplementation could be used as a safe and practical add-on therapy to stroke rehabilitation.

However, further studies will be required to test the efficacy of this approach in a post-treatment paradigm, in combination with common comorbidities and comedications, and finally validate in a confirmatory, multicenter preclinical study design before further translation to human stroke patients can be considered.

Rebecca Sadler recently earned a PhD degree from the Institute for Stroke and Dementia Research, Munich Cluster for Systems Neurology, in Munich, Germany.

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Is edible oil refining about to change?

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

Rebecca Guenard

Anyone familiar with edible oils knows there is an inherent level of trace containments regardless of the source. There are PCBs and dioxanes in marine oil and glycidyl fatty acid esters and MCPD in vegetable oils.

The more an oil is refined, the more it is exposed to higher temperatures and retention times, thus generating additional toxins that lead to further downstream processing to remove the contaminants.

Crude oil refineries have been testing different methods for contamination removal that maximize product retention while reducing energy costs. Their goal is impurity removal at low temperature and pressure. Short-path distillation and thin-film stripping are two techniques that have been gaining popularity as probable methods for achieving this goal. Recent reports indicate the processes could change the future of oil refining.

Deodorization is currently one of the last steps in refining. It uses steam, produced by high temperature and pressure, to strip away odors, impurities, and environmental contaminants from edible oils. However, the process also removes desirable compounds, like tocopherols. This naturally occurring oxidizing agent is typically added back in after deodorization.

Despite its tediousness, deodorization is a crucial purifying step that can reduce free fatty acid concentrations to below 1%. However, to achieve this conventional steam deodorization, most vegetable oils must be kept at nearly 250°C for up to 30 minutes. These conditions are not ideal for marine oils that lose micronutrients at such high temperatures. Desirable omega-3 free fatty acids (FFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), can degrade or be lost in the deodorization step. Since infrastructure costs for multiple processing facilities can be prohibitive, refineries want one purification technique for multiple oils. Many refineries are now opting for distillation methods.

In September, the Swiss company Nutriswiss announced that it had achieved the highest quality edible oil ever processed. The crude oil refiner, headquartered in Lyss, claims to have reduced yield loss, improved crystallization properties, preserved micronutrients, and lowered pollution concentrations with the installation of short-path distillation. In an article for bakeryandsnacks.com, a Nutriswiss spokesperson said the technology "gave them a major quality advantage when refining edible oils (https://tinyurl.com/shortpathdistillation)." The company says it worked with the German company, VTA, to design the deodorizer but did not provide any details (https://tinyurl.com/VTArefining).

Generally, short-path distillation works by heating the oil as it runs down the interior walls of a column. As impurities evaporate from the oil, they condense on a different surface a short distance away. The process is carried out under deep vacuum to minimize the collision and degradation of the vapor molecules. Mechanical rollers or wipers are used to spread the oil.

The inherent disadvantage of conventional short-path distillation methods is that it is a single-stage process that requires several passes or multiple columns in series to achieve the desired purity. The system requires significant initial investment and higher operating costs for the manufacturer—both in terms of energy consumption and processing time. Also, in certain applications, significant yield losses of as much as 15% have been reported when using molecular distillation for product purification.

Artisan Industries based in Stoughton, Massachusetts, USA, was recently awarded a US patent (US10472589, *Inform,* July/August 2020, page 38) for removing toxins from edible fats and oils with a process that combines concepts from short-path distillation and thin-film stripping (https://www.artisanind.com/). In an interview with Food Engineeringmag.com, senior vice president and chief technology officer, Perry Alasti said, "This novel patented proprietary technology's main advantages, for both short-path distillation and thin-film evaporation for physical de-acidification of edible oils, are lower operating temperatures and shorter processing times, enabling treatment of heat-sensitive oils without degradation (https://tinyurl.com/artisaninterview)."

The new deodorizer integrates steam stripping and separations technology. A feed stream of crude or chemically refined oils is preheated to 400–450°F. The preheated oil is introduced at the top of a column and runs down, while superheated steam is injected from the bottom of the column and flows in the opposite direction. The deodorizer operates under moderate vacuum conditions of 1to 5 Torr. By this means, a cleaner edible oil, stripped of FFAs and toxins, is collected at the base of the column. FFAs, along with the contaminates, are carried to the top of the column where they are condensed and removed from the process.

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According to Alasti, one advantage of the process is that temperatures near the bottom of the stripping column are lower than in conventional deodorizers and distillation equipment. The pressure remains consistent throughout the process, resulting in better separation and more complete stripping of contaminants. The oil runs through the column quickly while still achieving optimal purity.

The deodorizer operates on a variety of marine oils, in addition to flaxseed and algae oil, without destroying their nutrients. The company claims that 100% of the omega-3 fatty acids in these oils are retained and toxins, like PCBs, are reduced to a concentration less than 500 parts per trillion. The method can also be used to remove glycidyl esters and MCPDs from palm and soybean oils. Finally, Artisan suggests that the technology can be used downstream of existing deodorizers to remove process-generated contaminants.

Consumers have increased their consciousness about the impact of edible oils on health over the last decade. A once fringe market for unrefined oils has now become mainstream. Store shelves for specialty edible oils continue to expand with unique seed varieties, like pumpkin and walnut, as well as more cold-pressed versions of mainstays.

Government regulations drive change even faster. The European Union has recently reconsidered the amount of toxins allowed in edible oils. In January 2021, the European Commission will start to enforce a stricter maximum limit on MCPD in edible oils. In palm, olive pomace, and nut oils, the cap will be 2.5 milligrams per kilogram. For rapeseed, maize, sunflower, and soybean oil, MCPD is not to exceed 1.25 milligram per kilogram. For vegetable and fish oil used as food ingredients for small children, the requirements will be more stringent, in the microgram range.

Such pressure from regulating agencies and consumers are a driving force for innovating current refining methods. Another consideration is the desire to reduce energy consumption. Like most industries, edible oil refineries want to use energy in the most efficient way possible to benefit the environment, as well as their bottom line. For these reasons, refineries will start to adopt more mild processing methods. Technology that enables a shorter duration at high temperatures and operation at lower vacuum pressures will be an advantage.

Information

Removing toxins from edible fats and oils, Alasti, P., U.S. Patent 010472589B1, November 12, 2019.

Removal of organic environmental pollutants from fish oil by short-path distillation, Breivik, H. and Thorstad, O., *Lipid Technol. 17*: 55–58, 2005.

3-MCPD- and glycidyl esters can be mitigated in vegetable oils by use of short path distillation, Pudel, F., *Eur. J. Lipid Sci. Technol. 118*: 396–405, 2016.

Michigan latest US state to contemplate ban on PFASs in food packaging: Bill would also ban phthalates and bisphenols beginning in 2022

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

A new bill introduced in the Michigan state senate would ban the use of per-and polyfluoroalkyl substances (PFASs), phthalates, and bisphenols in food packaging.

If the measure ultimately is signed into law, Michigan would join Washington state and Maine in banning the class of synthetic chemicals from food packaging, further adding to the headwinds against the use of PFASs in fast food wrappers and related materials.

Senate Bill 1072 was introduced on September 1, 2020 by Michigan State Senator Jeff Irwin, a Democrat. It would prohibit the sale or distribution of food packaging containing PFASs, bisphenols, or phthalates beginning January 1, 2022.

Any ban, however, would be contingent on the results of an alternatives analysis and a determination that there are safer alternatives to PFASs, phthalates, and bisphenols available.

Irwin said Michigan's analysis could learn from similar assessments underway in Washington and Maine. Washington state plans to publish the results of its alternatives assessment for PFASs later this year.

A report to the Michigan legislature on potential alternatives would be due on January 1, 2021, according to the original text of the bill.

That leaves a short time frame for the bill to move through the state legislature, which is scheduled to see its two-year legislative cycle end around the third week of December, according to Irwin.

New York's legislature has also passed a bill that would ban PFASs in food packaging, but it would forgo an alternatives analysis in favor of an outright ban. That bill, which has not been signed into law, keeps with the suggestions in draft model legislation from the Toxics in Packaging Clearinghouse (TPCH), which calls for a ban on PFASs in all packaging, not just food packaging.

But Irwin said he is most focused on substances in food packaging.

He said he first heard of PFAS-containing fast food wrappers several years ago. And they are still present today many types of fast food packaging, according to a recent study by the nonprofit Ecology Center—in conjunction with Toxic-Free Future and the Mind the Store campaign of Safer Chemicals Healthy Families (SCHF).

"Industry could just decide to stop putting these harmful chemicals into food packaging," he said. "Unfortunately, that hasn't happened."

SALTY FOODS STUDY CASTS DOUBT ON EFFECTIVENESS OF REGULATORY FCM TESTING

The amount of salt in food can affect the extent to which chemicals in food contact materials (FCMs) migrate, according to a study involving scientists at the European Commission's Joint Research Centre.

The results suggest that, under some circumstances, current testing approaches for regulatory compliance may underestimate migration of substances from FCMs intended for use with salty foods.

In the EU, the plastic FCMs Regulation sets migration limits for certain additives. It also requires manufacturers of the materials to provide actors in their supply chains with documentary evidence of compliance, including assessment of migration.

The regulation provides detailed rules for migration testing, which is usually conducted using "simulants" designed to represent a particular food category.

Six official simulants are listed in the regulation, but none are specifically intended for salty foods.

Three JRC scientists, plus Emmanouil Tsochatzis at Aarhus University in Denmark, investigated the influence of high salinity on the migration of caprolactam and 2,4-DTBP from polyamide–polyethylene multilayer FCMs.

Caprolactam is the monomer of a type of polyamide widely used in kitchenware and a regulated additive under the plastic FCMs Regulation. 2,4-DTBP is a non-intentionally added substance originating from the degradation of an anti-oxidizing agent.

The group used "simulant A" from the regulation—ethanol at a concentration of 10%—and water, across four salinity levels, the highest of which was 15%.

The results suggested that salinity affected the migration of caprolactam, with the nature of the relationship flipping in terms of direction according to the medium: higher salinity was associated with reduced migration with water, but increased migration with simulant A. In contrast, it appeared to have no effect on 2,4-DTBP.

In a paper published in the *Journal of Chromatography B*, the scientists say that that polarity and affinity for water could be the reason for the difference between the two substances. Caprolactam is polar and hydrophilic, whereas 2,4-DTBP is nonpolar and hydrophobic.

Salinity may have an "important and crucial" effect on the migration of polar compounds, such as caprolactam, they conclude.

Furthermore, the results suggest a "potential" need to use different simulants when performing migration testing of packaging intended for use with salty foods.

Meet Linsen Liu

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

Fast facts

Name	Linsen Liu
Joined AOCS	1991
Education	Ph.D. in food science and technology, Iowa State University (Ames, Iowa, USA)
Job title	Vice president of sciences
Employer	Guittard Chocolate Co. (San Francisco, California, USA)

PROFESSIONAL

What's a typical day like for you?
Typically, my days involve leading cross-functional meetings to enable effective manufacturing, innovation, and production.

Flash back to when you were 10 years old. What did you want to be when you grew up?
I wanted to be an electrical engineer when I was in middle school. In fact, I taught myself basic electrical engineering and did electrical wiring in my parents' and neighbors' houses.

Why did you decide to do the work you are doing now?
In 1977, I was assigned by the Chinese government to study oil processing. My passion for fats and oils, however, was developed in graduate school under the late Professor Guoqi Han, who was one of the pioneering researchers into oil chemistry in China. Then, during my doctoral study at Iowa State University, the late Professor Earl Hammond broadened my view from oil chemistry to oil biochemistry and food sciences.

Is there an achievement or contribution that you are most proud of? Why?
I conducted a systematic study on chemical interesterification in the 1990s. The study was published in the *Journal of the American Oil Chemists' Society* (*81*: 331–337, 2004) and its key application was patented in US Patent 6238926B1. This study revealed the essential role of alpha-proton, the mechanism of byproduct formation, and a technique for monitoring interesterification; the technology was recognized by peers as a breakthrough and has been adopted by the global oils and fats industry.

What event, person, or life experience has had the most influence on the direction of your life?
I was born in China during the Great Starvation (1959–1962) and grew up during the Cultural Revolution (1966–1976) in Mao's [Chairman Mao Zedong, 1893–1976] communist China. Poverty and political madness might be the most striking events impacting my life. In middle school, I began studying electrical engineering and mechanics in the hope of having a better future life and dodging the political nonsense.

PERSONAL

How do you relax after a hard day of work?
I like to read *Bailey's Industrial Oil and Fat Products* and *Beckett's Industrial Chocolate and Manufacture and Use* in my spare time, both of which have given me the most joy professionally and intellectually. In fact, I have read all the editions of *Bailey's*; my favorite is the third edition.

What skill would you like to master?
I look forward to mastering the skill of fine-chocolate manufacturing because cocoa butter presents the most intriguing formulation challenges for an oil chemist with its variety of polymorphic crystal structures. As a technical leader, I have been optimizing innovation management, which is considered to be an emerging science and would improve the effectiveness and efficiency of the commercialization of technology.

What are you looking forward to in the coming months?
Becoming a chocolate maker and applying best practices in the commercialization of technology.

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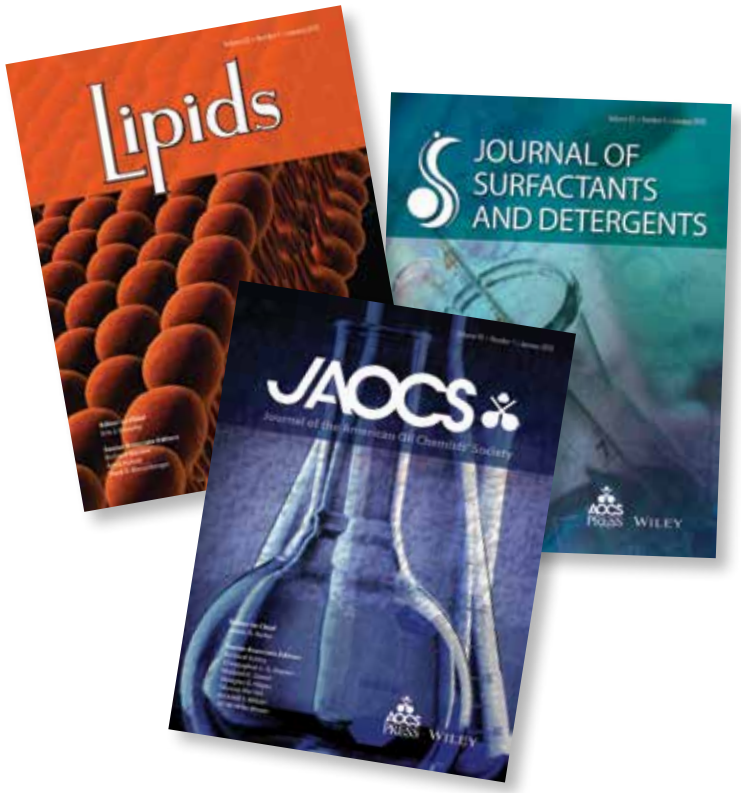
Young Scientists to Watch publish with AOCS Journals

The AOCS Young Scientists to Watch initiative is directed at scientists in the early stages of their career (younger than 36 years old or have earned their highest degree within the last 10 years) who are conducting transformative research in the area of fats, oils, oilseed proteins, and related materials. The recognition is given to feature scientists whose research "has significantly advanced scientific understanding within their discipline or holds substantial promise for such an impact in the near future."



A special collection of papers written by Young Scientists to Watch recipients and published in one of the three AOCS journals: *Journal of the American Oil Chemists' Society*, *Journal of Surfactants and Detergents*, and *Lipids has been established and is available at https://aocs.onlinelibrary.wiley.com/hub/young-scientists-to-watch.* These researchers will also be given priority to present at upcoming AOCS Annual Meetings.

Following are three papers from the collection written by Young Scientists to Watch recipients Ozan N. Ciftci, Nanjing Zhong, and Bingcan Chen which were published in the *Journal of the American Oil Chemists' Society* (*JAOCS*).

Effect of chemical structure of solid lipid matrix on its melting behavior and volumetric expansion in pressurized carbon dioxide
Junsi Yang and Ozan N.
First published: November 26, 2019
https://doi.org/10.1002/aocs.12305

Enzymatic production of diacylglycerols from high-acid soybean oil
Nanjing Zhong, Maomao Kou, Fenghuan Zhao, Kunpeng Yang, and Shaoyan Lin
First published: June 6, 2019
https://doi.org/10.1002/aocs.12245

Genotype x environmental effects on yielding ability and seed chemical composition of industrial hemp (*Cannabis sativa* L.) varieties grown in North Dakota, USA
Yang Lan, Fengchao Zha, Allen Peckrul, Bryan Hanson, Burton Johnson, Jiajia Rao, and Bingcan Chen
First published: October 4, 2019
https://doi.org/10.1002/aocs.12291

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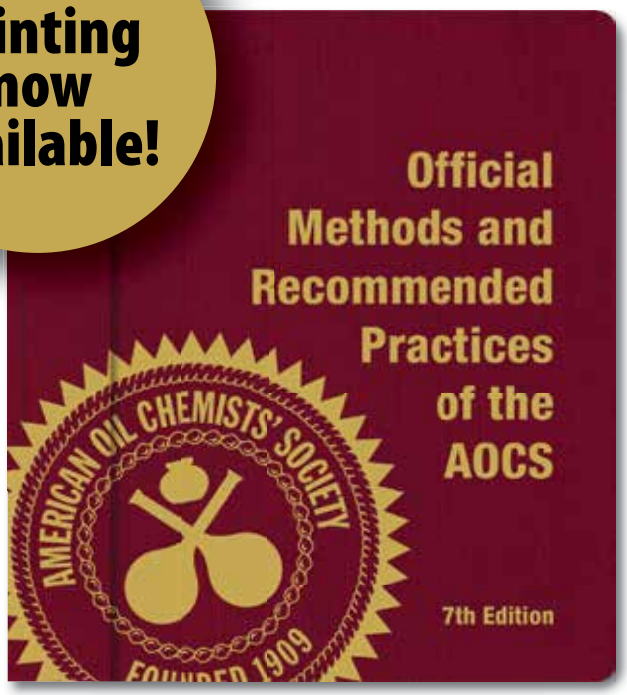

The second printing of the 7th edition of ***Official Methods and Recommended Practices of the AOCS*** is now available! The updated *AOCS Methods* has four new official methods and revisions to fifteen previously approved methods.

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ADDITIONS

AOCS Official Method Ca 17a-18
Determination of Trace Elements in Oil by Inductively Coupled Plasma Optical Emission Spectroscopy

Joint JOCS/AOCS Official Method Cd 29d-19
2-/3-MCPD Fatty Acid Esters and Glycidyl Fatty Acid Esters in Edible Oils and Fats by Enzymatic Hydrolysis

Joint JOCS/AOCS Recommended Practice Cd 29e-19
2-/3-MCPD Fatty Acid Esters and Glycidyl Fatty Acid Esters in Fish Oils by Enzymatic Hydrolysis

Joint JOCS/AOCS Official Method Ch 3a-19
Determination of the Composition of Fatty Acids at the 2-Position of Oils and Fats-Enzymatic Transesterification Method using *Candida antarctica* Lipase

REVISIONS

AOCS Standard Procedure Ba 6a-05
Crude Fiber in Feed by Filter Bag Technique

AOCS Official Method Cc 7-25
Refractive Index of Fats and Oils

AOCS Official Method Cd 26-96
Stigmastadienes in Vegetable Oils

AOCS Official Method Cd 27-96
Steroidal Hydrocarbons in Vegetable Oils

AOCS Official Method Cd 3d-63
Acid Value of Fats and Oils

AOCS Official Method Cd 29c-13
2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by GC/MS (Difference Method)

AOCS Official Method Ce 8-89
Tocopherols and Tocotrienols in Vegetable Oils and Fats by HPLC

AOCS Official Method Ch 3-91
Fatty Acids in the 2-Position in the Triglycerides of Oils and Fats

AOCS Official Method Ch 5-91
Specific Extinction of Oils and Fats, Ultraviolet Absorption

AOCS Analytical Guidelines Ch 7-09
International Trade Standard Applying to Olive and Olive-Pomace Oils

AOCS Official Method Ch 8-02
Wax Content by Capillary Column Gas-Liquid Chromatography

AOCS Procedure M 1-92
Determination of Precision of Analytical Methods

AOCS Procedure M 3-82
Surplus Status of Methods

AOCS Criteria M 5-09
Approved Chemists (Criteria)

AOCS Criteria M 6-09
Certified Laboratories (Criteria)

New and revised methods included in the 2020 Additions and Revisions may also be purchased individually as PDF downloads.

PATENTS

Cosmetic or pharmaceutical composition, to be applied topically

Albrecht, M., Kuhs GmbH, US10660831, May 26, 2020

A cosmetic or pharmaceutical composition, to be applied topically is described, which has a hydrophilic outer phase, at least one cosmetic and/or pharmaceutical active ingredient, and at least one carrier substance for the active ingredient. The carrier substance here forms such structures, which comprises at least two lamellar double membrane layers arranged one over another in the manner of a sandwich, wherein between adjacent double membrane layers, aligned parallel to each other, a layer of an inner phase is respectively arranged. The active ingredient is distributed in the double membrane layer and in the layer of the inner phase such that the layer of the inner phase contains the active ingredient in a concentration range between 2% by weight and 98% by weight and the double membrane layer contains the active ingredient in a concentration between 98% by weight and 2% by weight, respectively in relation to the total concentration of active ingredient, and the outer phase has no or almost no active ingredient.

Compositions and methods comprising medium-chain triglycerides for treatment of epilepsy

Pan, Y., *et al.*, Societe des Produits Nestle SA, US10668041, June 2, 2020

The invention provides compositions and methods for treatment of epilepsy in an animal. In one embodiment, a method for treating epilepsy in a companion animal can comprise administering to the companion animal a food composition comprising a medium-chain triglyceride (MCT), wherein the MCT is present in the food composition in an effective amount for reducing or preventing seizures when the food composition is administered to the companion animal.

Methods and compositions for improving cognitive function

French; S., *et al.*, Mars, Inc., US10695318, June 30, 2020

This invention relates to compositions, and methods of use thereof, for (i) enhancing executive cognitive function(s) (for example, decision making, planning, working memory, multitasking, judgment, numerical problem-solving, reading comprehension), and/or (ii) increasing blood flow in brain vasculature, comprising administering to a subject in need thereof, certain polyphenols such as flavanols, procyanidins, or pharmaceutically acceptable salts or derivatives thereof.

Solvent extraction of oil from distillers dried grains and methods of using extraction products

Bruinsma, K., *et al.*, Novita Nutrition, LLC, US10696921, June 30, 2020

A process for extraction of crude oil from distillers dried grain solubles and/or distillers dried grains and producing corn distillers meal that may be used as a livestock supplement is disclosed. For example, the corn distillers meal may be used as a crude protein supplement for use in a livestock feed diet or a poultry feed diet. The solvent extracted crude oil may be suitable for oleochemical processing for personal care and home care products, biodiesel production, and/or renewable diesel production from hydro-treating the extracted oil to make green diesel fuel.

Composition containing highly unsaturated fatty acid or alkyl ester thereof and a method for producing the same

Doisaki, N., *et al.*, Nippon Suisan Kaisha, Ltd., US10696924, June 30, 2020

To provide a composition comprising highly enriched PUFA or its alkyl esters while containing fatty acid esters of 3-MCPD at adequately low concentrations and to provide an efficient method for producing the composition. A composition that contains fatty acids or fatty acid alkyl esters as its major component, the composition containing highly unsaturated fatty acid or alkyl ester thereof, wherein the proportion of the highly unsaturated fatty acid in the constituent fatty acids of the composition is 50 area % or more and wherein the concentration of 3-MCPD as found upon analyzing the composition by American Oil Chemists' Society official method Cd 29b-13 assay A is less than 1.80 ppm.

Enzyme-based methods of separating protein from protein-rich material

Ju, L.-K., *et al.*, The University of Akron, US10696993, June 30, 2020

Improved enzyme-based methods of separating protein from protein-rich material are provided. A method can include utilizing a modeling equation to more effectively hydrolyze the various types of carbohydrates present in a protein-rich material. A method can include a fed-batch method of incrementally adding a protein-rich material, an enzyme broth, or both a protein-rich material and an enzyme broth. A method can also include partially or completely recycling the hydrolysate.

Process of producing cocoa shell powder

Bernaert, H., *et al.*, Barry Callebaut AG, US10701951, July 7, 2020

A process for producing powdered cocoa shells as a food ingredient, as replacer for cocoa powder, to impart coloration in food products, and as fat bloom inhibitor in cocoa-based products.

Soap compositions and methods

Smith, S.A., Vanguard Soap LLC, US10702491, US10702491

Natural soap compositions and methods of manufacturing the same having anti-microbial properties for treating and preventing diaper rash and other microbial infections. The soap compositions may contain one or more fatty acids with carbon length ranging from four (C4) to twenty-two (C22) and/or natural fatty acid mixtures of coconut oil, olive oil, and/or tall oil fatty acids which are saponified with lye. The saponification lye may be sodium or potassium hydroxide. In preferred embodiments, the soap compositions contain at least one of sodium or potassium caprate, sodium or potassium caprylate, or mixtures thereof, especially 55:45% caprylate to caprate. The soap compositions are effective at treating or preventing diaper rashes and other microbial infections associated with *Candida albicans* (Ca–yeast), *Pseudomonas aeruginosa* (Psa–a Gram negative bacteria), *Staphylococcus aureus* (Sa–a Gram positive bacteria), and *Aspergillus niger* (An–a mold).

Corrosion-resistant epoxidized vegetable oil interior coating

McVay, R., *et al.*, PPG Industries Ohio, Inc., US10703920, July 7, 2020

A coating composition comprising epoxidized vegetable oil, an amine terminated polyamide and a silicone resin is disclosed. Substrates coated at least in part with such coatings are also disclosed.

Lipid extraction processes

Home, N., *et al.*, Aker Biomarine Antarctic AS, July 7, 2020

The present invention provides improved processes for extracting and preparing lipids from biological sources for use in pharmaceuticals, nutraceuticals, and functional foods.

Processes for the manufacture and use of pancreatin

Blume, H., *et al*, Abbott Products GmbH, US10704037, July 7, 2020

A process for the manufacture and use of pancreatin in which the concentration of one or more biological contaminants is reduced, such as viruses and/or bacteria, through heating the pancreatin at a temperature of at least 85°C. for a period of less than about 48 hours.

Brassica plants yielding oils with a low total saturated fatty acid content

Zheng, H., *et al.*, Cargill, Inc., US10709079, July 7, 2020

Brassica plants producing oils with a low total saturated fatty acid content and methods for producing such plants are described. The oils have a low total saturated fatty acid in combination with a low, mid, or high oleic acid content.

Storage-stable enzyme granules

Hede, P.D., *et al.*, Novozymes A/S, US10711262, July 14, 2020

The storage stability of enzyme granules for detergents can be improved by incorporating a polyamine having a molecule with at least 10% w/w of nitrogen wherein at least 50% of the N atoms are present as amines.

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

Lamprecht, B.A., *et al.*, POET Research, Inc., US10711221, July 14, 2020

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

Pharmaceutical wound-healing composition

Prabhune, A.A., *et al.*, Council of Scientific and Industrial Research, US10709743, July 14, 2020

The present invention discloses a biodegradable and biocompatible pharmaceutical composition comprising silk Sericin, sophorolipid, a gelling or thickening agent, and one or more pharmaceutically acceptable carriers or excipients for faster wound healing and limit scarring.

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

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PCP Protein and Co-Products
S&D Surfactants and Detergents

Review Articles

Enzymatic biodiesel production by hydroesterification using waste cooking oil as feedstock

Costa, M.J., *et al., Chem. Eng. Pro.—Process Intensification 157,* November 2020, https://doi.org/10.1016/j.cep.2020.108131.

Biodiesel production was investigated by hydroesterification using enzymatic catalysis in both hydrolysis and esterification reactions. Soybean oil resulting from food frying processes (WCO) was used as feedstock. Lipase from *Geotrichum candidum* was produced by submerged fermentation and used without prior purification as biocatalyst in WCO hydrolysis, which was optimized using a factorial design. A complete hydrolysis of WCO (44.1% mass fraction) was obtained after 80 min of reaction at 40 °C, performed in an emulsifier-free system with no buffer (40 °C and 900 rpm). In a subsequent step, FFA obtained from the hydrolysis reaction were esterified (40 °C, 200 rpm) using ethanol as acyl-acceptor (1:1.5) and *Pseudomonas fluorescens* lipase (PFL) immobilized on styrene-divinylbenzene (SDB) as biocatalyst (15% m/v). This biocatalyst was recycled for 6 cycles with no significant activity losses. The biodiesel composition was found by gas chromatography, and proton nuclear magnetic resonance (^{1}H NMR) spectra and Fourier transform infrared spectroscopy (FTIR) were used to verify the quality and purity of biodiesel. Enzymatic biodiesel achieved FAEE content of 94.1 ± 0.5% and no traces of monoacyglycerol (MAG), diacylglycerol (DAG), triacylglycerol (TAG) or free glycerol were observed, indicating that the hydroesterification route produced high-quality biodiesel, despite the fact that WCO was used as feedstock

An overview on advancements in biobased transesterification methods for biodiesel production: oil resources, extraction, biocatalysts, and process intensification technologies

Shashi Kant Bhatia, S.K, *et al., Fuel* 285, February 1, 2021, https://doi.org/10.1016/j.fuel.2020.119117.

Biodiesel is a non-toxic renewable energy source that is gaining attention globally owing to its direct applicability in preexisting engines without any modification. Various technologies from laboratory scale to industrial scale have been developed, and many plants have been established for biodiesel production using various feedstocks. Using biobased technology in biodiesel production is advantageous as these methods generate less waste and are considered ecofriendly. This article mainly discusses the availability of various oil resources—edible, non-edible, waste cooking oils (WCO)—and advancements in technology related to oil extraction. Specifically, biobased methods, such as immobilized enzymes (matrix) and heterogeneous catalysts (derived from biomass), reported to catalyze the transesterification reaction for biodiesel production are discussed in detail. Biodiesel production using conventional technologies results in low yield and purity and is time-consuming. Newly introduced process intensification technologies (microreactor, membrane reactor, microwave, reactive distillation, and centrifugal contractor) to overcome these issues are also discussed. The need to develop integrated process technologies for biodiesel production to make the process more economical is emphasized.

Original Articles

BIO PRO IOP *Yarrowia lipolytica* as a metabolic engineering platform for the production of very long-chain wax esters

Gao, Q., *et al., J. Agric. Food Chem.*. Just Accepted Manuscript, https://doi.org/ 10.1021/acs.jafc.0c04393.

The oleaginous yeast *Yarrowia lipolytica* is an attractive cell factory platform strain and can be used for sustainable production of high-value oleochemical products. Wax esters (WEs) have good lubricating property and are usually used as a base for production of advanced lubricants and emollient oils. In this study, we reported the metabolic engineering of *Y. lipolytica* to heterologously biosynthesize high-content very long-chain fatty acids (VLCFAs) and fatty alcohols, and efficiently esterify them to obtain very long-chain WEs. Co-expression of fatty acid elongases from different sources in *Y. lipolytica* could yield VLCFAs with carbon chain lengths up to 24. Combining with optimization of the central metabolic modules could further enhance the biosynthesis of VLCFAs. Furthermore, through screening of heterologous fatty acyl reduc-

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tases (FAR), we enabled high-level production of fatty alcohols. Genomic integration and heterologous expression of wax synthase (WS) and FAR in a VLCFAs-producing *Y. lipolytica* strain yielded 95–650 mg/L WEs with carbon chain lengths from 32 to 44. Scaled-up fermentation in 5-L laboratory bioreactors significantly increased the production of WEs to 2.0 g/L, the highest content so far in yeasts. This study contributes to the further efficient biosynthesis of VLCFAs and their derivatives.

EAT LOQ Effect of natural extracts obtained from food industry by-products on nutritional quality and shelf life of chicken nuggets enriched with organic Zn and Se provided in broiler diet

Martinez, L., *et al.*, *Poultry Sci.* 99: 1491–1501, 2020, https://doi.org/10.1016/j.psj.2019.11.008.

This study investigated the influence of an organic mineral-supplemented broiler diet on the quality of nuggets. The resulting chicken nuggets were enriched with inorganic and organic forms of Zn and Se. The nuggets were processed by incorporating extracts from food industry by-products (*rosemary* [RH and RL], hydroxytyrosol [HYT], pomegranate [P], grape [GS], and *Harpagophytum* [H]). The physiochemical, microbiological, and sensory characteristics of the chicken nuggets were evaluated over a 12-month period of frozen storage. The addition of natural extracts did not affect the pH, proximate composition, or color (CIELab) of the nuggets among samples. However, significative differences were found between month of analysis (range from pH 6.16 to 6.63; luminosity from 62.51 to 84.74; redness from 0.16 to 7.14; and yellowness from 10.80 to 33.77). In addition, the combination of phenolic compounds with Zn and Se retarded microbial growth and reduced protein and lipid oxidation, thus maintaining the sensory quality and extending the shelf life of this product. For instance, microbial growth in samples with a combination of R_L + GS was 75% what it was in the control sample (C), while microbial growth in samples that incorporated R_H + P or HYT + P + H was 50% less

than in C. Incorporating organic minerals Zn and Se alone reduced microbial deterioration by 15%. This mix was significantly effective at reducing the oxidative reactions of lipids and proteins by 40% and 50%, as measured after 9 and 12 mo of frozen storage, respectively. The addition of the natural extracts and Zn and Se did not adversely affect the acceptability of the meat product.

EAT LOQ Environmental life cycle assessment of different biorefinery platforms valorizing olive wastes to biofuel, phosphate salts, natural antioxidant, and an oxygenated fuel additive (triacetin)

Khounani, Z., *et al.*, *J. Clean. Prod.* 278: 123916, 2021, https://doi.org/10.1016/j.jclepro.2020.123916.

The olive agro-industry is a global, large-scale chemical- and energy-intensive agricultural activity in which byproducts and residues are not generally used and end up as waste. Therefore, designing and implementing innovative platforms based on circular bioeconomy and zero-discharge principles are critical to enhancing the sustainability of the olive supply chain system. We recently designed and compared two olive agro-biorefinery approaches with conventional olive oil production (i.e., olive/olive oil production, including olive cultivation and oil extraction). The first agro-biorefinery system included olive cultivation and oil extraction from fruit and pomace for edible use, with the remaining pomace post-oil extraction used as animal feed. The second agro-biorefinery system included olive cultivation, olive oil extraction, olive pomace oil extraction, and converting the pomace oil to biodiesel. Crude biodiesel glycerol was also valorized into two different phosphate salts and an oxygenated fuel additive (namely triacetin), while olive oil leaves were converted into a natural antioxidant for biodiesel. The performance of this natural antioxidant was also investigated and compared with the most promising synthetic antioxidant used by the biodiesel industry (i.e., propyl gallate). The environmental performance of the two biorefinery systems were compared using the IMPACT 2002+ method. Overall, both agro-biorefineries outperformed conventional olive oil production with respect to all four endpoint damage categories. Compared to conventional olive oil production, the production of 1 ton of olive oil in the first and second agro-biorefinery systems was associated with respective sav-

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ings of 10.62% and 4.08% in the climate change damage category, 6.73% and 11.21% in primary energy consumption, and 12.01% and 1.55% in damage to human health. Implementation of the two circular bioeconomy approaches also led to respective savings of 31.28% and 26.38% in the ecosystem quality damage category for Sc-2 and Sc-3. Overall, the first and second agro-biorefinery scenario achieved respective savings of 12.27% and 4.49% in the weighted endpoint damage categories of olive oil production. These findings revealed that transitioning from a linear economy-oriented olive agri-food system to a bioeconomy-oriented olive agro-biorefinery, would not only provide a wide spectrum of value-added bioproducts, but would also mitigate the environmental impacts from each ton of olive oil produced.

LOQ H&N Freeze-dried extract from olive leaves: valorization, extraction kinetics, and extract characterization

Kashaninejad, M., *et al.*, *Food Bioprod. Process. 124*: 196–207, 2020, https://doi.org/10.1016/j.fbp.2020.08.015.

Valorization of olive leaves (OL) in a biorefinery should include extraction of bioactive compounds, given the high content of extractives from this by-product. Extraction of bioactive compounds from Spanish OL (cultivar "Serrana de Espadán") was studied by conventional and ultrasound-assisted extraction (UAE). Faster extraction was observed by UAE, although similar final extraction yield was reached by both technologies. The best extraction solvent was an 80% ethanol hydroalcoholic mixture at a ratio of 20 mL per gram of dried OL (DOL). At these conditions, the highest content of oleuropein and luteolin-7-O-glucoside was determined as 31 ± 2 and 4.1 ± 0.2 mg/$g_{DO,}$ respectively. The power law and the Weibull models fit the total phenolic compounds extraction kinetics quite well. The major soluble carbohydrate was mannitol, with a content of 4.48 ± 0.09 mg/g_{DOL} in the extract. The influence of OL source was also studied, and it was concluded that the leaves collected as factory waste presented the highest phenolic yield and antioxidant capacity. The optimum extract was freeze-dried, resulting in a solid power with more than 11% of oleuropein and 17% of mannitol. Antioxidant activity of the freeze-dried extract was preserved for two months.

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EAT LOQ Development of pea flour-based active films produced through different homogenization methods and their effects on lipid oxidation

Yildiz, E., *et al.*, *Food Hydrocoll. 111*: 106238, 2021, https://doi.org/10.1016/j.foodhyd.2020.106238.

The objective of this study was to investigate the effects of gallic acid incorporation and homogenization (high-speed homogenization and ultrasonication) on the physicochemical characteristics of pea flour-based active films and the activity of the films against lipid oxidation. The water vapor permeability results of the active films increased by approximately 16% with the addition of gallic acid. Furthermore, active films had higher elongation and flexibility due to the plasticizing effect of gallic acid and an increase in the free volume of the film structure. Although the phenolic content of the films prepared by ultrasonication was higher than the ones prepared by high-speed homogenization, their antioxidant activities were the same. The films were immersed in a model food, olive oil, and stored at 40°C for 2 weeks. Peroxide value, TBARS, p-anisidine value, and totox analysis were carried out to test the effects of films on lipid oxidation. Active films reduced the formation of primary lipid oxidation products, hydrogen peroxides, up to 28%. In addition, the use of active films reduced totox values up to 20% as compared to control samples. Regardless of the homogenization method, gallic acid incorporation reduced olive oil oxidation significantly. Therefore, gallic acid incorporated pea flour films can be used as a packaging material to minimize the oxidation of susceptible foods.

LOQ H&N Chemical and biological activities of faveleira (*Cnidoscolus quercifolius Pohl*) seed oil for potential health applications

Ribeiro, P.P.C., *et al*, *Food Chem. 337*: 127771, 2021, https://doi.org/10.1016/j.foodchem.2020.127771.

Faveleira (*Cnidoscolus quercifolius*) is an emerging Brazilian plant, with seeds rich in edible oil. This study investigates physicochemical properties, chemical composition, thermal and oxidative stability, *in vitro* and *in vivo* toxicity, antioxidant, antinociceptive, and anti-inflammatory activities of faveleira seed oil. It was observed that the oil has low acidity, value of peroxide, chlorophyll, carotenoids, beta-carotene, and high concentrations of unsaturated fatty acids. In addition to presenting thermal and oxidative stability and high total phenolic content, vanillin, eugenol, and quercetin were predominating. The oil showed no toxicity *in vitro* and *in vivo*, and presented antioxidant, anti-inflammatory, and antinociceptive activities. These findings provide relevant and appropriate conditions for processing of faveleira seed oil as functional food.

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